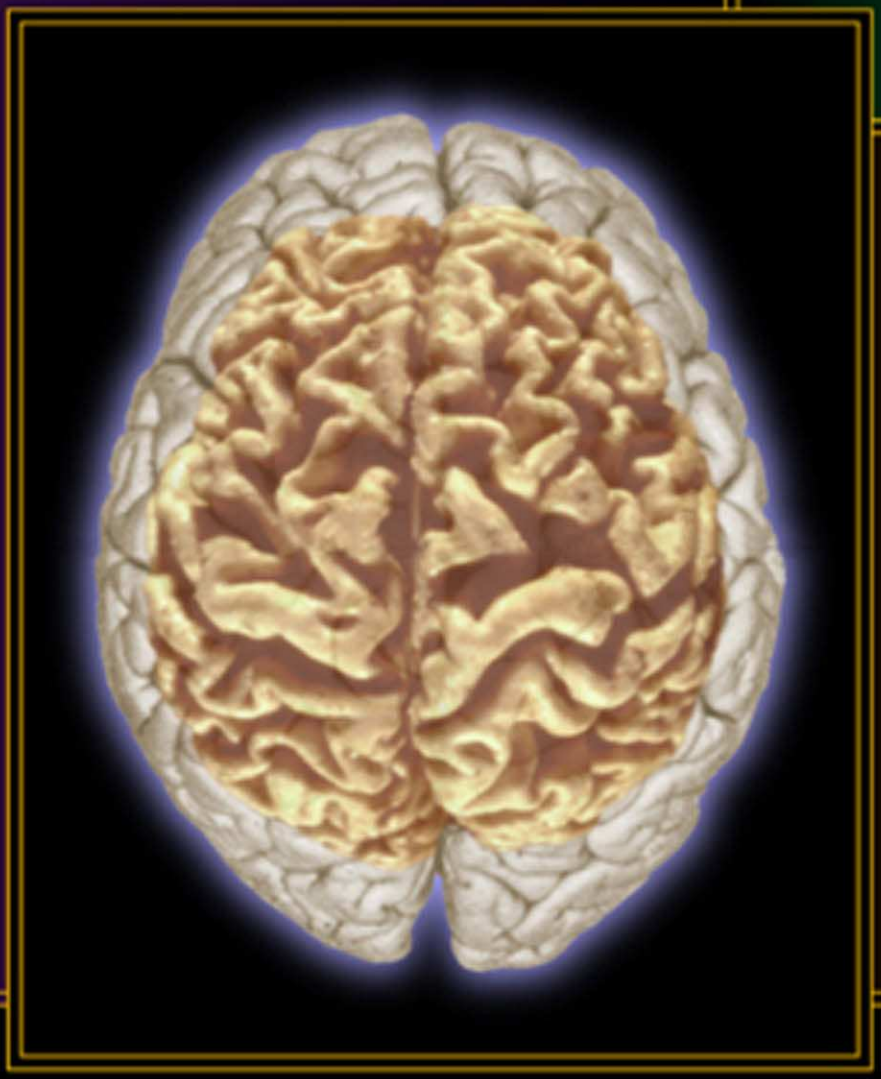

Functional Neurobiology of Aging

Patrick R. Hof
Charles V. Mobbs

FUNCTIONAL NEUROBIOLOGY OF AGING

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FUNCTIONAL NEUROBIOLOGY OF AGING

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Foreword

The present volume is an innovative resource on aging in the nervous system that reflects the considerable maturation of this new field. The authors are leading researchers of the many topics selected. The scope of the topics is broad, but while the editors have sought to be comprehensive, they did not sacrifice depth in the choice of some focused topics about which a great deal is known. This is altogether a commendable effort that should find wide use in academic courses and by the many researchers who have begun to recognize the importance of aging processes in many age-related conditions, conditions that have tended to be studied as specific disease entities but without considering the interactions of the disease with the changes of "usual" aging being taken into account.

Some historical perspectives seem pertinent here. One precursor of this book is James Birren's landmark monograph *Psychology of Aging* (1964), which synthesized the small but serious literature on cognitive and behavioral changes of normal aging in humans in relation to anatomical findings. Birren emphasized the general slowing of mental processes that was progressive across middle-age to later ages during normal aging, as distinct from pathological changes of senility. This slowing, which was observed in rodents and humans, might be attributed to the integrative level of complex circuits, because the conduction properties of axons did not show much change, for example, in the sciatic nerve of old rats (Birren and Wall, 1956). Neuron loss, which was generally assumed to be a major factor in brain aging, is now thought to be largely due to cerebrovascular disease or Alzheimer's disease.

Two other scholarly achievements in the biology of aging that interacted with Birren's book were Alex Comfort's *The Biology of Senescence*, which progressed through three editions (1957, 1964, and 1979), and the two editions of Bernard Strehler's *Time, Cells, and Aging* in 1962 and 1977. These three books are properly regarded as landmarks, and required heroic efforts to synthesize diverse sets of scattered and difficult information. We must mourn the recent death of Alex Comfort and praise the continued vitality of Jim Birren and Bernie Strehler. These books are part of the deep foundation upon which the present volume may be seen to rest. The books also had major importance for me when I was trying to design a Ph.D. project on aging under Alfred Mirsky at the Rockefeller University. Thesis projects were then often discussed with the august faculty of Rockefeller. I recall Peyton Rous saying something like "Why are you wasting your time on a subject like that? Everyone knows that aging is mainly about cancer and vascular disease." Perhaps Rous was thinking of Cazali's aphorism, "A man is as old as his arteries" (Critchley, 1931). But, by ferreting out the strongest papers cited by Birren, Comfort, and Strehler, I did convince Mirsky of biological mechanisms at work in aging that could not be accounted for by vascular disease or cancer, and so he supported my thesis project on the molecular endocrinology of responses to cold stress in aging mice (Finch *et al.*, 1969).

Thus began my own career in the neurobiology of aging, which led to the great pleasure of my working with Charles Mobbs, Giulio Pasinetti, and many other of the present authors who have built this field into its present thriving state. Of course, we must still keep in mind Peyton Rous's concerns about vascular contributions to brain aging!

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References

Birren, J. E. (1964) "The Psychology of Aging," Prentice Hall, New York.

Birren, J. E., and Wall, P. D. (1956). Age changes in conduction velocity, refractory period, number of fibers, connective tissue space, and blood vessels in sciatic nerve of rats. *J. Comp. Neurol.* **104**, 1–6.

Comfort, A. "The Biology of Senescence," 1st ed., 1957, Routledge and Kegan Paul; 2nd ed., 1964, Elsevier; 3rd ed., 1979, Elsevier.

Critchley, M. (1931). The neurology of old age. *Lancet*, 1331–1337.

Finch, C. E., Foster J. R., and Mirsky, A. E. (1969). Aging and the regulation of cell activities during exposure to cold. *J. Gen. Physiol.* **54**, 690–712.

Strehler, B. L. "Time, Cells, and Aging," 1st ed., 1962; 2nd ed., 1977. Academic Press, San Diego

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Preface

This book has been developed to meet our own needs, but is based on our perception that many others have also found these needs unmet by available resources. First, teaching our course on the neurobiology of aging, as well as tutorials, laboratory rotations, and similar exercises, was made much more difficult without a central source for the material contained in this book. Those teaching courses in neurology, neuropathology, and gerontology often face a similar problem. Second, like other researchers studying the neurobiology of aging, as well as physicians treating patients with age-related diseases, we have often needed to obtain an overview of areas outside our immediate area of expertise, but there has been no comprehensive source for such overviews. The present book was thus designed to address each of these needs and to be suitable as both a textbook for advanced students and a reference book for professionals.

Until recently the best general reference for those studying the neurobiology of aging would have been a text on neuropathology or neurology. Although there is obviously some overlap between the present book and such sources, these are not fully adequate for the purposes discussed above. Many neuropathological processes occur early in life and thus relevance to the aging brain is often unclear. Perhaps more important, a key concern in understanding the aging of any system is appreciating the difference between pathological and nonpathological processes. While this difference was formerly the subject of some controversy, there is now general agreement that the distinction between pathological and nonpathological processes is central to understanding and treating age-related impairments of any system, including neuronal systems. Therefore, the editors have worked closely with the contributors to clarify which age-related impairments are pathological and which are not. The nature of this distinction is made clear in many of the chapters, but often lies in the observation that pathological processes are not universal. Thus, as described in several chapters, the incidence of diseases peaks at some age, but the development of nonpathological processes continues to increase with age. This distinction has been particularly troublesome for Alzheimer's disease, the incidence of which peaks very late in life. Most investigators now feel that even for this disease, the incidence declines in extremely old populations.

To enhance the utility of the book, the chapters have been organized into four large sections, based on neurobiological functions that are most vulnerable during aging, a scheme that also provides the basis of the title of the book. Although clearly the information in the present book could be organized many different ways, we have chosen the functional approach to emphasize the relationship between observed age-related changes in neuronal properties and functional decline; thus we hope to avoid a mere cataloging of age-correlated changes. While perhaps other functional schemes could be proposed, our aim was to produce the most comprehensive resource on the neurobiology of aging available, and the scheme chosen was sufficiently broad to comfortably embrace this wealth of material. An additional introductory section was included to develop certain concepts particularly pertinent to aging in the specific context of age-related impairments in neurobiological function.

We acknowledge the role of Dr. James Roberts, whose vision instigated the process that led to this book; Dr. John Morrison, who was a constant source of encouragement and support; and the terrific staff of Academic Press, especially Craig Panner, Hilary Rowe, and Lori Asbury for their patience with the vagaries of academics and for actually crystallizing a process into a book. Finally, we take this opportunity to express our deepest gratitude to the superb scientists who have actually written this book. We have been fortunate to have had the cooperation of many of the leading investigators in the neurobiology of aging, and the quality of the present volume clearly attests to the conscientious efforts of these writers in producing a major resource for the field. We are all too aware that the effort of writing these chapters has necessarily distracted these scientists from their main job of creating new knowledge and deeply appreciate that despite this circumstance they made the time to impart their knowledge and wisdom to the rest of us.

Patrick R. Hof
Charles V. Mobbs

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SECTION I

Overview

A. Introduction to Concepts in Aging Research
(CHAPTERS 1–3)

B. Epidemiology of Neural Aging
(CHAPTER 4)

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Jack E. Riggs

1

Age-Specific Rates of Neurological Disease

I. Introduction

Is aging "normal" or "pathological?" The emerging answers to this simple question are so complex that the question has essentially been rendered semantic. Additionally, training biases profoundly affect the perspective from which this question is viewed and investigated. For example, clinicians typically view disease as a pathological process amenable to therapeutic intervention, but perceive aging (or more appropriately, senescence) as an inevitable process with limited prospects for significant manipulation. Clouding the question even further is the observation that many diseases are inseparably linked to aging.

Taking advantage of the varied perspectives, clues regarding the nature of aging can, should, and are being sought using a multitude of approaches. Epidemiological studies of aging and disease traditionally give descriptive measures of disease burden and attempt to identify risk factors associated with aging-related disorders. Analysis and interpretation of age-specific disease rates, however, are relatively underappreciated vantage points from which to view aging. A preliminary note of caution is warranted. Age-specific rates deal with populations. Consequently, conclusions derived from the analysis of age-specific rate dynamics are applicable to populations, not to individuals, and especially not to the molecular processes involved in aging. This limitation, however, may have some powerful practical advantages. This chapter focuses on age-specific rates of neurological disease. The major neurological disorders associated with aging are Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, primary malignant brain tumors, and stroke.

II. Age-Specific Rates

An age-specific rate (R_x) is defined as the number of occurrences of a certain event at a specific age (N_x) divided by the total number of individuals (P_x) alive at that age. This relationship can be expressed as

$$R_x = \frac{N_x}{P_x}. \quad (1)$$

The two commonly measured age-specific rates, incidence and mortality rates, are often used to measure and compare disease burden over time as they are assumed to be independent of changes in the age structure of a population (Hennekens and Buring, 1987), i.e., an age-specific mortality rate should be independent of the relevant age group population size (Fig. 1.1). For example, the mortality rate from primary malignant brain tumor among U.S. men aged 85 years and older should not be dependent on whether there are 1000 or 1,000,000 individuals in that age group.

III. Age-Specific Rates of Neurological Disease

Epidemiological studies in the elderly of developed nations over recent decades have documented an increasing frequency of Alzheimer's disease (Plum, 1979; Katzman, 1986; Fox, 1989; Centers for Disease Control, 1991), amyotrophic lateral sclerosis (Durrleman and Alpérovitch, 1989; Lilienfeld *et al.*, 1989; Chancellor and Warlow, 1992), Parkinson's disease (Duvoisin and Schweitzer, 1966; Kurtzke and Murphy, 1990; Lilienfeld *et al.*, 1990; Chio *et al.*, 1993c; Clarke, 1993), and primary malignant brain tumor (Davis and Schwartz, 1988; Helseth *et al.*, 1988; Boyle *et al.*, 1990; Greig *et al.*, 1990; Davis *et al.*, 1991). Unlike these neurodegenerative disorders, the burden of stroke, especially as measured by mortality, has declined significantly in recent decades (Whisnant, 1984; Klag *et al.*, 1989).

A. Alzheimer's Disease

Age-specific incidence rates of Alzheimer's disease increase exponentially with age (Sayetta, 1986; Hebert *et al.*, 1995; Gao *et al.*, 1998; Jorm and Jolley, 1998). This exponential rate of increasing incidence slows down after the eighth or ninth decade of life (Gao *et al.*, 1998), although there is no actual decline in the incidence rate (Fig. 1.2). Age-specific mortality rates from Alzheimer's disease also increase exponentially with increasing age, but fail to maintain that exponential rate of increase at an earlier age than that seen with age-specific

FIG. 1.1. An age-specific mortality rate is generally considered to be independent of the relevant age group population size; i.e., an age-specific mortality rate should not depend on whether the relevant age group population size is 1000 or 1,000,000.

incidence rates (Imaizumi, 1992). A limitation of Alzheimer's disease mortality data is that dementia is not consistently reported on death certificates (Ganguli and Rodriguez, 1999).

B. Amyotrophic Lateral Sclerosis

Age-specific incidence rates of amyotrophic lateral sclerosis also demonstrate approximately exponential increases with age until the seventh or eighth decade of life (Kahana *et al.*, 1976; Kurtzke, 1982; Leone *et al.*, 1987; Lopez-Vega *et al.*, 1988; Durrleman and Alpérovitch, 1989; Tysnes *et al*., 1991; Brooks, 1996). The age-specific incidence pattern of amyotrophic lateral sclerosis is similar to that seen in Alzheimer's disease (Fig. 1.2). Amyotrophic lateral sclerosis age-specific mortality rates also increase exponentially with increasing age and (as with Alzheimer's disease) fail to maintain that exponential rate of increase at an earlier age than that seen with age-specific incidence rates (Leone *et al.*, 1987; Riggs, 1990b; Chio *et al.*, 1993b; Neilson *et al.*, 1994). Unlike the situation with Alzheimer's disease, mortality due to amyotrophic lateral sclerosis is consistently reported on death certificates (Hoffman and Brody, 1971; Chio *et al.*, 1993a).

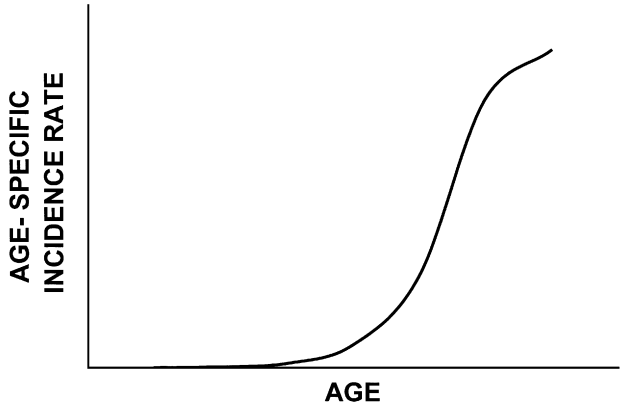

FIG. 1.2. Age-specific mortality rates for most aging-related neurologic disorders demonstrate a similar pattern. Age-specific mortality rates will rise initially at an exponential rate with increasing age. After some age, age-specific mortality rates will increase at a slower rate, plateau, or decline.

C. Parkinson's Disease

Age-specific incidence rates of Parkinson's disease also increase rapidly with increasing age, then decrease after the eighth or ninth decade of life (Morens *et al.*, 1996). This age-specific incidence pattern is also similar to that seen in Alzheimer's disease and amyotrophic lateral sclerosis (Fig. 1.2). Age-specific mortality rates of Parkinson's disease also increase exponentially with increasing age, but again fail to maintain that exponential rate of increase at an earlier age than that seen with age-specific incidence rates (Riggs, 1990c; Bonifati *et al.*, 1993; Imaizumi, 1995). A limitation of Parkinson's disease mortality data is that parkinsonism may not be consistently reported on death certificates as 15 to 25% of patients are only identified in screening survey studies (de Rijk *et al.*, 1995, 1997).

D. Primary Malignant Brain Tumor

Age-specific incidence rates of primary malignant brain tumor also demonstrate rapid increases with age until the seventh or eighth decade of life and then drop off or decline (Sutherland *et al.*, 1987; Helseth *et al.*, 1988; Sant *et al.*, 1988; Ahsan *et al.*, 1995; Polednak and Flannery, 1995; Kuratsu and Ushio, 1997). The age-specific incidence pattern of primary malignant brain tumor is similar to that seen in Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease (Fig. 1.2). Age-specific mortality rates of primary malignant brain tumor also increase exponentially with increasing age and also fail to maintain that exponential rate of increase after an age that is less than the peak incidence rate (Riggs, 1991a, 1995a).

E. Stroke

Age-specific incidence rates of stroke show a dramatic exponential increase with increasing age (Robins and Baum, 1981). No decline in the exponential rate of increase in stroke incidence occurs before the ninth decade of life. Age-specific mortality rates of stroke also increase exponentially with increasing age (Riggs, 1990d). Unlike neurodegenerative disorders, age-specific stroke mortality does not deviate from increasing exponentially with increasing age before the ninth decade of life (Riggs, 1990d).

IV. Age-Specific Rates and Mortality Dynamics

Age-specific rates of neurological disease can provide more than just a descriptive measure of the societal burden of neurological disorders in an aging population. Age-specific rates are a window of opportunity for understanding mortality dynamics associated with aging at the population level.

A. Deviation from Gompertzian Mortality Dynamics

Gompertz (1825) initially described the exponentially increasing risk of mortality with increasing age. Age-specific mortality, however, is consistently lower than that predicted

by a constant exponential increase at older ages (Juckett and Rosenberg, 1993). This observation is true for both age-specific general mortality and age-specific mortality from many neoplasms and degenerative disorders associated with aging (Riggs, 1993c). The basis for this deviation from a constant exponential increase between age-specific mortality and increasing age may relate to the definition of age-specific mortality rate, which is the number of individuals dying from a particular disease at a specific age divided by the number of individuals alive at that age [Eq. (1)]. The numerator includes individuals susceptible to that particular disease as evidenced by the fact that their death was attributed to that disease. The denominator includes all individuals, both susceptible and nonsusceptible, alive at a specific age. Human populations are quite heterogeneous in their susceptibilities to various diseases. For example, some individuals are inherently more prone to developing Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, or primary malignant brain tumor than are others.

The rate of exponential increase in age-specific mortality with increasing age in amyotrophic lateral sclerosis (Riggs, 1990b), Parkinson's disease (Riggs, 1990c), and primary malignant brain tumor (Riggs, 1991a) is much greater than the rate of exponential increase in age-specific general mortality with increasing age (Riggs, 1990a). Therefore, individuals susceptible to these neurological disorders in the population are depleted at a faster rate than is the general population. As a mathematical consequence, age-specific mortality rates must deviate from constant exponential (or Gompertzian) dynamics after a certain age (Fig. 1.2). The age at which this deviation occurs varies for different disorders depending on the relative size of the susceptible population subset and the rate at which that population subset is depleted. Thus, deviation of Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, and primary malignant brain tumor age-specific mortality rates at older ages from that predicted by Gompertzian dynamics is primarily a mathematical phenomenon rather than an indicator of failure to describe the biological relationship between aging and mortality (Riggs, 1993c).

B. The Increasing Burden of Neurodegenerative Disease

The importance of neurological disease in old age is not merely a reflection of the dramatic increase in the number of elderly individuals. The frequency of neurological disorders in the elderly has also increased dramatically. As examples of this rising burden of neurological disease, mortality rates among U.S. men aged 85 years and older increased 328% for amyotrophic lateral sclerosis between 1977 and 1986 (Riggs, 1990b), 411 for Parkinson's disease between 1955 and 1986 (Riggs, 1990c), and 924% for primary malignant brain tumor between 1962 and 1987 (Riggs, 1991a). Does this increasing frequency and burden of neurologic disease in the elderly reflect better case ascertainment from improved diagnostic capabilities, greater interest and knowledge among physicians, and increased environmental pathogenic influences associated with industrialization (Katzman, 1986; Davis and Schwartz, 1988; Durrleman and Alperovitch, 1989; Fox, 1989; Lilienfeld *et al.*, 1989, 1990; Boyle *et al.*, 1990; Davis *et al.*, 1991; Chancellor and Warlow, 1992; Modan *et al.*, 1992; Clarke, 1993)? These factors, although valid, may not entirely account for the increasing frequency of neurological disease in the elderly.

The aging population has had a significant impact on the societal burden from several neurological disorders such as Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, and primary malignant brain tumor. This increased burden of neurologic disease in the elderly is the result of more than just the dramatically increasing number of elderly individuals. The profound demographic change in the age structure of populations in developed nations is primarily the result of increasing survival. ''Differential survival'' over time, and its effect of less selective culling of the surviving population gene pool, is an additional explanation for the increasing frequency of several neurologic diseases associated with senescence.

From a historical perspective, old age or senescence should be considered an unnatural state. Under primitive conditions, it is quite uncommon to live to old age. Medawar (1952) made this point eloquently in his classic lecture delivered at University College London in 1951 when he stated that senescence ''is in a real and important sense an artefact of domestication; that is, something revealed and made manifest by the most unnatural experiment of prolonging an animal's life.'' In several respects, humankind can be considered the most domesticated member of the animal kingdom. Advancing civilization has been associated with a fivefold increase in human life expectancy since the stone age and a corresponding increase in the burden of disorders of senescence (Riggs, 1993b). Refining this position, a recent *Lancet* editorial (Editorial, 1993) stated that modern ''civilization . . . is not the cause of our chronic diseases . . . it merely unveiled what our genes had lurking in store for us for centuries, if not millennia, as we now live long enough to see these genes massively expressing themselves.''

In the United States in 1900, there were only 3 million people over 65 years old and just 72,000 individuals over 85 years old (Olshansky *et al.*, 1993). By 1996, those corresponding numbers had increased to 33.3 million and 2.2 million (Olshansky *et al.*, 1993). Currently, the oldest old, consisting of those individuals aged 85 years and older, are the fastest growing segment of the population (Suzman *et al.*, 1992). Indeed, between 1951 and 1989, the size of this population subset in the United States grew at a staggering annual rate of 3.14% in men and 4.94% in women (Riggs, 1996a).

Age-specific mortality rates provide an important clue to understanding the increasing burden of neurological disease in the elderly. Because age-specific rates are generally assumed to be independent of the age structure of a population, mortality rates among individuals aged 85 years and older from amyotrophic lateral sclerosis, Parkinson's disease, or primary malignant brain tumor would generally be considered to be independent of the size of that population subset. However, mortality rates among individuals aged 85 years and older (and also other elderly age groups) from amyotrophic lateral sclerosis (Riggs, 1990b), Parkinson's disease (Riggs, 1990c), and primary malignant brain tumor (Riggs, 1991a, 1995a) were directly correlated with the population size of that age group (Riggs, 1996a). Thus, mortality rates from these neurological diseases among ''the oldest old'' were directly

FIG. 1.3. Age-specific mortality rates (per 100,000) from primary malignant brain tumor among U.S. men aged 85 years and over during the years 1962 to 1989 (adapted from data in Riggs, 1995a). Note the direct correlation between the age-specific mortality rate and the relevant age group population size (in 100,000's).

correlated with the size of that age group population subset. Similarly, increasing mortality in the elderly from many other diseases, such as lung cancer (Riggs, 1995b), ovarian cancer (Riggs, 1995c), and multiple myeloma (Riggs, 1995d), demonstrated an increasing dependency with increasing age on increasing age group population size. Thus, in certain aging-related diseases, age-specific mortality rates are a function of age group population size at older ages (Fig. 1.3). This relationship can be expressed as

$$(R_x) = f(P_x). \tag{2}$$

This dependence of age-specific mortality rates on age group population size suggests that something intrinsic about the elderly population is changing, which may account for the observed increasing burden of disease. That intrinsic "something" may be changing genetic susceptibility at the population level. Despite being matched for age and gender, age-specific mortality rate comparisons can be misleading if populations are not also matched genetically (Riggs, 1994). Is it possible that sufficient differences in genetic susceptibility to certain neurological diseases between successive elderly population cohorts could occur over the span of just a few years to have contributed to the substantially increasing burden of those disorders?

Charles Darwin (1859) deduced that in the "struggle for existence, it follows that any being, if it vary however slightly in any manner profitable to itself, under the complex and sometimes varying conditions of life, will have a better chance of survival." Accordingly, survival is not random, and competition for survival will result in "survival of the fittest" (or differential survival). Evolution, by means of differential survival (or, more accurately, differential reproduction), and natural selection occur over a very long time scale. However, the selective culling effect of differential survival on the surviving gene pool of an aging population cohort occurs immediately (Riggs, 1996b). When an individual in a population dies, the remaining population gene pool of that age cohort is immediately altered (Riggs, 1996b). As a result of differential survival, the surviving gene pool, and hence disease susceptibilities, of a population cohort will become progressively altered. The decreasing frequency of the apolipoprotein E $\epsilon 4$ allele with aging (Davignon *et al.*, 1987; Cauley *et al.*, 1993) provides direct evidence that differential survival does alter the surviving gene pool. As adversity to survival in developing nations decreases, individuals survive longer, and the aggregate genetic susceptibility to diseases, including certain neurological disorders, among aging survivors will change. Indeed, if the aggregate gene pool of a surviving elderly cohort did not change as members of that cohort died, this would imply that disease and death occur randomly with respect to genetic influences. The thesis that differential survival in human populations impacts on disease patterns is based on two fundamental principles. First, human populations are genetically heterogeneous. Second, genetic factors influence disease susceptibility. Both of these principles have been verified repeatedly by molecular genetic investigations. Indeed, human populations are genetically heterogeneous in their susceptibility to Alzheimer's disease (Katzman and Saitoh, 1991), amyotrophic lateral sclerosis (Mulder *et al.*, 1986), Parkinson's disease (Kondo and Kurland, 1973), and primary malignant brain tumor (Choi *et al.*, 1970).

The increasing age structure of the world's population primarily reflects enhanced survival due to declining mortality (Olshansky *et al.*, 1993). Enhanced survival has resulted in a several hundred percent increase in the population size of the oldest age groups in the United States over the past four decades (Riggs, 1996a). The magnitude of these demographic changes is sufficient to have contributed significantly to the increasing burden and frequency of neurological disease in the elderly population. As more members of a given cohort survive into old age, there has been less "selection" with respect to the surviving gene pool and a resultant increased genetic propensity to the disorders of senescence (Medawar, 1952; Riggs, 1992a, 1996b).

C. Longitudinal Gompertzian Analysis

Gompertz (1825) described a mathematical relationship between human aging and mortality. Gompertz noted that mortality rose exponentially with increasing age. Because mortality rates are expressed conventionally as deaths per 100,000 population, the Gompertz relationship may be expressed as

$$R_x = R_o(10)^{\alpha x}, \tag{3}$$

where R_x is the mortality rate at age x, R_o is the extrapolated death rate at birth, and α is the slope of the exponential term. The Gompertz equation becomes a linear function when expressed logarithmically:

$$\log R_x = \alpha x + \log R_o. \tag{4}$$

Strehler and Mildvan (1960) introduced the following modification to the Gompertz relationship:

$$B = \frac{\alpha}{\log(K/R_o)}. \tag{5}$$

In this equation, Strehler and Mildvan (1960) defined B as the fractional loss of vitality and K as a proportionality constant that relates the vitality of an organism and the magnitude of

environmental challenges to the mortality rate. Rearranging Eq. (5) yields

$$\alpha = -B(\log R_o) + B\,(\log K). \tag{6}$$

Implicit in the Strehler–Mildvan modification of the Gompertz model of aging and mortality [Eq. (5)], assuming that both B and K are constant, is the prediction of a negative linear relationship between α and log R_o and that annual age-specific mortality rate distributions will intersect at a single, fixed point (Riggs, 1990a). Moreover, this intersect point will occur at the point where age equals $1/B$ and log R_x equals log K. Longitudinal Gompertzian analysis has validated this prediction using mortality data for many aging-related degenerative disorders (Riggs, 1990b,c) and neoplasms (Riggs, 1991a,b,c). Because disease-specific values of B have been constant for every disorder studied, B has been postulated to represent some aggregate measure of genetic influences on age-specific mortality (Riggs, 1991b,c, 1992c). The Strehler–Mildvan modification of the Gompertzian mortality rate distribution conforms to thermodynamic interpretation (Lestienne, 1988). Using a thermodynamic perspective, Atlan (1968) demonstrated that the rate of decline of vitality with age (the quantity B introduced by Strehler and Mildvan) can be conceptualized in terms of entropy. Consequently, longitudinal Gompertzian analysis can be used to determine B, which is an aggregate measure of the rate of increase in genomic entropy for those genetic factors involved in aging-related mortality. Associated with declining mortality, the values of K for cervical cancer (Riggs, 1992b), emphysema (Riggs, 1992c), and stroke (Riggs and Myers, 1994) have declined significantly. Associated with increasing mortality, the value of K for non-Hodgkin's lymphoma has increased significantly (Riggs, 1993a). Consequently, K is not necessarily constant and has been postulated to represent some aggregate measure of environmental influences on age-specific mortality (Riggs, 1992b,c, 1993a). Thus, longitudinal Gompertzian analysis suggests that age-specific mortality rate distributions are determined by the interaction of genetic and environmental influences.

1. Parkinson's Disease Mortality

Parkinson's disease mortality is particularly useful in illustrating the method of longitudinal Gompertzian analysis. Age-specific mortality rates in the United States due to Parkinson's disease from 1955 to 1988 have been determined previously (Riggs, 1990c, 1991d, 1992d) from federal sources (National Center for Health Statistics, 1955–1988). Deaths were recorded for the following age groups (in years); less than 1, 1–4, 5–9, 10–14, 15–19, 20–24, 25–29, 30–34, 35–39, 40–44, 45–49, 50–54, 55–59, 60–64, 65–69, 70–74, 75–79, 80–84, and 85 and over. In the analysis of these data, the mortality rate for each age group was assigned to the mean age of the group. By using the number of deaths in each age group and population size estimates of the corresponding age group, annual age-specific Parkinson's disease mortality rates were determined.

The initial step in longitudinal Gompertzian analysis is to determine if mortality increases exponentially with age (Riggs, 1990a). Parkinson's disease age-specific mortality rates plotted on a logarithmic scale for U.S. men in 1988 are shown in Fig. 1.4. Parkinson's disease log R_x appears to increase linearly between age 45 and 80 years (Fig. 1.4). Using linear regression to analyze the relationship between log R_x and age between age 45 and 80 years yields an r-squared value greater than 0.98 (where r is the correlation coefficient of the linear regression). Therefore, in 1988 the relationship between Parkinson's disease log R_x and age among U.S. men aged 45 to 80 years was highly linear and, thus by definition, Gompertzian. From Eq. (3), the values for α and log R_o are also derived using linear regression. For Parkinson's disease mortality among U.S. men in 1988 between age 45 and 80 years, the following relationship is derived:

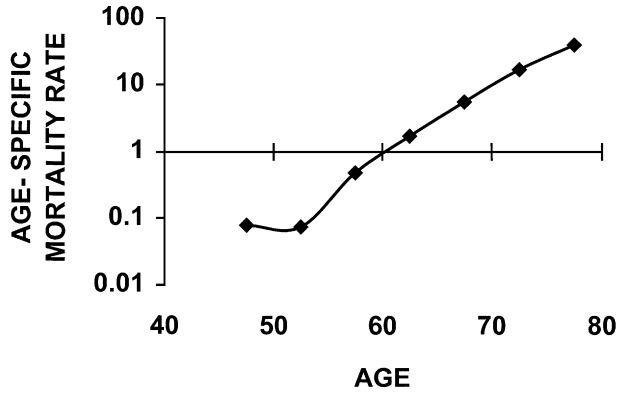

FIG. 1.4. Age-specific Parkinson's disease mortality rates (per 100,000). among U.S. men in 1988 plotted on a logarithmic scale. Note the essentially constant exponential rate of increase in age-specific mortality rates between ages 45 and 80 years.

$$\log R_x = 0.107421x - 6.58931. \tag{7}$$

Values of α and log R_o for Parkinson's disease mortality among U.S. men between 1955 and 1988 have been determined previously (Riggs, 1990c, 1991d, 1992d).

The next step in longitudinal Gompertzian analysis is to determine the relationship between α and log R_o for different years (Riggs, 1990a). The plot of α versus log R_o for Parkinson's disease among U.S. men between 1955 and 1988 (Fig. 1.5) demonstrates the linear negative relationship predicted by the Strehler–Mildvan modification of the Gompertz relationship. Using linear regression to analyze the relationship between α and log R_o (Fig. 1.5), the following equation regarding Parkinson's disease mortality among U.S. men is derived:

$$\alpha = -0.01365 \log R_o + 0.016998. \tag{8}$$

The r-squared value for the linear regression defining Eq. (8) is greater than 0.98. A potential mathematical explanation for this highly linear negative correlation between α and log R_o is that when data from repeated studies that are actually measuring the same phenomenon are analyzed using linear regression, a natural negative linear relationship between the slope and intercept values will emerge spontaneously (Riggs and Hobbs, 1998). However, if this mathematical explanation was the reason for this observed linear negative relationship, the sequence of annual α and log R_o values would be random.

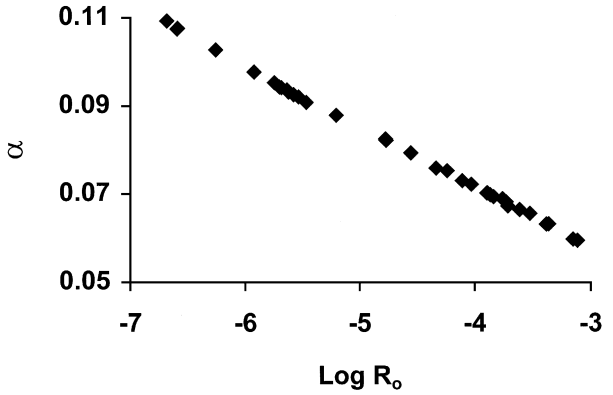

FIG. 1.5. Plot of annual pairs of α and log R_o values for Parkinson's disease mortality among U.S. men aged 45 to 80 years from 1955 to 1988. Note the negative linear relationship between α and log R_o.

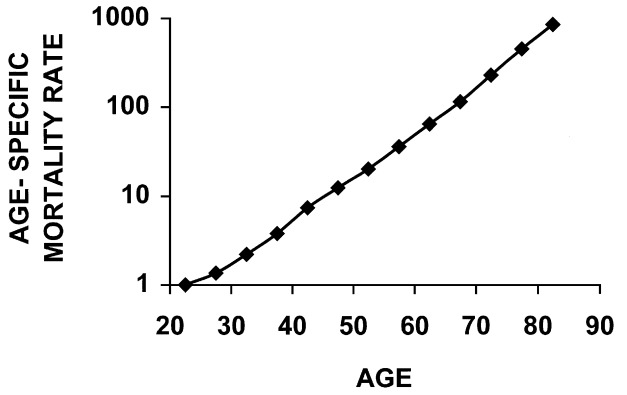

FIG. 1.6. Age-specific stroke mortality rates (per 100,000) among U.S. white men in 1988 plotted on a logarithmic scale. Note the constant exponential rate of increase in age-specific mortality rates between ages 20 and 85 years.

However, the sequence annual α and log R_o data points are not random, but rather are highly ordered (Riggs and Hobbs, 1998).

Because Eq. (8) is in the same format as Eq. (6), the value for B with respect to Parkinson's disease mortality among U.S. men is directly determined. Accordingly, the value of B for Parkinson's disease mortality in U.S. men was 0.01365. Thus, the annual rate of increase in "genomic entropy" between age 45 and 80 years with respect to Parkinson's disease mortality was 1.365% for U.S. men.

2. Stroke Mortality

Longitudinal Gompertzian analysis provides an insightful method of analyzing the trend of declining stroke mortality. Age-specific mortality rates among white men in the United States due to stroke from 1951 to 1988 have been determined previously (Riggs and Myers, 1994) from federal sources (National Center for Health Statistics, 1955–1988). Age-specific stroke mortality rates plotted on a logarithmic scale for U.S. white men in 1988 increase linearly between age 20 and 85 years (Fig. 1.6). Using linear regression to analyze the relationship between log R_x and age between age 20 and 85 years yields an r-squared value greater than 0.99 (where r is the correlation coefficient of the linear regression). Therefore, in 1988 the relationship between log R_x and age for stroke mortality among U.S. white men between age 20 and 85 years was highly linear and, thus by definition, Gompertzian. From Eq. (4), the values for α and log R_o are also derived by linear regression analysis. For example, for stroke mortality among U.S. white men in 1988 between age 20 and 85 years, the following relationship is derived:

$$\log R_x = 0.049429x - 1.23334. \tag{9}$$

Linear regression analysis of the relationship between log R_x from stroke and age between age 20 and 85 years for U.S. white men for the years 1951 through 1988 yields corresponding α and log R_o values for each year (Riggs and Myers, 1994). The corresponding r-squared value for each pair of α and log R_o was greater than 0.99 for every year.

The next step in longitudinal Gompertzian analysis is to determine the relationship between α and log R_o for the different years (Riggs, 1990a). The plot of α versus log R_o for U.S. white men aged 20 to 85 years for the years 1951 through 1988 (Fig. 1.7) does not demonstrate the linear negative relationships seen in many Gompertzian diseases (Fig. 1.5). However, the pattern displayed in Fig. 1.7 is not random. When the annual pairs of α and log R_o values for mortality due to stroke among U.S. white men are connected sequentially (Fig. 1.8), a distinctive pattern is seen. This same pattern was seen when cervical cancer (Riggs, 1992b) and emphysema (Riggs, 1992c) mortalities were analyzed by longitudinal Gompertzian analysis. The pattern in Fig. 1.8 is produced by a function that has a constant slope and a decreasing Y intercept (Riggs, 1992b,c). The slope of the function that relates α and log R_o, from Eq. (6), is equal to $-B$. Because B is always constant, a new value C can be introduced for the term B (log K) in Eq. (6) and be expressed by the equation:

$$C = B(\log K), \tag{10}$$

where C is equal to the varying "Y intercept" in Fig. 1.8. Consequently, Eq. (6) can be written as

$$\alpha = -B(\log R_o) + C. \tag{11}$$

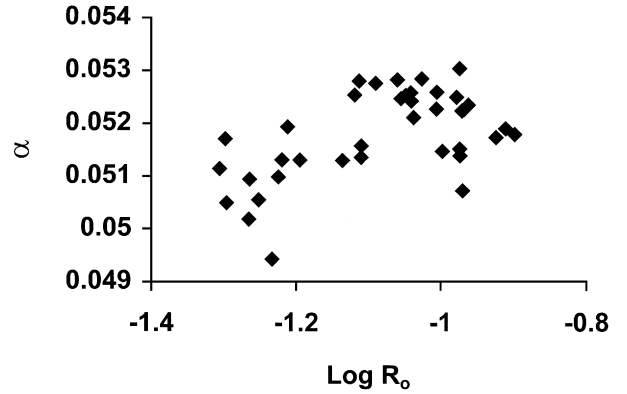

FIG. 1.7. Plot of annual pairs of α and log R_o values for stroke mortality among U.S. white men aged 20 to 85 years from 1951 to 1988. Note the apparent lack of any relationship between α and log R_o.

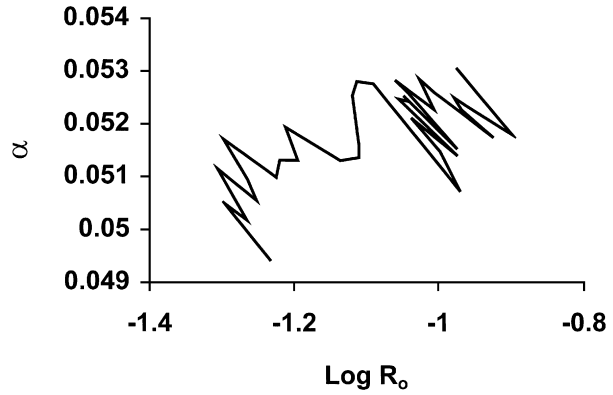

FIG. 1.8. Plot of annual pairs of α versus log R_o for stroke mortality among U.S. white men aged 20 to 85 years from 1951 to 1988 when data pairs (as shown in Fig. 1.7) are connected sequentially. Note that this distinctive nonrandom pattern can be produced by a function interrelating α and log R_o, which has a constant negative linear slope, as shown in Fig. 1.5, and a decreasing Y intercept. The 1951 data point is at the upper right. The 1988 data point is at the lower left.

A method of determining B when C is changing over time was defined in a study using longitudinal Gompertzian analysis of cervical cancer mortality (Riggs, 1992b). The slope [which from Eq. (11) is equal to $-B$] between consecutive pairs of α and log R_o values for stroke mortality in U.S. white men was calculated (Riggs and Myers, 1994). The slope for year N is defined by the following equation (Riggs, 1992b)

$$(-B)_N = \frac{\alpha_{N+1} - \alpha_N}{(\log R_o)_{N+1} - (\log R_o)_N}. \qquad (12)$$

The annual plots of $-B$ for stroke mortality among U.S. white men is shown in Fig. 1.9. As can be seen, there is an underlying dominant or most frequent value of $-B$. From Fig. 1.9, it is also apparent that there are outlier values of $-B$. The basis for these outlier values is inherent in the definition of slope, which is defined as the change in Y (or α) divided by the change in X (or log R_o). As the change in X becomes small, the absolute value of the slope may become large. Thus, the outlier values of $-B$ in Fig. 1.9 occur at points when there were small changes in consecutive values of log

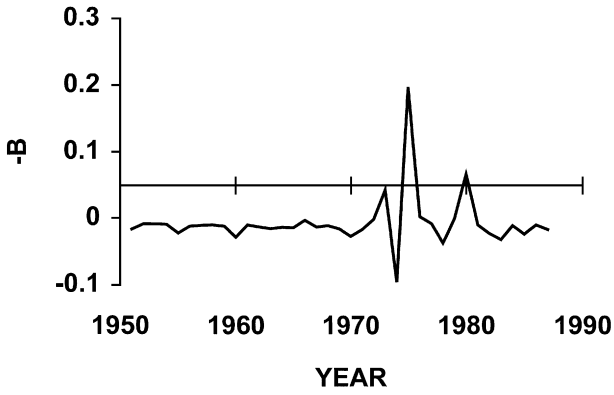

FIG. 1.9. Plot of $-B$ for mortality from stroke among U.S. white men aged 20 to 85 years from 1951 to 1987.

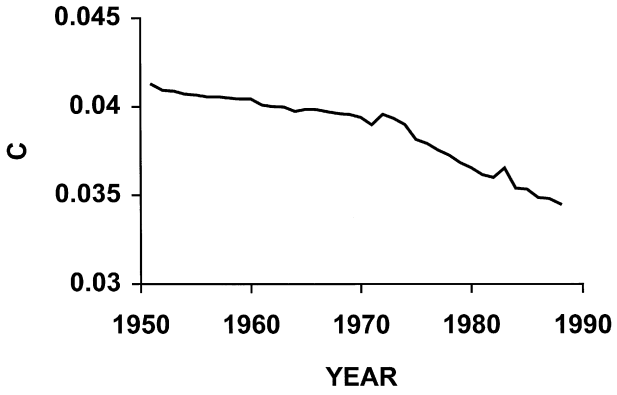

FIG. 1.10. Plot of C for mortality from stroke among U.S. white men aged 20 to 85 years from 1951 to 1988.

R_o. Because the values of $-B$ tend to be small and outlier values of $-B$ can be large, the mean is not a good method of determining the underlying dominant value of $-B$. Instead, the median value of $-B$ is used as the best estimate for the underlying constant value of $-B$ (Riggs, 1992b). The median value of $-B$ for stroke mortality among U.S. white men aged 20 to 85 years was -0.01211 (Riggs and Myers, 1994). Accordingly, the value of B for stroke mortality among U.S. white men was 0.01211. Thus, the annual rate of increase in "genomic entropy" between age 20 and 85 years with respect to stroke mortality was 1.211% for U.S. white men.

Annual values of C for stroke mortality among U.S. white men aged 20 to 85 years between 1951 and 1988 were determined using this median value of $-B$ and Eq. (11). The annual values of C are shown in Fig. 1.10. Thus, age-specific stroke mortality rates among U.S. white men aged 20 to 85 years between 1951 and 1988 conformed to the dynamics predicted by the Strehler–Mildvan model of aging and mortality in which the value of log K (or C) was declining over time. Between 1951 and 1988, etiopathogenic influences (as reflected by C values) among U.S. white men with respect to stroke mortality declined 16.4%.

V. Commentary

Newtonian mechanics was used to assist humankind to successfully navigate to the Moon and back. Newtonian mechanics, however, does not provide insight as to the "true" nature of gravity. Indeed, Newtonian mechanics ignores the heterogeneous atomic composition of large masses and the quantum mechanical interactions between the atoms making up those masses. Newtonian mechanics, nevertheless, is a very functional and practical perspective from which to observe and gain insight into gravitational forces, despite the fact that the "true" nature of the force of gravity remains unknown.

The relationship between age-specific rates and aging may be similar. The molecular interactions involved in aging are so complex that understanding the "true" nature of senescence will be extremely difficult. However, examination and analysis at the macroscopic level of age-specific rates may provide a very functional and practical perspective from which to observe and gain insight into the process of aging.

Finally, is aging ''normal'' or ''pathological?'' Perhaps the ''simple'' correct answer to this complex question is that aging is both ''normal'' and ''pathological''.

References

Ahsan, H., Neugut, A. I., and Bruce, J. N. (1995). Trends in incidence of primary malignant brain tumors in USA, 1981–1990. *Int. J. Epidemiol.* **24**, 1078–1085.

Atlan, H. (1968). Strehler's theory of mortality and the second principle of thermodynamics. *J. Gerontol.* **23**, 196–200.

Bonifati, V., Vanacore, N., Bellatreccia, A., and Meco, G. (1993). Mortality rates for Parkinsonism in Italy (1969 to 1987). *Acta Neurol. Scand.* **87**, 9–13.

Boyle, P., Maisonneuve, P., Saracci, R., and Muir, C. S. (1990). Is the increased incidence of primary malignant brain tumors in the elderly real? *J. Natl. Cancer. Inst.* **82**, 1594–1596.

Brooks, B. R. (1996). Clinical epidemiology of amyotrophic lateral sclerosis. *Clin. Neurol.* **14**, 399–420.

Cauley, J. A., Eichner, J. E., Kamboh, M. I., Ferrell, R. E., and Kuller, L. H. (1993). Apo E allele frequencies in younger (age 42–50) vs older (age 65–90) women. *Genet. Epidemiol.* **10**, 27–34.

Centers for Disease Control. (1991). Mortality from Alzheimer's disease—United States, 1979–1987. *JAMA, J. Am. Med. Assoc*. **265**, 313, 317.

Chancellor, A. M., and Warlow, C. P. (1992). Adult onset motor neuron disease: Worldwide mortality, incidence, and distribution since 1950. *J. Neurol. Neurosurg. Psychiatry* **55**, 1106–1115.

Chio, A., Magnani, C., Oddenio, E., Tolardo, G., and Schiffer, D. (1993a). Accuracy of death certificate diagnosis of amyotrophic lateral sclerosis. *J. Epidemiol. Commun. Health* **46**, 517–518.

Chio, A., Magnani, C., and Schiffer, D. (1993b). Amyotrophic lateral sclerosis mortality in Italy, 1958 to 1987: A cross-sectional and cohort study. *Neurology* **43**, 927–930.

Chio, A., Magnani, C., Tolardo, G., and Schiffer, D. (1993c). Parkinson's disease mortality in Italy, 1951 through 1987. Analysis of an increasing trend. *Arch. Neurol*. **50**, 149–153.

Choi, N. W., Schuman, L. M., and Gullen, W. H. (1970). Epidemiology of primary central nervous system neoplasms. II: Case-control study. *Am. J. Epidemiol.* **91**, 467–485.

Clarke, C. E. (1993). Mortality from Parkinson's disease in England and Wales 1921-89 *J. Neurol., Neurosurg. Psychiatry.* **56**, 690–693.

Darwin, C. (1859). ''On the Origin of Species.'' John Murray, London.

Davignon, J., Bouthillier, D., Nestruck, A. C., and Sing, C. F. (1987). Apolipoprotein E polymorphism and atherosclerosis: Insight from a study in octogenarians. *Trans. Am. Clin. Climatol. Assoc*. **99**, 100–110.

Davis, D. L., and Schwartz, J. (1988). Trends in cancer mortality: US white males and females, 1968–83. *Lancet* **1**, 633–636.

Davis, D. L., Ahlbom, A., Hoel, D., and Percy, C. (1991). Is brain cancer mortality increasing in industrial countries? *Am. J. Ind. Med*. **19**, 421–431.

de Rijk, M. C., Breteler, M. M., Graveland, G. A., Ott, A., Grobbee, D. E., van der Meche, F. G., and Hofman, A. (1995). Prevalence of Parkinson's disease in the elderly: The Rotterdam Study. *Neurology* **45**, 2143–2146.

de Rijk, M. C., Tzourio, C., Breteler, M. M., Dartigues, J. F., Amaducci, A., Lopez-Pousa, S., Manubens-Bertran, J. M., Alperovitch, A., and Rocca, W. A. (1997). Prevalence of parkinsonism and Parkinson's disease in Europe: The EUROPARKINSON Collaborative Study European Community Concerted Action on the Epidemiology of Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* **62**, 10–15.

Durrleman, S., and Alpérovitch, A. (1989). Increasing trend of ALS in France and elsewhere: Are the changes real? *Neurology* **39**, 768–773.

Duvoisin, R. C., and Schweitzer, M. D. (1966). Paralysis agitans mortality in England and Wales, 1855–1962. *Br. J. Prev. Soc. Med*. **20**, 27–33.

Editorial. (1993). Rise and fall of diseases. *Lancet* **341**, 151–152.

Fox, P. (1989). From senility to Alzheimer's disease: The rise of the Alzheimer's disease movement. *Milbank Mem. Fund Q*. **67**, 58–102.

Ganguli, M., and Rodriguez, E. G. (1999). Reporting of dementia on death certificates: A community study. *J. Am. Geriatr. Soc*. **47**, 842–849.

Gao, S., Hendrie, H. C., Hall, K. S., and Hui, S. (1998). The relationships between age, sex, and the incidence of dementia and Alzheimer's disease: A meta-analysis. *Arch. Gen. Psychiatry* **55**, 809–815.

Gompertz, B. (1825). On the nature of the function expressive of the law of human mortality. *Philos. Trans. R. Soc. London* **115**, 513–585.

Greig, N. H., Ries, L. G., Yanik, R., and Rapoport, S. I. (1990). Increasing annual incidence of primary malignant brain tumors in the elderly. *J. Natl. Cancer Inst*. **82**, 1621–1624.

Hebert, L. E., Scherr, P. A., Beckett, L. A., Albert, M. S., Pilgrim, D. M., Chown, M. J., Funkenstein, H. H., and Evans, D. A. (1995). Age-specific incidence of Alzheimer's disease in a community population. *JAMA, J. Am. Med. Assoc*. **273**, 1354–1359.

Helseth, A., Langmark, F., and Mork, S. J. (1988). Neoplasms of the central nervous system in Norway. II. Descriptive epidemiology of intracranial neoplasms 1955–1984. *Acta Pathol. Microbiol. Immunol. Scand.* **96**, 1066–1074.

Hennekens, C. H., and Buring, J. E. (1987). ''Epidemiology in Medicine.'' Little, Brown, Boston.

Hoffman, P. M., and Brody, J. A. (1971). The reliability of death certificate reporting for amyotrophic lateral sclerosis. *J. Chronic Dis*. **24**, 5–8.

Imaizumi, Y. (1992). Mortality rate of Alzheimer's disease in Japan: Secular trends, marital status, and geographical variations. *Acta Neurol. Scand*. **86**, 501–505.

Imaizumi, Y. (1995). Longitudinal Gompertzian analysis of Parkinson's disease mortality in Japan, 1950–1993. *Mech. Ageing Dev* **85**, 15–83.

Jorm, A. F., and Jolley, D. (1998). The incidence of dementia: A meta-analysis. *Neurology* **51**, 728–733.

Juckett, D. A., and Rosenberg, R. (1993). Comparison of the Gompertz and Weibull functions as descriptors for human mortality distributions and their intersections. *Mech. Ageing Dev.* **69**, 1–31.

Kahana, E., Alter, M., and Feldman, S. (1976). Amyotrophic lateral sclerosis: A population study. *J. Neurol*. **212**, 205–213.

Katzman, R. (1986). Alzheimer's disease. *N. Engl. J. Med*. **314**, 964–973.

Katzman, R., and Saitoh, T. (1991). Advances in Alzheimer's disease. *FASEB J*. **5**, 278–286.

Klag, M. J., Whelton, P. K., and Seidler, A. J. (1989). Decline in US stroke mortality, demographic and antihypertensive treatment. *Stroke* **20**, 14–21.

Kondo, K., and Kurland, L. T. (1973). Parkinson's disease, genetic analysis and evidence of a multifactorial etiology. *Mayo Clin. Proc*. **48**, 465–475.

Kuratsu, J., and Ushio, Y. (1997). Epidemiological study of primary intracranial tumours in elderly people. *J. Neurol. Neurosurg. Psychiatry* **63**, 116–118.

Kurtzke, J. F. (1982). Epidemiology of amyotrophic lateral sclerosis. *Adv. Neurol*. **36**, 281–302.

Kurtzke, J. F., and Murphy, F. M. (1990). The changing patterns of death rates in parkinsonism. *Neurology* **40**, 42–49.

Leone, M., Chandra, V., and Schoenberg, B. S. (1987). Motor neuron disease in the United States, 1971 and 1973–1978: Patterns of mortality and associated conditions at the time of death. *Neurology* **37**, 1339–1343.

Lestienne, R. (1988). On the thermodynamical and biological interpretation of the Gompertzian mortality rate distribution. *Mech. Ageing Dev.* **42**, 197–214.

Lilienfeld, D. E., Chan, E., Ehland, J., Godbold, J., Landrigan, P. J., Marsh, G., and Perl, D. P. (1989). Rising mortality from motoneuron disease in the USA, 1962–84. *Lancet* **1**, 710–712.

Lilienfeld, D. E., Chan, E., Ehland, J., Godbold, J., Landrigan, P. J., Marsh, G., and Perl, D. P. (1990). Two decades of increasing mortality from

Parkinson's disease among the US elderly. *Arch. Neurol*. (*Chicago*) **47**, 731–734.

Lopez-Vega, J. M., Calleja, J., Combarros, O., Polo, J. M., and Berciano, J. (1988). Motor neuron disease in Cantabria. *Acta Neurol. Scand*. **77**, 1–5.

Medawar, P. B. (1952). "An Unsolved Problem of Biology." H. K. Lewis, London.

Modan, B., Wagener, D. K., Feldman, J. J., Rosenberg, H. M., and Feinleib, M. (1992). Increased mortality from brain tumors: A combined outcome of diagnostic technology and change of attitude toward the elderly. *Am. J. Epidemiol*. **135**, 1349–1357.

Morens, D. M., Davis, J. W., Grandinetti, A., Ross, G. W., Popper, J. S., and White, L. R. (1996). Epidemiologic observations on Parkinson's disease: Incidence and mortality in a prospective study of middle-aged men. *Neurology* **46**, 1044–1050.

Mulder, D. W., Kurland, L. T., Offord, K. P., and Beard, C. M. (1986). Familial adult motor neuron disease: Amyotrophic lateral sclerosis. *Neurology* **36**, 511–517.

National Center for Health Statistics, Centers for Disease Control, U.S. Public Health Service, U.S. Department of Health and Human Services. (1951–1988). "Vital Statistics of the United States." U.S. Government Printing Office, Washington, DC.

National Center for Health Statistics, Centers for Disease Control, U.S. Public Health Service, U.S. Department of Health and Human Services. (1955–1988). "Vital Statistics of the United States." U.S. Government Printing Office, Washington, DC.

Neilson, S., Robinson, I., and Alperovitch, A. (1994). Rising amyotrophic lateral sclerosis mortality in France 1968–1990: Increased life expectance and inter-disease competition as an explanation. *J. Neurol*. **241**, 448–455.

Olshansky, S. J., Carnes, B. A., and Cassel, C. K. (1993). The aging of the human species. *Sci. Am*. **268**, 6–52.

Plum, F. (1979). Dementia: An approaching epidemic. *Nature (London)* **279**, 372–373.

Polednak, A. P., and Flannery, J. T. (1995). Brain, other central nervous system, and eye cancer. *Cancer (Philadelphia)* **75**(Suppl. 1), 330–337.

Riggs, J. E. (1990a). Longitudinal Gompertzian analysis of adult mortality in the U.S., 1900–1986 *Mech. Ageing Dev.* **54**, 235–247.

Riggs, J. E. (1990b). Longitudinal Gompertzian analysis of amyotrophic lateral sclerosis mortality in the U.S., 1977–1986: Evidence for an inherently susceptible population subset. *Mech. Ageing Dev.* **55**, 207–220.

Riggs, J. E. (1990c). Longitudinal Gompertzian analysis of Parkinson's disease mortality in the U.S., 1955–1986: The dramatic increase in overall mortality since 1980 is the natural consequence of deterministic mortality dynamics. *Mech. Ageing Dev.* **55**, 221–233.

Riggs, J. E. (1990d). Longitudinal Gompertzian analysis of stroke mortality in the U.S., 1951–1986: Declining stroke mortality is the natural consequence of competitive deterministic mortality dynamics. *Mech. Ageing Dev.* **55**, 235–243.

Riggs, J. E. (1991a). Longitudinal Gompertzian analysis of primary malignant brain tumor mortality in the U.S., 1962–1987: Rising mortality among the elderly is the natural consequence of competitive deterministic dynamics. *Mech. Ageing Dev.* **60**, 225–241.

Riggs, J. E. (1991b). Longitudinal Gompertzian analysis of prostate cancer mortality in the U.S., 1962–1987: A method of demonstrating relative environmental, genetic, and competitive influences upon mortality. *Mech. Ageing Dev.* **60**, 243–253.

Riggs, J. E. (1991c). Longitudinal Gompertzian analysis of pancreatic cancer mortality in the U.S., 1962–1987: Distinguishing between competitive and environmental influences upon evolving mortality patterns. *Mech. Ageing Dev.* **61**, 197–208.

Riggs, J. E. (1991d). Parkinson's disease: An epidemiologic method for distinguishing between symptomatic and neuroprotective treatments. *Clin. Neuropharmacol*. **14**, 489–497.

Riggs, J. E. (1992a). Aging and mortality: Manifestations of natural "non-selection." *Mech. Ageing Dev.* **62**, 127–135.

Riggs, J. E. (1992b). Longitudinal Gompertzian analysis of cervical cancer mortality in the U.S., 1962–1987: A method of quantitatively demonstrating changing environmental influences upon deterministic mortality dynamics. *Mech. Ageing Dev.* **63**, 117–129.

Riggs, J. E. (1992c). Longitudinal Gompertzian analysis of emphysema mortality in the U.S., 1962–1987: The differing basis for evolving mortality patterns in men and women. *Mech. Ageing Dev.* **64**, 161–175.

Riggs, J. E. (1992d). Cigarette smoking and Parkinson disease: The illusion of a neuroprotective effect. *Clin. Neuropharmacol*. **15**, 88–99.

Riggs, J. E. (1993a). Longitudinal Gompertzian analysis of non-Hodgkin's lymphoma mortality in the U.S., 1979–1988: Demonstrating the environmental basis for rising overall mortality *Mech. Ageing Dev.* **67**, 65–78. 35.

Riggs, J. E. (1993b). Stone-age genes and modern lifestyle: Evolutionary mismatch or differential survival bias. *J. Clin. Epidemiol*. **46**, 1289–91.

Riggs, J. E. (1993c). The Gompertz function: Distinguishing mathematical from biological limitations. *Mech. Ageing Dev.* **69**, 33–36.

Riggs, J. E. (1994). Age-specific mortality rate comparisons: Is there a fallacy? *J. Clin. Epidemiol*. **47**, 1463–1464.

Riggs, J. E. (1995a). Rising primary malignant brain tumor mortality in the elderly, a manifestation of differential survival. *Arch. Neurol. (Chicago)* **52**, 571–575.

Riggs, J. E. (1995b). Increasing lung cancer mortality rates in the elderly: A manifestation of differential survival. *Regul. Toxicol. Pharmacol*. **21**, 370–374.

Riggs, J. E. (1995c). Rising ovarian cancer mortality in the elderly: A manifestation of differential survival. *Gynecol. Oncol*. **58**, 64–67.

Riggs, J. E. (1995d). Increasing multiple myeloma mortality rates in the elderly: Demonstration of increasing dependency with increasing age upon age group population size. *Mech. Ageing Dev.* **81**, 131–138.

Riggs, J. E. (1996a). Changing demographics and neurologic disease in the elderly. *Neurol. Clin*. **14**, 477–486.

Riggs, J. E. (1996b). Differential survival, natural selection, and the manifestation of senescence. *Mech. Ageing Dev.* **87**, 91–98.

Riggs, J. E., and Hobbs, G. R. (1998). Nonrandom sequence of slope-intercept estimates in longitudinal gompertzian analysis suggests biological relevance. *Mech. Ageing Dev.* **100**, 269–275.

Riggs, J. E., and Myers, E. J. (1994). Defining the impact of prevention and improved management upon stroke mortality. *J. Clin. Epidemiol*. **47**, 931–939.

Robins, M., and Baum, H. M. (1981). The National Survey of Stroke Incidence. *Stroke* **12** (Suppl. 1), I45–I57.

Sant, M., Crosignani, P., Bordo, B. M., Nicola, G., Bianchi, M., and Berrino, F. (1988). Incidence and survival of brain tumors: A population-based study. *Tumori* **74**, 243–252.

Sayetta, R. B. (1986). Rates of senile dementia, Alzheimer's type, in the Baltimore Longitudinal Study. *J. Chronic Dis*. **39**, 271–286.

Strehler, B. L., and Mildvan, A. S. (1960). General theory of mortality and aging. *Science* **132**, 14–21.

Sutherland, G. R., Florell, R., Louw, D., Choi, N. W., and Sima, A. A. (1987). Epidemiology of primary intracranial neoplasms in Manitoba, Canada. *Can. J. Neurol. Sci*. **14**, 586–592.

Suzman, R. M., Willis, D. P., and Manton, K. G. (1992). "The Oldest Old." Oxford University Press, New York.

Tysnes, O. B., Vollset, S. E., and Aarli, J. A. (1991). Epidemiology of amyotrophic lateral sclerosis in Hordaland county, western Norway. *Acta. Neurol. Scand*. **83**, 280–285.

Whisnant, J. P. (1984). The decline of stroke. *Stroke* **15**, 160–168.

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Charles V. Mobbs and John W. Rowe

2

Nature versus Nurture in the Aging Brain

The relative contribution of genotype to many phenotypes is not constant, but changes with age. Twin studies have suggested that the contribution of genotype to general health increases until about age 70 and then decreases dramatically thereafter. In families in which age-related neurological diseases occur in a Mendelian autosomal dominant pattern (including Huntington's disease), penetrance of the disease-causing allele increases from 0% in childhood to 100% by about 70 years of age. However, for most diseases such familial cases constitute only a minority of the total; thus the relative contribution of genotype to the total incidence of most diseases decreases after about age 70. Similarly, the contribution of genotype to nonpathological age-related neurobiological impairments also appears to peak at about 70 years of age and then decreases. Thus after age 70, environmental effects increasingly dominate most age-related impairments in neurobiological function.

I. Introduction

A fundamental concern of biological research is to account for variations between individuals. In general this concern is addressed by assessing the relative contributions of genotype and environment to phenotype. However, individual variation of many phenotypes is significantly accounted for by age. It is often assumed that age is essentially a proxy for the cumulative effects of environment and thus that the effect of age on a phenotype is in fact a reflection of environment. In turn, this reasoning would imply that with respect to phenotype during aging, the relative effect of environment should increase and the relative effect of genotype should decrease. To address this hypothesis, it is necessary to assess how stable individual variations are during aging. For example, eye color is completely stable with age, and completely accounted for by genotype at all ages, whereas body weight is unstable with age in two respects: the body weight of each individual changes with age and the variability of body weight increases with age. Although individual differences in body weight are largely due to genotype, it is of interest to assess if age-related changes in body weight (both in average and in variability) are due to genotype or cumulative environmental effects.

Clearly in the case of autosomal dominant age-related genetic diseases, the coupling between genotype and phenotype, e.g., penetrance, increases with age. For example, Huntington's disease is an autosomal dominant disease with complete penetrance, as every individual bearing the disease-causing allele eventually develops the disease (if the individual lives long enough) and no individual without that allele develops the disease. However, at birth there is no phenotypic difference between individuals bearing the mutation for Huntington's disease and normal individuals; the phenotype only develops during aging, and by age 70 there is almost 100% concordance between genotype and phenotype, with the environment playing essentially no role. Thus penetrance of the Huntington's disease allele increases from 0 to 100% during aging. Furthermore, cumulative environmental effects during aging may depend largely on genotype. For example, as described later, practice enhances skill, and a high-fat diet induces obesity, but the effect of these manipulations depends largely on genotype. Thus when individuals share a common environment, the effect of genotype may become more accentuated during aging even for environmentally induced phenotypes. It has therefore become clear that assessing the coupling between genotype and phenotype during aging requires a careful analysis of the effect of age on genetic penetrance. As will be described later, such careful analysis in human twin studies has indicated that the relative contribution of genotype to phenotype often increases with age to a peak at around 70 years of age, after which phenotype is increasingly determined by environment. Interestingly, this pattern applies even to neurobiological functions, including cognitive functions, in which it might be hypothesized that effects of experience would monotonically increase throughout the life span.

These considerations also suggest that the effects of genotype may be revealed by environment. A classic example of this strong interaction between environment and heredity in a neuropathological process is the disease of phenylketonuria, first described by Fölling in 1934. Individuals homozygous

for the disease allele of the phenylalanine hydroxylase gene develop phenylketonuria, a disease leading to severe mental retardation, on a normal diet. However, if maintained on a diet free of phenylalanine, the disease will not develop (discussed in more detail in Rowe & Kahn, 1998, p. 219). As with diet-induced obesity and many other pathological processes, this example indicates that a unitary concept of ''environment'' is of limited value, since the relative contribution of environment and heredity depends profoundly on the specific nature of both environment and heredity. Specifically with respect to the interaction between environment and heredity during aging, a key consideration is that ''environment'' actually changes with age, for example due to profound age-related differences in exposure to drugs. Nevertheless, despite these complications, there has been remarkable agreement concerning the relationship between environment and heredity during aging in typical human (at least Western) environments.

II. Genotype, Environment, and General Health

One way to assess the relative contributions of genotype and environment on phenotype is to analyze the cause of individual variations of the phenotype across a population. Viewed statistically, age-related phenotypes can be analyzed by assessing the amount of variance that is accounted for by genotype, environment, and age, and the shared variance of these factors. A common approach to such an analysis involves examining the shared variance of a phenotype in monozygotic compared to dizygotic twins. In the Swedish Adoption/Twin Study of Aging supported by the MacArthur Foundation Study of Successful Aging, Harris *et al*. (1992) examined individual differences in objectively determined chronic illnesses as well as in self-rated health in monozygotic and dizygotic Swedish twins who were reared together or apart. The burden of chronic illness was assessed by a rating scale that was developed and validated to reflect ''constricted homeostasis'' (Rowe, 1985) as an indicator of general decline of physiological function. Thus this parameter was designed to reflect a broad range of functions, including cardiovascular, respiratory, neurologic, metabolic, and other functions. Individuals were divided into four age groups: less than 50 years old, 50–59 years old, 60–69 years old, and greater than 70.

A key initial observation of this study was that while *average* health scores decreased monotonically with age, *differences* in the health between individuals also increased at least as much with age. The effect of age on health was in contrast to the effect of age on height and weight in this study, as differences in height and weight between individuals were greater in the group aged 50–59 years than in the group aged less than 50 years, but after 60 years of age individual differences actually began to decrease so that the lowest individual variation for both height and weight was found in the oldest group. One interpretation of these observations is that while the health of many individuals deteriorated markedly during aging, other individuals maintained relatively good health as they aged. This interpretation was supported by the observation that the optimum score for objective health was observed in individuals at all age ranges, but the worst reported score for health increased with the age of the group. In contrast, effects of age on height and weight were relatively uniform. Thus a critical question was to determine what accounted for the individual variation in health during aging, which allowed some individuals to age with little impact on health [referred to as ''successful aging'' (Rowe and Kahn, 1997)], whereas other individuals developed severe health problems. Specifically, a key question was to assess the relative contribution of genotype and environment on health during aging.

This question was addressed by comparing the health during aging of monozygotic twins reared together or reared apart with the health of dizygotic twins reared together or reared apart, using standard statistical methods. For example, objective health in monozygotic twins reared apart was highly correlated at ages below 50, between 50 and 59, and between 60 and 69, but was not significantly correlated over the age of 70 years. Essentially similar results were obtained in twins reared together. Detailed analysis indicated that the relative contribution of genotype to objective health roughly doubled from the youngest group to the group aged 60–69, but after the age of 70 there was no longer a significant effect of genotype on objective health. These data suggest that objective health is significantly influenced by genotype (not surprisingly) and that the effect of genotype increases with age up until the age of about 70 years (surprisingly), after which the effects of environment become more important and the relative contribution of genotype to phenotype decreases dramatically.

Because effects of environment, unique to each individual, must surely accumulate with age, it may seem counterintuitive that effects of genotype could increase with age at all. This apparent conundrum can be resolved in the context of the following considerations. First, the effect of genes for age-related genetic diseases must, by definition, increase with age. Furthermore, because some effects of genes involve exacerbating effects of environment, cumulative environmental effects may be exaggerated as a function of genotype. Finally, the incidence rate of familial forms of diseases peaks earlier than the incidence rate of sporadic or nonfamilial forms. As described later, the net effect of these phenomena is that the concordance between genotype and phenotype increases with age, as the phenotype of genetic diseases develops, but then the relative contribution of genotype to phenotype decreases with age with increased incidence rates of sporadic forms of diseases and nondisease impairments. Nevertheless, these observations regarding general health may not apply to specific diseases. For example, the relative influence of genotype to death from coronary heart disease increases monotonically with age (Marenberg *et al*., 1994).

III. Motor Systems

As described in detail in later chapters of this book, motor system functionality decreases monotonically with age. In humans, this decline includes the development of several age-related diseases of motor systems (including Huntington's disease and Parkinson's disease) superimposed on universal but gradual impairments in neuromuscular functions. The incidence of each disease peaks at a characteristic age (for Huntington's disease around age 40, for Parkinson's disease

around age 70) and then begins to decline, whereas after this age the universal age-related impairments constitute an increasingly important component of the total variance in motor function. As the incidence of disease increases, the contribution of disease to individual variation in motor function also increases, and to the extent that risk of disease is primarily genetic, the genotype contributes substantially to phenotype during this time. However, as the incidence of motor diseases decreases (after about age 70), there is also a decline in the relative contribution of motor disease genes to age-related impairments in motor function.

A. Huntington's Disease

The age-specific incidence rate of Huntington's disease peaks at around age 40 (Greenamyre and Shoulson, 1994), whereas the overall prevalence in most populations is about 10 per 100,000 population (Conneally, 1984). (For mechanistic analysis, the incidence the rate, reflecting the rate of new cases, is more informative than prevalence, the total number of cases, as incidence specifically reflects age-specific vulnerability to developing the disease, whereas prevalance reflects a complex set of factors, including integrated incidence rate and longevity after development of the disease.) Monozygotic twins are essentially 100% concordant in the development of Huntington's disease, demonstrating the primary contribution of genotype to the risk of developing Huntington's disease (Sudarsky *et al*., 1983). More recent work has demonstrated that Huntington's disease is caused by a variable expansion of a CAG repeat producing a polyglutamine stretch in the gene product, huntingtin (Lunkes *et al*., 1998). Characterization of the allele for Huntington's disease has made it possible to definitively test for the relationship between genotype and Huntington's phenotype. Thus Kremer *et al*. (1994) studied 1007 late middle-aged patients who were clinically diagnosed with Huntington's disease from 565 families and 113 controls with other age-related neurological diseases. Of the 1007 diagnosed patients, 995 were found to bear from 36 to 121 CAG repeats, whereas none of the patients with other neurological diseases were found to bear these repeats. Thus by late middle age the concordance between genotype and Huntington's phenotype is essentially 100%.

Nevertheless, at relatively young ages (under age 20 years), there is little concordance between genotype and Huntington's phenotype, as at these young ages only about 10% of individuals who express CAG repeats in the huntingtin gene exhibit Huntington's phenotype. Therefore, with respect to incidence, Huntington's disease represents an extreme form of the coupling between genotype and age-related phenotype, in which the coupling increases from very low below the age of 20 years (at which age the great majority of individuals bearing the CAG repeat do not exhibit the Huntington's phenotype) to essentially 100% concordance by age 70 years (at which age almost every individual who bears the CAG repeat would have developed the disease). By the same token, however, the relative contribution of the CAG repeat to phenotypic variation in the whole population increases with age as the incidence of the disease peaks at about 40 years of age, but then begins to decline as the incidence of Huntington's disease decreases.

However, other aspects of the phenotype exhibit a more subtle relationship to genotype. For example, the number of CAG repeats can vary widely, from fewer than 30 to more than 100; the number of CAG repeats is highly (inversely) correlated with age of onset of Huntington's disease early in life, but the strength of this correlation decreases with age (Crauford and Dodge, 1993; Kremer *et al*., 1993). Therefore the coupling between genotype and the age of onset of Huntington's disease decreases with age.

B. Parkinson's Disease

Parkinson's disease is about 10-fold more prevalent than Huntington's disease, and the incident rate of Parkinson's disease peaks later than that of Huntington's disease, at about 75 years of age, after which incidence rate begins to decline (Martilla, 1987). In marked contrast to the perfect concordance for Huntington's disease in identical twins, several studies have failed to observe any concordance for Parkinson's disease in identical twins (Lilienfeld, 1994). At a minimum, this observation clearly indicates a much lower overall contribution of genotype to Parkinson's phenotype than for Huntington's phenotype. However, several families have been studied in which the Parkinson's disease follows a dominant Mendelian pattern of inheritance (Golbe *et al*., 1996), and in several different families this led to the identification of an allele of α-synuclein as the genetic basis of the disease in these families. (Polymeropoulos *et al*., 1997). In another group of families in which Parkinson's disease is inherited in an autosomal recessive Mendelian pattern, mutations in the gene coding for a novel protein, named parkin, account for the appearance of the Parkinson's phenotype (Hattori *et al*., 1998; Kitada *et al*., 1998). Nevertheless, mutations in α-synuclein and parkin only account for a very small subset of all cases of familial Parkinson's disease (Vaughan *et al*., 1998), and thus of an even smaller subset of all cases of Parkinson's disease.

Clearly the coupling of genotype to phenotype is much lower, and the genetic basis much more complex, in Parkinson's disease than in Huntington's disease. However, Parkinson's disease is not only much more common than Huntington's disease, it is a much more heterogeneous syndrome, and thus plausibly involves a more heterogeneous set of pathophysiological processes. For those forms of Parkinson's disease for which a single gene defect has been defined, the coupling between genotype and phenotype behaves as it does in Huntington's. Thus within kindreds in which α-synuclein mutations are common, at young ages there is no concordance between mutations in α-synuclein and Parkinson's phenotype, whereas by age 70 there is a very high concordance between genotype and phenotype. However, in the population as a whole, this relationship is less evident (and indeed not detected at all in twin studies), as α-synuclein mutations only account for a small proportion of all cases of Parkinson's disease (in contrast to Huntington's disease, all of whose cases are accounted for by mutations in a single gene).

A key phenomenon for interpreting these data is that the incidence rate of familial forms of Parkinson's disease peaks earlier than in sporadic or nonfamilial forms. Thus mutations in parkin lead to juvenile onset Parkinson's disease, whose incidence peaks at around 20 years of age, and the incidence

of Parkinson's disease due to mutations in α-synuclein peaks at around 50 years of age. In contrast, the incidence rate of Parkinson's disease in the population as a whole peaks around 70 years. Because twin studies indicated that genotype makes little contribution to the late-onset (and most common) form of the disease, taken together these data imply that the contribution of genotype to Parkinson's phenotype increases with age up until about age 50 and then begins to decline such that by age 70, there is little contribution of genotype to phenotype (Langston, 1998). Thus a major question is the extent to which genotype accounts for nondisease phenotype during aging. Twin studies have indicated that although psychomotor speed declines with age, the effect of genotype and possibly early environment continues to dominate this phenotype during aging, at least up until age 67, whereas in contrast, effects of exercise were minimal (Simonen *et al*., 1998). Nevertheless, the effect of age on the penetrance of genotype on psychomotor function has not been elucidated in detail after the age of 70 years.

IV. Cognitive Function

Although genetic effects on motor diseases increase with age before they decrease, it might be hypothesized that cognitive functions are more likely to reflect cumulative experience during aging, and thus the contribution of genotype might be less for cognitive functions. However, as described later, effects of genotype are probably at least as great on cognitive functions during aging as for motor functions.

A. Alzheimer's Disease

In contrast to Parkinson's disease, twin studies have demonstrated a significant genetic contribution to the risk of developing Alzheimer's disease (Breitner *et al*., 1993, 1995; Bergem, 1994; Gallo and Breitner, 1995; Raiha *et al*., 1996, 1997; Bergem *et al*., 1997; Gatz *et al*., 1997; Plassman and Breitner, 1997; Rubinsztein, 1997). For example, in a study of Swedish twins, concordance between monozygotic twins was 67%, compared to only 22% for dizygotic twins (Gatz *et al*., 1997). Conclusions from twin studies have been corroborated in family studies. For example, offspring whose parents had both been diagnosed with Alzheimer's disease had a 47% chance of developing Alzheimer's disease by age 65, far higher than the risk of the general population at that age (Bird *et al*., 1993). Similarly, analysis of 70 kindreds with evidence of hereditary forms of Alzheimer's disease indicated that offspring whose parents had Alzheimer's disease had a lifetime risk of developing the disease by age 87 of 64%, again, far higher than the risk in age-matched controls (Farrer *et al*., 1990). Interestingly, the risk for offspring in families with early-onset Alzheimer's disease was only 53%, compared to a remarkable 86% for offspring in families with late-onset Alzheimer's disease (Farrer *et al*., 1990). Thus at least within these kindreds, there is evidence of increased penetrance of genotype during aging. However, the incidence rate of Alzheimer's disease appears to decrease after age 90 (Lautenschlager *et al*., 1996). Because of the relatively small number of individuals alive at these very advanced ages, it is not yet known if the effect of genotype on the risk of Alzheimer's disease may decline after age 90.

As with Parkinson's disease, allelic variations in several specific genes (presenilin 1, presenilin 2, β-amyloid precursor, and apolipoprotein E) have been implicated in the etiology of Alzheimer's disease (Cruts and Van Broeckhoven, 1998; see the review by O'Malley and Tanzi in the present volume). Thus how aging influences the penetrance of these genes is of great interest. Campion *et al*. (1999) addressed this question in a particularly interesting recent study that examined the genetic basis of early-onset autosomal dominant Alzheimer's disease in the entire population of the city of Rouen. In this study, early-onset autosomal dominant Alzheimer's disease was defined by the occurrence of Alzheimer's disease before the age of 61 years in three generations of a given family. Thirty-four such families were observed in Rouen, with a population of about 500,000. In 56% of such families, allelic variations in presenilin 1 were observed, and in 15% of such families, allelic variations of the β-amyloid precursor were observed. In contrast, in nine families that did not exhibit an early-onset form of the Alzheimer's disease, such allelic variations were not observed. These data suggest that the penetrance of presenilin 1 and β-amyloid precursor mutations reaches 100% at relatively young ages (around age 60). However, because the incidence of Alzheimer's disease before the age of 60 is less than 1% of the incidence at age 80–90, this clearly demonstrates that the coupling between presenilin 1 (or β *N*-amyloid precursor) and Alzheimer's disease phenotype peaks at around age 60 and then returns to negligible by age 80. In contrast to the (eventual) complete penetrance of the presenilin and β-amyloid alleles, alleles of the apolipoprotein E gene are never completely penetrant, but nevertheless account for a much larger proportion (possibly 20%) of cases of Alzheimer's disease (Slooter *et al*., 1998). Nevertheless, the effect of apolipoprotein E genotype peaks at around age 70 and then declines (but is significant even at 90 years of age) (Farrer *et al*., 1997; Slooter *et al*., 1998). Thus, taken together, evidence suggests that the coupling between genotype and Alzheimer's disease phenotype peaks by 70 years of age and then, as with other age-related diseases, the role of genotype begins to decline.

B. Nonpathological Age-Related Changes in Cognitive Functions

Although Alzheimer's disease causes devastating loss of cognitive function in affected individuals, far more individuals experience much milder cognitive impairments that are apparently not associated with disease (Botwinick, 1978). Because the effects of genotype on performance in standardized intelligence tests have been examined in great detail in young populations, several studies have used similar methodologies to assess effects of aging on the contribution of genotype to performance on these tests (Plomin *et al*., 1994; Finkel *et al*., 1995, 1998; McClearn *et al*., 1997; Emery *et al*., 1998). For example, Plomin et al. (1994), as part of the Swedish Adoption/Twin Study of Aging, examined cognitive functions in 112 pairs of twins (both monozygotic and dizygotic) reared apart and in 111 matched pairs of twins reared together. The age of the twins was 64.1 ± 7.5 (mean $\pm$ SD) years. At this age, the average heritability of general cognitive function (a

composite of spatial, verbal, memory, and speed of processing performances, after removal of effects of age and gender) was 80%. In a detailed review of studies from both the Swedish Adoption/Twin Study of Aging and the Minnesota Twin Study of Adult Development and Aging, Finkel *et al*. (1995) concluded that both sets of studies suggested that the heritability of general cognitive function is about 80% throughout adulthood, compared to estimates of about 50% during childhood and adolescence, but evidence suggested a possible decrease in heritability after age 70 years. A further analysis of the Swedish twin study appeared to corroborate this result, as in Swedish twins over the age of 80, the heritability of general cognitive function was estimated to be about 62% (McClearn *et al*., 1997). Other studies have also suggested that the heritability of general cognitive function increases from about 50% in childhood and adolescence to about 80% in adulthood (McCartney *et al*., 1990; McGue *et al*., 1993). It should be noted that although aging influences the heritability of general cognitive function, the effect of age on cognitive function itself is more specific to specific subsystems. In general, functions reflecting knowledge improve with age, whereas functions reflecting speed of processing and memory are impaired with age. Thus aspects of cognition reflected by the Wechsler subscales of information, vocabulary, and comprehension are relatively unimpaired or even improve with age in nondemented individuals, whereas cognitive functions reflected by the subscales of block design, picture arrangement, and digit symbol tend to deteriorate robustly with age (Botwinick, 1978). Interestingly, the heritability of general cognitive function during aging is greater than the heritability of any of the functions reflected by subscales, which has been interpreted to indicate that the ''nature of the genetic influence in the cognitive domain appears to be more general than specific'' (Plomin *et al*., 1994). Thus, in contrast to the effect of age on the heritability of general cognitive function, the heritability of memory function alone is reported to be stable with age (Finkel and McGue, 1998).

V. Genotype Influences Cumulative Effect of Environment

Because cognitive function is defined by experience, it may seem surprising that the heritability of cognitive function can increase with age at all (although decreasing after the age of 70). One resolution of this apparent paradox is that the influence of experience may be enhanced by genotype. For example, in a twin study examining the effect of genotype on the acquisition of a motor skill, Fox *et al*. (1996) reported that while genotype influenced the initial performance of the skill, the effect of genotype on the enhancement of the skill by practice was even greater. These investigators concluded that ''the effect of practice is to decrease the effect of environmental variation (previous learning) and increase the relative strength of genetic influences on motor performance.'' Similarly, it seems plausible that genotype may influence the cumulative effect of experience on general cognitive functions. However, after the age of 70 it appears that these genetic effects have reached their peak, and the effects of unique experiences come to dominate.

A similar phenomenon may influence neuroendocrine functions during aging. For example, it has been reported in males that the heritability of body mass index is about 46% in males aged 46–59 years old and 61% in males 60–76 years old (Herskind *et al*., 1996). As with other phenotypes, the mechanism of this increasing penetrance on body mass index is unclear. However, work in rodents suggests a possible mechanism. Two strains of mice, C57BL/6J and A/J, have similar body weights throughout life if fed a standard laboratory chow, which is very low in fat. In contrast, when fed a diet high in fat, C57BL/6J gain a substantial amount of body weight and, after several months, develop diabetes, whereas A/J mice, while consuming the same amount of the diet, stay relatively thin and normoglycemic (Surwit *et al*., 1988). Thus in the presence of one environment (characterized by a low-fat diet), there is little effect of genotype on body weight or blood glucose, whereas in a different environment (characterized by a high-fat diet), the effect of genotype (which initially is very small) increases substantially as body weight and blood glucose increase over time in C57BL/6J mice, but not A/J mice. Furthermore, the increase of heritability of body weight over time on a high-fat diet appears to be mediated through a neuroendocrine mechanism, as the high-fat diet induces weight-reducing responses in the hypothalamus of A/J mice, but not in C57BL/6J mice (Bergen *et al*., 1999).

Age-related impairments in sensory functions may also involve genetic exacerbation of environmental insults. For example, C57BL/6J mice exhibit gradual age-related impairments in auditory function, leading to essentially complete loss of hearing by old age, whereas CBA and other strains of mice retain largely normal auditory function until old age; this effect of genotype is now known to be due to a recessive gene whose penetrance increases with age (see Willott, this volume). Of particular interest, however, is that this recessive gene also greatly potentiates noise-induced hearing loss (Erway *et al*., 1996). Thus, while the effect of genotype on hearing function clearly increases with age in mice, part of the mechanism by which this occurs may involve exacerbation of environment, as is the case in diet-induced obesity. Similarly, the concordance of age-related macular degeneration is 100% in monozygotic twins but only 42% in dizygotic twins (Meyers *et al*., 1995); thus again the effects of genotype on macular degeneration increase with age. Numerous environmental effects of macular degeneration have been observed (including smoking and diabetes), so it will be of interest to assess if the genetic effects involve exacerbation of these deleterious effects.

VI. Summary

Although genotype in humans contributes increasingly to many age-related impairments up until about age 70, it is important to emphasize that the contribution of genotype to phenotype begins to decline after age 70, and for many phenotypes the effect of environment is at least as great as the effect of genotype. Furthermore, it is now appreciated that simple changes in lifestyle, such as moderate exercise, can have a profound beneficial effect, especially on impairments that occur after the age of 70 (Rowe and Kahn, 1997). In addition,

as described earlier, age-related increases in the heritability of genetic diseases may involve a genetic mechanism that exacerbates deleterious environmental effects. Thus, burgeoning advances in genomics should not only stimulate further analysis of the genetic basis of age-related impairments, they should equally inform further analysis of (potentially more tractable) environmental influences. While initially the interaction between heredity and environment will be most easily determined in analyzing age-related diseases, ultimately the effects of these interactions on nonpathological age-related impairments may be of even greater general significance.

References

Bergem, A. L. (1994). Heredity in dementia of the Alzheimer type. *Clin. Genet*. **46**, 144–149.

Bergem, A. L., Engedal, K., and Kringlen, E. (1997). The role of heredity in late-onset Alzheimer disease and vascular dementia. A twin study. *Arch. Gen. Psychiatry* **54**, 264–270.

Bergen, H. T., Mizuno, T., Taylor, J., and Mobbs, C. V. (1999). Resistance to diet-induced obesity is associated with increased proopiomelanocortin mRNA and decreased neuropeptide Y mRNA in the hypothalamus. *Brain. Res.* **850**, 198–203.

Bird, T. D., Nemens, E. J., and Kukull, W. A. (1993). Conjugal Alzheimer's disease: Is there an increased risk in offspring? *Ann. Neurol.* **34**, 396–399.

Botwinick, J. (1978). "Aging and Behavior." Springer, New York.

Breitner, J. C., Gatz, M., Bergem, A. L., Christian, J. C., Mortimer, J. A., McClearn, G. E., Heston, L. L., Welsh, K. A., Anthony, J. C., Folstein, M. F., and Brandt, J. (1993). Use of twin cohorts for research in Alzheimer's disease. *Neurology* **43**, 261–267.

Breitner, J. C., Welsh, K. A., Gau, B. A., McDonald, W. M., Steffens, D. C., Saunders, A. M., Magruder, K. M., Helms, M. J., Plassman, B. L., Folstein, M. F., and Brandt, J. (1995). Alzheimer's disease in the National Academy of Sciences-National Research Council Registry of Aging Twin Veterans. III. Detection of cases, longitudinal results, and observations on twin concordance. *Arch. Neurol. (Chicago)* **52**, 763–771.

Campion, D., Dumanchin, C., Hannequin, D., Dubois, B., Belliard, S., Puel, M., Thomas-Anterion, C., Michon, A., Martin, C., Charbonnier, F., Raux, G., Camuzat, A., Penet, C., Mesnage, V., Martinez, M., Clerget-Darpoux, F., Brice, A., and Frebourg, T. (1999). Early-onset autosomal dominant Alzheimer disease: Prevalence, genetic heterogeneity, and mutation spectrum. *Am. J. Hum. Genet*. **65**, 664–670.

Conneally, P. M. (1984). Huntington's disease: Genetics and epidemiology. *Am. J. Hum. Genet*. **36**, 506–526.

Crauford, D., and Dodge, A. (1993). Mutation and age at onset in Huntington's disease. *J. Med. Genet*. **30**, 1008–1111.

Cruts, M., and Van Broeckhoven, C. (1998). Molecular genetics of Alzheimer's disease. *Ann. Med*. **30**, 560–565.

Emery, C. F., Pedersen, N. L., Svartengren, M., and McClearn, G. E. (1998). Longitudinal and genetic effects in the relationship between pulmonary function and cognitive performance. *J. Gerontol.* **53**, P311–P317.

Erway, L. C., Shiau, Y. W., Davis, R. R., and Krieg, E. F. (1996). Genetics of age-related hearing loss in mice. III. Susceptibility of inbred and F1 hybrid strains to noise-induced hearing loss. *Hear. Res*. **93**, 181–187.

Farrer, L. A., Myers, R. H., Cupples, L. A., St. George-Hyslop, P. H., Bird, T. D., Rossor, M. N., Mullan, M. J., Polinsky, R., Nee, L., and Heston, L. (1990). Transmission and age-at-onset patterns in familial Alzheimer's disease: Evidence for heterogeneity. *Neurology* **40**, 395–403.

Farrer, L. A., Cupples, L. A., Haines, J. L., Hyman, B., Kukull, W. A., Mayeux, R., Myers, R. H., Pericak-Vance, M. A., Risch, N., and van Duijn, C. M. (1997). Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA, J. Am. Med. Assoc*. **278**, 1349–1356.

Finkel, D., and McGue, M. (1998). Age differences in the nature and origin of individual differences in memory: A behavior genetic analysis. *Int. J. Aging Hum. Dev.* **47**, 217–239.

Finkel, D., Pedersen, N. L., McGue, M., and McClearn, G. E. (1995). Heritability of cognitive abilities in adult twins: Comparison of Minnesota and Swedish data. *Behav. Genet*. **25**, 421–431.

Finkel, D., Pedersen, N. L., Plomin, R., and McClearn, G. E. (1998). Longitudinal and cross-sectional twin data on cognitive abilities in adulthood: The Swedish Adoption/Twin Study of Aging. *Dev. Psychol*. **34**, 1400–1413.

Fölling, A. (1934). Über Ausscheidung von Phenylbreztraubensäure in den Haien als Stoffwechselanomalic in Verbindung mit Imbezillität. *Hoppe-Seyler Z.* **227**, 169–176.

Fox, P. W., Hershberger, S. L., and Bouchard, T. J. (1996). Genetic and environmental contributions to the acquisition of a motor skill. *Nature (London)* **384**, 356–358.

Gallo, J. J., and Breitner, J. C. (1995). Alzheimer's disease in the NAS-NRC Registry of aging twin veterans. IV. Performance characteristics of a two-stage telephone screening procedure for Alzheimer's dementia. *Psychol. Med*. **25**, 1211–1219.

Gatz, M., Pedersen, N. L., Berg, S., Johansson, B., Johansson, K., Mortimer, J. A., Posner, S. F., Viitanen, M., Winblad, B., and Ahlbom, A. (1997). Heritability for Alzheimer's disease: The study of dementia in Swedish twins. *J. Gerontol.* **52**, M117–M1125.

Golbe, L. I., Di Iorio, G., Sanges, G., Lazzarini, A. M., La Sala, S., Bonavita, V., and Duvoisin, R. C. (1996). Clinical genetic analysis of Parkinson's disease in the Contursi kindred. *Ann. Neurol*. **40**, 767–775.

Greenamyre, J. T., and Shoulson, I. (1994). Huntington's disease. *In* "Neurodegenerative Diseases" (D. B. Calne, ed.), pp. 685–704. Saunders, Philadelphia.

Harris, J. R., Pedersen, N. L., McClearn, G. E., Plomin, R., and Nesselroade, J. R. (1992). Age differences in genetic and environmental influences for health from the Swedish Adoption/Twin Study of Aging. *J. Gerontol*. **47**, P213–P220.

Hattori, N., Matsumine, H., Asakawa, S., Kitada, T., Yoshino, H., Elibol, B., Brookes, A. J., Yamamura, Y., Kobayashi, T., Wang, M., Yoritaka, A., Minoshima, S., Shimizu, N., and Mizuno, Y. (1998). Point mutations (Thr240Arg and Gln311Stop) in the Parkin gene. *Biochem. Biophys. Res. Commun*. **249**, 754–758.

Herskind, A. M., McGue, M., Sorensen, T. I., and Harvald, B. (1996). Sex and age specific assessment of genetic and environmental influences on body mass index in twins. *Int. J. Obes. Relat. Metab. Disord*. **20**, 106–113.

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y., and Shimizu, N. (1998). Mutations in the parkin gene cause autosomal recessive juvenile Parkinsonism. *Nature (London)* **392**, 605–608.

Kremer, B., Squitieri, F., Telenius, H., Andrew, S. E., Theilmann, J., Spence, N., Goldberg, Y. P., and Hayden, M. R. (1993). Molecular analysis of late onset Huntington's disease. *J. Med. Genet*. **30**, 991–995.

Kremer, B., Goldberg, P., Andrew, S. E., Theilmann, J., Telenius, H., Zeisler, J., Squitieri, F., Lin, B., Bassett, A., and Almqvist, E. (1994). A worldwide study of the Huntington's disease mutation. The sensitivity and specificity of measuring CAG repeats. *N. Engl. J. Med.* **330**, 1401–1406.

Langston, J. W. (1998). Epidemiology versus gentics in Parkinson's disease: Progress in resolving an age-old debate. *Ann. Neurol*. **44**, S45–S52.

Lautenschlager, N. T., Cupples, L. A., Rao, V. S., Auerbach, S. A., Becker, R., Burke, J., Chui, H., Duara, R., Foley, E. J., Glatt, S. L., Green, R. C., Jones, R., Karlinsky, H., Kukull, W. A., Kurz, A., Larson, E. B., Martelli, K., Sadovnick, A. D., Volicer, L., Waring, S. C., Growdon, J. H., and Farrer, L. A. (1996). Risk of dementia among relatives of

Alzheimer's disease patients in the MIRAGE study: What is in store for the oldest old? *Neurology* **46**, 641–650.

Lilienfeld, D. E. (1994). An epidemiological overview of amyotrophic lateral sclerosis, Parkinson's disease, and dementia of the Alzheimer's type. *In* "Neurodegenerative Diseases" (D. B. Calne, ed.), pp. 399–425. Saunders, Philadelphia.

Lunkes, A., Trottier, Y., and Mandel, J. L. (1998). Pathological mechanisms in Huntington's disease and other polyglutamine expansion diseases. *Essays Biochem*. **33**, 149–163.

Marenberg, M. E., Risch, N., Berkman, L. F., Floderus, B., and de Faire, U. I. (1994) Genetic susceptibility to death from coronary heart disease in a study of twins. *N. Engl. J. Med*. **333**, 1041–1046.

Martilla, R. J. (1987). Epidemiology. *In* "Handbook of Parkinson's Disease" (W. Koller, ed.), pp. 55–50. Dekker, New York.

McCartney, M., Harris, M. J., and Bernieri, F. (1990). Growing up and growing apart: A developmental metanalysis of twin studies. *Psychol. Bull*. **107**, 226–237.

McClearn, G. E., Johansson, B., Berg, S., Pedersen, N. L., Ahern, F., Petrill, S. A., and Plomin, R. (1997). Substantial genetic influence on cognitive abilities in twins 80 or more years old. *Science* **276**, 1560–1563.

McGue, M., Bouchard, T. J., Iaconon, W. G., and Lykken, D. T. (1993). Behavioral genetics of cognitive ability: A life-span perspective. *In* "Nature, Nurture, and Psychology" (R. Plomin and G. E. McClearn, eds.), pp. 59–76. American Psychological Association, Washington, DC.

Meyers, S. M., Greene, T., and Gutman, F. A. (1995). A twin study of age-related macular degeneration. *Am. J. Ophthalmol*. **120**, 757–766.

Plassman, B. L., and Breitner, J. C. (1997). The genetics of dementia in late life. *Psychiatr. Clin. North Am*. **20**, 59–76.

Plomin, R., Pedersen, N. L., Lichtenstein, P., and McClearn, G. E. (1994). Variability and stability in cognitive abilities are largely genetic later in life. *Behav. Genet*. **24**, 207–215.

Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E. S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W. G., Lazzarini, A. M., Duvoisin, R. C., Di Iorio, G., Golbe, L. I., and Nussbaum, R. L. (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* **276**, 2045–2047.

Raiha, I., Kaprio, J., Koskenvuo, M., Rajala, T., and Sourander, L. (1996). Alzheimer's disease in Finnish twins. *Lancet* **347**, 573–578.

Raiha, I., Kaprio, J., Koskenvuo, M., Rajala, T., and Sourander, L. (1997). Alzheimer's disease in twins. *Biomed. Pharmacother.* **51**, 101–104.

Rowe, J. W. (1985). Health care of the elderly. *N. Engl. J. Med*. **312**, 827–835.

Rowe, J. W., and Kahn, R. L. (1998). "Successful Aging." Saunders, Philadelphia.

Rubinsztein, D. C. (1997). The genetics of Alzheimer's disease. *Prog. Neurobiol*. **52**, 447–454.

Simonen, R. L., Videman, T., Battie, M. C., and Gibbons, L. E. (1998). Determinants of psychomotor speed among 61 pairs of adult male monozygotic twins. *J. Gerontol.* **53**, M228–M234.

Slooter, A. J., Cruts, M., Kalmijn, S., Hofman, A., Breteler, M. M., Van Broeckhoven, C., and van Duijn, C. M. (1998). Risk estimates of dementia by apolipoprotein E genotypes from a population-based incidence study: The Rotterdam Study. *Arch. Neurol. (Chicago)* **55**, 964–968.

Sudarsky, L., Myers, R. H., and Walshe, T. M. (1983). Huntington's disease in monozygotic twins reared apart. *J. Med. Genet*. **20**, 408–411.

Surwit, R. S., Kuhn, C. M., Cochrane, C., McCubbin, J. A., and Feinglos, M. N. (1988). Diet-induced type II diabetes in C57BL/6J mice. *Diabetes* **37**, 1163–1167.

Vaughan, J., Durr, A., Tassin, J., Bereznai, B., Gasser, T., Bonifati, V., De Michele, G., Fabrizio, E., Volpe, G., Bandmann, O., Johnson, W. G., Golbe, L. I., Breteler, M., Meco, G., Agid, Y., Brice, A., Marsden, C. D., and Wood, N. W. (1998). The alpha-synuclein Ala53Thr mutation is not a common cause of familial Parkinson's disease: A study of 230 European cases. European Consortium on Genetic Susceptibility in Parkinson's Disease. *Ann. Neurol*. **44**, 270–273.

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B. Jane Keck and Joan M. Lakoski

3

Neurochemistry of Receptor Dynamics in the Aging Brain

I. Introduction

Alterations in central nervous system (CNS) function are intrinsic to aging processes in many species. Numerous investigations have been conducted to identify changes in synaptic processes that are associated with specific age-related disorders. Various neurotransmitter systems have been shown to undergo changes as a function of aging. For example, there is a loss of dopaminergic cells and a corresponding increase in neurotransmitter synthesis with aging (Greenwood *et al*., 1991). Other researchers have shown that declines in striatal noradrenaline, dopamine, and serotonin concentrations occur with age and suggest that these changes correspond to alterations in cognitive function (Tanila *et al*., 1994). Additional studies using aged nonhuman primates have demonstrated reductions in a variety of neurotransmitters, including acetylcholine, norepinephrine, and serotonin (Beal *et al*., 1991).

This chapter focuses on the neurochemistry of changes that occur in various receptor systems in the aging brain. The serotonin (5-hydroxytryptamine; 5-HT) neurotransmitter system, in particular, has been shown to be differentially regulated as a function of age (Steinbusch *et al*., 1990; Johnson *et al*., 1993). Because numerous age-related changes are observed in the serotonergic system, a neurochemical system with diffuse neuronal connections in the CNS, we will specifically refer to this neurotransmitter system throughout the chapter to exemplify various facets of receptor-related changes in the aging brain. For example, the serotonergic system has been implicated in several neurobehavioral processes, including anxiety (Jolas *et al*., 1995), depression (Delgado *et al*., 1990; Cowen, 1993; Stockmeier *et al*., 1998), pain transmission (Yaksh and Wilson, 1979; Crisp and Smith, 1989), and mood and cognition (Altman *et al*., 1990; Bliss and Collingridge, 1993). In fact, the quality of these behaviors is often disrupted with advanced age (Timiras, 1994) and is associated with a decline in serotonergic neuronal function. Additionally, several reports of increased 5-HT turnover (Petkov *et al*., 1987; Steinbusch *et al*., 1990) and decreased 5-HT levels or release (Schlicker *et al*., 1989; Ko *et al*., 1997) in the CNS with aging indicate the emergence of age-induced changes in the neurochemical balance of the 5-HT neurotransmitter system.

In a broader context, this chapter highlights fundamental issues and approaches used to address the neurochemical aspects of receptor expression and function. Whether assessment is made of receptor structure, localization, or distribution, these indices are regulated in a dynamic fashion. Indeed, neurochemical analysis of receptor expression is linked to continual processes of receptor-specific turnover, up- and down-regulation, and kinetics of receptor recovery, as well as modulation by paracrine and autocrine factors, which include a variety of hormone and cytokines. We will endeavor to provide insight into the numerous issues and research strategies that link the neurochemistry of receptors to normal and pathological processes in the aging CNS. This snapshot of the dynamics of receptors in the aging brain can serve as a model for similar issues to be examined in peripheral tissues.

The following sections describe various findings of alterations in receptor turnover, signal transduction, neuromodulatory regulation, and the relevance of these changes to the aging brain. We will first, however, explore the age-related changes that occur in receptor density for several specific neurotransmitter receptor systems (i.e., serotonin, dopamine, adrenaline, and acetylcholine).

II. Receptor Density and Function

The density of receptors in a given membrane and their ability to recover from insult (e.g., hypoxic injury or neurotoxic exposure) are critical factors in the ability of the aging brain to adapt to and subsequently respond to pharmaceutical agents that interact specifically with these receptors. For example, we have found that the density of hippocampal 5-HT_{1A} receptors is decreased with aging (Keck and Lakoski, 2000). It is important to recognize that changes in receptor number may reflect alterations in the rates of receptor synthesis and turnover. Thus, it is also vital to consider the importance of modifications in

other indices, such as receptor recovery, when treating disorders such as depression and anxiety, which are associated with altered 5-HT_{1A} receptor function (Charney *et al*., 1990; Meltzer, 1990).

The serotonin receptor subtype 5-HT_{1A}, in particular, is significant due to its implication in disorders of mood; the functional importance of this receptor has been validated by the effectiveness of 5-HT_{1A} receptor agonists/antagonists in the treatment of anxiety and depression, respectively (Fernández-Guasti and López-Rubalcava, 1990; Martin *et al*., 1990). Changes in 5-HT_{1A} receptor density with age are variable; human studies report a decline in 5-HT_{1A} receptor density (Dillon *et al*., 1991) and mRNA abundance (Burnet *et al*., 1994), whereas investigations using the rat do not consistently demonstrate losses in 5-HT_{1A} receptor number. Yamaguchi and Yamagata (1991) showed no significant change in the number of cortical 5-HT_{1A} receptors with age, yet observations in our own laboratory indicate a slight decrease in receptor density in the hippocampus associated with aging (Keck and Lakoski, 2000). Interestingly, deficiencies of 5-HT_{1A} receptors have been observed in cortical tissue from patients diagnosed with the age-related disorder Alzheimer's disease (Middlemiss *et al*., 1986). This diversity in age-specific changes in basal 5-HT_{1A} receptor number may reflect neuroanatomical differences, between rats and humans, in radioligand binding using the agonist 8-hydroxy-2(di-*n*-propylamino)-tetralin ([^{3}H]8-OH-DPAT), which selectively binds to 5-HT_{1A} receptor sites (Duncan *et al*., 1998).

Despite conflicting binding data, age-dependent changes in 5-HT_{1A} receptor function are well documented and include the attenuation of 5-HT_{1A} receptor agonist-induced reduction of 5-hydroxyindoleacetic acid (Robson *et al*., 1993), declines in 5-HT_{1A} receptor-mediated circadian rhythmicity (Penev *et al*., 1995), decreases in the functional adaptation of the 5-HT_{1A} receptor following exposure to a subchronic stressor (de Castro *et al*., 1996), and a loss of responsiveness to an irreversible inactivating agent, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ; Keck and Lakoski, 1996a). Correspondingly, 5-HT_{1A} receptors from old animals display distinct patterns of recovery and turnover following irreversible inactivation compared to their young counterparts (Keck and Lakoski, 2000; see later). Moreover, studies of corticosteroid hormone modulation of the 5-HT_{1A} receptor in the aging hippocampus demonstrated an age-dependent loss in the ability of high and low levels of corticosterone pretreatment to down- and upregulate the density of 5-HT_{1A} receptor agonist binding, respectively (Maines *et al*., 1998). Similar observations have been noted for the serotonin transporter in which there was an age-related loss in the ability of corticosterone treatment to induce a decline in binding density in hippocampal tissue (Maines *et al*., 1999). Thus, in the hippocampus, an age-dependent change in 5-HT_{1A} receptor/ corticosteroid interaction was revealed.

Another 5-HT receptor subtype, the 5-HT_{2A} receptor, has become increasingly important since the discovery and use of therapeutically effective atypical antipsychotic medications for the treatment of mood disorders and psychosis. Several *in vitro*, *in vivo*, postmortem, and positron emission tomography (PET) findings confirm a decline in 5-HT_{2A} receptor binding with advancing age (Druse *et al*., 1997; Baeken *et al*., 1998; Meltzer *et al*., 1998). For example, a study utilized PET scans in young and elderly individuals to test for cortical alterations in 5-HT_{2A} receptor-binding density. Even after correcting for effects of cerebral atrophy, a significant loss (57%) in specific binding was demonstrated in the elderly group for this binding site relative to the young control group (Meltzer *et al*., 1998). These findings underscore the role of 5-HT_{2A} receptors in the etiology of mood changes in the elderly and may have important therapeutic implications for the treatment of disorders such as geriatric depression.

As a consequence of aging, there are a variety of anatomical and physiological changes in brain dopamine (DA)-binding proteins. Araki *et al*. (1997) demonstrated a decrease in radioligand binding (using [^{3}H]mazindol) to the DA transporter. The clinical importance of age-related changes in the classical DA receptors (D_1 and D_2) is exemplified by the resultant motor disturbances associated with altered receptor number/function. It has been suggested that the decreased motor abilities associated with normal aging or neurodegenerative diseases, such as Parkinson's disease, result from alterations in striatal D_1 and D_2 receptors (Cross and Rossor, 1983; Joseph *et al*., 1983; Rinne *et al*., 1991; Roth and Joseph, 1994; Turjanski *et al*., 1995). In fact, a large body of evidence supporting this theory reveals substantial declines in D_2 receptor densities in both humans (D. F. Wong *et al*., 1984, 1997; W. F. Wong *et al*., 1997) and rodents (O'Boyle and Waddingron, 1984; Tajuddin and Druse, 1996) and demonstrates a loss of D_2 receptor-containing neurons (Han *et al*., 1989) with aging. Additionally, researchers have uncovered a variety of age-related changes in D_2 receptor mRNA, including a reduction in receptor mRNA levels (Mesco *et al*., 1991; Della Vedova *et al*., 1992), and a decrease in the rate of D_2 receptor mRNA synthesis (Mesco *et al*., 1993). Together, these findings strengthen the implied relationship among aging, DA receptor modification, and disease-related motor dysfunction.

Like the dopaminergic and serotonergic receptor systems, acetylcholine (ACh) receptors undergo age-related alterations. Studies of muscarinic (M) cholinergic receptor subtypes M_1 and M_2 have shown an age-specific pattern of binding density (Biegon *et al*., 1989). Declines in nicotinic ACh receptors have also been shown in aged humans (Marutle *et al*., 1998), and postmortem investigations of cortical and hippocampal tissues revealed age-related decreases in mRNA expression for nicotinic receptor subunits α_4 and β_2 (Tohgi *et al*., 1998). These findings, exhibiting decreases in nicotinic acetylcholine receptor transcription during aging, suggest that such changes may be important contributors to the cognitive decline seen in the elderly (Tohgi *et al*., 1998). Additionally, Buyukuysal and associates (1998) reported a reduction in the stimulated release of ACh as well as a decline in muscarinic ACh receptor density with advancing age. A corroborating report by Vannuchi *et al*. (1997) showed a decline in ACh neurotransmitter release in aged rats and an associated decline in cognitive performance. These reports lend further support for a relationship between cholinergic decline and cognitive dysfunction and point to the muscarinic receptor as a possible target for medications used to treat memory problems associated with aging (Vannuchi *et al*., 1997).

Similar to cholinergic receptors, adrenergic receptors are also described to decline in number with aging. Using quanti-

tative autoradiographic analyses and ^{125}I-labeled pindolol binding as an indicator of β-adrenergic receptor density, a significant region-specific loss was demonstrated in the aging rat brain (Miller and Zahniser, 1988). A report by Gould and Bickford (1997) supports an age-related loss in the adrenergic receptor system function. More specifically, these investigators showed that in aged rats there is a deficiency in β-adrenergic signal transduction. Together, these data indicate that with aging there is a significant decline in β-adrenergic receptor number and function.

It is clear that with aging there is a myriad of alterations in receptor numbers that occur in the mammalian CNS. As changes in receptor density are critical aspects to the changing neurochemistry of neurotransmitter system functioning, these age-induced alterations in receptors will likely extend into physiological, behavioral, and cognitive ramifications.

III. Receptor Turnover

The practical importance of analysis of changes in receptor turnover with aging has already been introduced briefly in terms of older individuals' abilities to adapt to neurological damage. The capability to recover quickly, or at all, from receptor loss/cellular injury could impact the overall progression of and recovery from an illness. A fast rate of receptor turnover might enhance the recuperation process, especially if the illness had an associated loss of receptor number or receptor function. Similarly, a slow turnover of receptors following insult might decrease the likelihood of recovery. Furthermore, the development or discovery of agents that enhance receptor turnover (e.g., neurotrophic factors, estrogen) might prove very useful for the treatment of trauma-related processes in young individuals, as well as progressive neurodegenerative disease processes in the elderly.

A. Approaches to Investigate Receptor Inactivation and Turnover

In investigations using animal models, various neurotoxins and drug agents have been used to examine (1) the sensitivity of ''aged'' receptors to the damaging effects of a toxin and (2) the recovery of various receptors (Riekkinen *et al*., 1992; Zawia *et al*., 1992). In general, these studies have shown a loss in receptor plasticity with aging and a low rate of receptor regeneration (Greenberg and Weiss, 1979; Pitha *et al*., 1982; Zhou *et al*., 1984). The alkylating agent EEDQ (Battaglia *et al*., 1987; Cox *et al*., 1993; Gozlan *et al*., 1994; Pinto and Battaglia, 1994; Raghupathi *et al*., 1996), which acts to irreversibly block specific receptors, is one such agent that has been used frequently to investigate age-related changes in receptor turnover. The EEDQ molecule noncompetitively inactivates several G-protein-coupled receptor sites, including serotonergic (Pinto and Battaglia, 1993; Gozlan *et al*., 1994; Keck and Lakoski, 1996b; Ni *et al*., 1997; Kettle *et al*., 1999), dopaminergic (Henry and Roth, 1984), α-adrenergic (Adler *et al*., 1985), β-adrenergic (Neve and Molinoff, 1986), and muscarinic (Norman and Creese, 1986) receptors. This neurotoxicant irreversibly inactivates receptors by producing a receptor-linked mixed carbonic anhydride, which, in turn, forms an irreversible bond with nucleophilic groups in the ligand-binding pocket of the binding site (Belleau *et al*., 1969). Subsequently, the kinetics of receptor recovery can be established and compared in young adult and old animal models. Results of these types of experiments contribute much needed information regarding the effects of aging on the functional capacity of neurotransmitter systems following a neurotoxic insult.

Of the serotonergic receptor subtypes, the 5-HT$_{1A}$ receptor is the most sensitive to inactivation by EEDQ (Gozlan *et al*., 1983, 1994). This receptor subtype has been shown to be negatively coupled to adenylate cyclase via pertussis toxin-sensitive guanine nucleotide-binding proteins (Zgombick *et al*., 1989). Pretreatment with WAY 100,635, a selective 5-HT$_{1A}$ receptor antagonist, protects these binding sites (over 70%) from inactivation by EEDQ (Gozlan *et al*., 1994) and confirms that the irreversible inactivator interacts directly at the ligand recognition site of the 5-HT$_{1A}$ receptor. Therefore, EEDQ administration has been a very appropriate pharmacological tool to examine 5-HT$_{1A}$ receptor recovery with aging (Keck and Lakoski, 1997).

In our own work, we have investigated the kinetics of receptor recovery for the aging 5-HT$_{1A}$ receptor (Keck and Lakoski, 1996b, 1997, 2000). Our findings have provided an interesting framework in which to characterize age-associated changes in the recovery profile of the 5-HT$_{1A}$-binding site in the CNS. We examined the kinetics of 5-HT$_{1A}$ receptor recovery with aging and discovered that, unlike D_2 receptors or α_1- and α_2-adrenoceptors, hippocampal 5-HT$_{1A}$ receptors demonstrate a faster rate of recovery in old rats compared to young adult rats. Dopaminergic and α-adrenergic receptors, however, have been shown to undergo a delayed rate of recovery in aged animals (see later; Henry and Roth, 1984; Leff *et al*., 1984; Zhou *et al*., 1984; Henry *et al*., 1987). In our studies, 5-HT$_{1A}$ receptor density in the hippocampus returned to vehicle control levels at an earlier time point in old, 22-month rats compared to young, 3-month rats. The turnover of hippocampal 5-HT$_{1A}$ receptors was nearly three times faster in old rats versus young adult rats (Keck and Lakoski, 2000). From these findings, we surmise that a cooperative relationship exists between age-dependent increases in 5-HT$_{1A}$ receptor turnover and increases in the turnover of the 5-HT neurotransmitter itself (Petkov *et al*., 1987; Steinbusch *et al*., 1990).

Numerous studies of toxin-induced degradation of the dopaminergic receptor system have demonstrated age-related differences in the rate of recovery of dopamine receptors, especially the D_2 receptor. Typically, senescent rats have been shown to exhibit lower rates of dopamine receptor turnover, production, and degradation compared to mature rats (Henry and Roth, 1984; Leff *et al*., 1984; Norman *et al*., 1987; Battaglia *et al*., 1988) following treatment with EEDQ. An age-related decline in receptor synthesis following EEDQ treatment also correlated with a reduced recovery in motor function (Henry *et al*., 1987). Results from other investigations showing that the rates of recovery for both D_1 and D_2 receptor proteins were higher in young rats than in older age groups (Kula *et al*., 1992; Crawford *et al*., 1994) lend additional support for an age-related alteration in the recovery pattern of dopaminergic receptors.

For adrenergic receptors, the recovery of [^{3}H]prazocin and [^{3}H]rauwolscine binding, which labels α_1- and α_2-adreno-

ceptors, respectively, was delayed significantly in aged compared to young rats (Zhou *et al*., 1984). Similar to the β-adrenoreceptor, the *de novo* synthesis of new receptor proteins was demonstrated to be low in senescent animals (Pitha *et al*., 1982).

Hence, a general theme has unfolded that indicates an overall decline in the ability of aged receptors to recover from neurotoxic injury, with an exception being 5-HT_{1A} receptors in senescent rats which have been shown to recover at a faster rate compared to the same receptor populations in young rats.

IV. Receptor/Effector Coupling Processes

The topic of age-related changes in receptor-linked signal transduction mechanisms requires an entire book of its own. However, we have included here a short description of some of the relevant findings in the serotonergic system with aging to serve as a cursory introduction to the large realm of alterations that can occur in receptor/effector coupling mechanisms. In the serotonergic system, age-induced changes in receptor/effector coupling mechanisms and metabolism suggest that the 5-HT receptor function is altered with aging (Robson *et al*., 1993).

From our early studies with EEDQ, we discovered an age-related decrease in 5-HT_{1A} receptor affinity following EEDQ treatment. Using EEDQ, we hypothesized that a subpopulation of low-affinity 5-HT_{1A} receptors, perhaps G-protein-uncoupled, was unmasked. Previous work by other researchers indicated that [^{3}H]8-OH-DPAT, the agonist radioligand used in our studies, labeled both high and low affinity-binding sites (Mongeau *et al*., 1992; Nénonéné *et al*., 1994). The high affinity site was perceived to be G-protein coupled, as the addition of the nonhydrolyzable GTP analog GppNHp converted this binding site to a low affinity state (Mongeau *et al*., 1992; Nénonéné *et al*., 1994). Nénonéné *et al*. (1994) also demonstrated that the low affinity [^{3}H]8-OH-DPAT-binding site was insensitive to GppNHp, implying that this binding site was uncoupled from its G-protein.

Our own research findings showing age- and brain-region specific changes following treatment with EEDQ (Keck and Lakoski, 2000) may reflect similar alterations in the affinity of the radioligand [^{3}H]8-OH-DPAT for 5-HT_{1A} receptors and/or in the ability of these receptors to interact with their corresponding G-proteins. Because EEDQ evidently interacts specifically with receptors that are coupled to G-proteins and because subpopulations of 5-HT_{1A} receptors in specific brain tissues are insensitive to EEDQ blockade in aged rats, our findings demonstrating an age-dependent decrease in affinity for the same pool of receptors following EEDQ treatment imply that an increase in the proportion of low affinity/G-protein-uncoupled sites (unaffected by EEDQ administration) emerges with aging (see also Keck and Lakoski, 1996a). The density of [^{3}H]8-OH-DPAT binding is not affected by EEDQ in these brain regions in old rats, yet treatment with the irreversible inactivator produces concomitant decreases in affinity. Future binding studies using a radiolabeled antagonist selective for the 5-HT_{1A} receptor (such as [^{3}H]p-MPPF; Kung *et al*., 1996) would be useful to confirm these observations.

Age-associated changes in the affinity for 5-HT_{1A} receptors in the hippocampus, frontal cortex, and amygdala following EEDQ may be especially important, as it is well known that these brain areas play a role in memory (Eichenbaum *et al*., 1992) and general cognitive function, two related abilities that are often compromised with aging. Likewise, deficiencies of 5-HT_{1A} receptors have been observed in the cortices of patients with age-related diseases such as Alzheimer's disease (Middlemiss *et al*., 1986). One explanation for the age-related decline in 5-HT_{1A} receptor affinity following EEDQ treatment may be that age-dependent changes in the conformation of these receptors account for difficult interactions among receptor proteins, G-protein-coupled effectors, and EEDQ. For example, it has been shown that sulfhydryl groups are needed to assure efficient coupling between the receptor and its G-protein (Kitamura and Nomura, 1987). These nucleophilic groups may also be crucial for the alkylation process induced by EEDQ at the target receptor. An age-related decrease in the number or accessibility of sulfhydryl groups (Reader *et al*., 1995) or EEDQ-sensitive carboxylic groups at the receptor site, perhaps coupled with an increase in the number of receptors that are dissociated from the G-protein, could affect the affinity of this agent for the 5-HT_{1A} receptor. These mechanisms may also contribute to the limited effect of EEDQ treatment on 5-HT_{1A} receptor binding observed in the frontal cortex and amygdala of old rats.

Multiple signal transduction processes are also coupled to the expression and function of other 5-HT receptor subtypes as well as a plethora of dopaminergic, adrenergic, and cholinergic receptors. In addition to the classical role of coupling to G-proteins commonly found with metabotropic receptors, it is important to acknowledge that age-dependent changes in other signal transduction components, ranging from expression of adenylate cyclase to calcium-mediated sequestering processes, will also impact the ability of the aged receptor to effectively and efficiently transduce signaling information.

V. Neuromodulatory Regulation of Receptors

Age-related changes in the hormonal regulation of receptor number and function are vital forces in the changing responsiveness of CNS tissues to neurotransmitter effects. For example, the loss of estrogen in the aged female has been recognized to alter the effects of the neurotransmitter 5-HT in a variety of ways. Estrogen replacement therapy increases the efficacy of fluoxetine (a selective serotonin reuptake inhibitor) in the treatment of depression in geriatric females (Schneider *et al*., 1997; McCusker *et al*., 1998; Rubinow *et al*., 1998). This gonadal hormone has been shown to produce increases in tryptophan hydroxylase mRNA expression (Pecins-Thompson *et al*., 1996), 5-HT levels in the plasma, blood and platelets (Malyszko *et al*., 1995; Blum *et al*., 1996), and excretion of the serotonin metabolite 5-hydroxyindole acetic acid (5-HIAA; Lippert *et al*., 1996; Mueck *et al*., 1997). These data support a general role for estrogen in the enhancement of serotonergic activity.

The contribution of estrogen to 5-HT_{1A} receptor expression has also been investigated by several research groups. For instance, the hyperphagic response elicited by the selective

5-HT_{1A} receptor agonist 8-OH-DPAT was less prominent in the presence of estrogen (Salamanca and Uphouse, 1992; Uphouse *et al*., 1994; Maswood and Uphouse, 1997). In another study, Maswood *et al*. (1995) suggested that 8-OH-DPAT was less effective in reducing tissue concentrations of the serotonin metabolite 5-HIAA due to the presence of estrogen. Furthermore, Lakoski (1988, 1989) reported that the ability of 8-OH-DPAT to reduce the firing of dorsal raphe neurons was reduced by estrogen. All of these studies suggest an inhibitory role of estrogen on the function of 5-HT_{1A} receptors. Still others have reported an enhanced activity of the 5-HT_{1A} receptor following estrogen. Chronic estradiol increased the functional response to 8-OH-DPAT by enhancing the ability of the agonist to increase serum corticosterone levels *in vivo* (Matsuda *et al*., 1991). Also, estradiol treatment has been shown to selectively potentiate the ability of centrally administered 8-OH-DPAT to decrease heart rate (Alper and Schmitz, 1996), enhance the inhibition of adenylyl cyclase activity by 5-HT (Clarke and Maayani, 1990), and increase the hypothermic response to 8-OH-DPAT in ovariectomized rats (Young *et al*., 1993). While the density of 5-HT receptor-binding sites varies with estrous cycle (Biegon *et al*., 1980; Al-Dahan *et al*., 1994), data describing changes in 5-HT_{1A} receptor binding or mRNA concentration following treatment with estrogen remain inconsistent (Biegon *et al*., 1982, 1983; Biegon and McEwen, 1982; Clarke and Maayani, 1990; Sumner and Fink, 1993; Frankfurt *et al*., 1994). Although the effects of estrogen on 5-HT_{1A} receptor density and/or function is uncertain (i.e., increased versus decreased), an interaction among this ovarian hormone and 5-HT_{1A} receptors is well documented (Maswood *et al*., 1995; Cologer-Clifford *et al*., 1996; McKittrick and McEwen, 1996).

Evidence shows that estrogen is capable of producing neurotrophic effects on neurons and receptors. Estrogen treatment increases the growth and arborization of neuronal processes (neurites) on dendrites and axons of developing neurons (Nichizuka and Arai, 1981; Hammer and Jacobson, 1984; Stanley *et al*., 1986). Moreover, while estrogen promotes the expansion of neurites primarily during development, following injury to adult brain regions (e.g., deafferentation or axotomy), the growth-promoting attributes of estrogen are expressed anew and estrogen is again capable of influencing dendritic differentiation and synapse development (Matsumoto and Arai, 1981). Similarly, treating cultured neurons damaged by glutamate with estrogen protects against cell death and free-radical buildup (Behl *et al*., 1997). Ultimately, estrogen-induced enhancement of receptor function will be beneficial in treating disorders of the CNS involving receptor dysfunction.

The findings just described lead to the intriguing hypothesis that estrogen supplementation may prevent or improve degenerative symptoms associated with a loss of serotonergic cells, receptors, or function observed in disorders such as Alzheimer's disease (Aletrino *et al*., 1992; Palmer and DeKosky, 1993; Storga *et al*., 1996; Baldereschi *et al*., 1998). Indeed, estrogen therapy has been shown to augment cognitive functioning in the postmenopausal woman (Jacobs *et al*., 1998), protect against the development of dementia in Parkinson's disease (Marder *et al*., 1998), improve attention, orientation, mood, and social interaction in females with senile dementia of the Alzheimer's type (Fillit *et al*., 1986), and enhance verbal memory in women with Alzheimer's disease (Asthana *et al*., 1996). In the future, consistent data illustrating a change in the degree of 5-HT_{1A} receptor mRNA expression following estrogen administration would be useful in the development of new treatments for aging female populations undergoing degenerative processes (e.g., Alzheimer's disease). In essence, estrogen and other growth factors, such as nerve growth factor, may work together to decrease the vulnerability of adult serotonergic neurons to disease processes or aging and may increase their compensatory responses following neurodegenerative injury.

VI. Future Directions

Future studies that examine age-induced changes in neurotransmitters and the corresponding onset of neurodegenerative diseases will provide information about the relationship between aging receptors and age-related declines in cognition, anxiety, depression, and Alzheimer's disease. A practical goal of current researchers should be to investigate the age-dependent role of molecular changes in the expression of neurotransmitter receptors and their subtypes in the etiology of these behavioral processes. Indeed, there is a great need for additional information regarding the molecular substrates of neurotransmitter-related behaviors in the aging animal. As the involvement of multiple neurotransmitter systems in aging processes becomes more fully understood, particularly at the level of gene regulation, new approaches for the treatment of cognitive and mood disorders in aged populations will ensue.

Tremendous advances have been made in our understanding of the fundamental biological processes that govern the dynamics of receptor expression and function. However, the future will be even brighter as image analysis techniques are developed to visualize receptor dynamics across the life span. The enhancements in image resolution that can be obtained *in vivo* are providing the first steps toward monitoring receptors in an organism on a continual basis throughout the health span. Thus, the temporal limitation of our current approaches, which often make it cumbersome and difficult to correlate the neurochemical profile of a given receptor subtype with a behavioral and/or genetic trait, holds great promise for resolution in the coming years.

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References

Adler, C. H., Meller, E., and Goldstein, M. (1985). Recovery of α_2-adrenoceptor binding and function after irreversible inactivation by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). *Eur. J. Pharmacol*. **116**, 175–178.

Al-Dahan, M. I., Jalilian Tehrani, M. H., and Thalmann, R. H. (1994). Regulation of gamma-aminobutyric $acid_B$ ($GABA_B$) receptors in cerebral cortex during the estrous cycle. *Brain Res*. **640**, 33–39.

Aletrino, M. A., Vogels, O. J. M., Van Domburg, P. H. M. F., and Ten Donkelaar, H. J. (1992). Cell loss in the nucleus raphes dorsalis in Alzheimer's disease. *Neurobiol. Aging* **13**, 461–468.

Alper, R. H., and Schmitz, T. M. (1996). Estrogen increases the bradycardia elicited by central administration of the serotonin$_{1A}$ agonist 8-OH-DPAT in conscious rats. *Brain Res*. **716**, 224–228.

Altman, H. J., Normile, H. J., Galloway, M. P., Ramirez, A., and Azmitia, E. C. (1990). Enhanced spatial discrimination learning in rats following 5,7-DHT-induced serotonergic deafferentation of the hippocampus. *Brain Res*. **518**, 61–66.

Araki, T., Kato, H., Shuto, K., Fujiwara, T., and Itoyama, Y. (1997). Effect of aging on dopaminergic receptors and uptake sites in the rat brain studied by receptor autoradiography. *J. Neurol. Sci*. **148**, 131–137.

Asthana, S., Craft, S., Baker, L. D., Raskind, M. A., Avery, E., Lofgreen, C., Wilkinson, C. W., Falzgraf, S., Veith, R. C., and Plymate, S. R. (1996). Transdermal estrogen improves memory in women with Alzheimer's disease. *Soc. Neurosci. Abstr.* **22**, 200.

Baeken, C., D'haenen, H., Flamen, P., Mertens, J., Terriere, D., Chavatte, K., Boumon, R., and Bossuyt, A. (1998). ^{123}I-5-I-R91150, a new single-photon emission tomography ligand for 5-HT2A receptors: Influence of age and gender in healthy subjects. *Eur. J. Nucl. Med*. **25**, 1617–1622.

Baldereschi, M., DiCarlo, A., Lepore, V., Bracco, L., Maggi, S., Grigoletto, F., Scarlato, G., and Amaducci, L. (1998). Estrogen-replacement therapy and Alzheimer's disease in the Italian Longitudinal Study on Aging. *Neurology* **50**, 996–1002.

Battaglia, G., Norman, A. B., and Creese, I. (1987). Differential serotonin$_2$ receptor recovery in mature and senescent rat brain after irreversible receptor modification: Effect of chronic reserpine treatment. *J. Pharmacol. Exp. Ther.* **243**, 69–75.

Battaglia, G., Norman, A. B., and Creese, I. (1988). Age-related differential recovery rates of rat striatal D-1 dopamine receptors following irreversible inactivation. *Eur. J. Pharmacol*. **145**, 281–290.

Beal, M. F., Walker, L. C., Storey, E., Segar, L., Price, D. L., and Cork, L. (1991). Neurotransmitters in neocortex of aged rhesus monkeys. *Neurobiol. Aging* **12**, 407–412.

Behl, C., Skutella, T., Lezoualc'h, F., Post, A., Widmann, M., Newton, C. J., and Holsboer, F. (1997). Neuroprotection against oxidative stress by estrogens: Structure-activity relationship. *Mol. Pharmacol*. **51**, 535–541.

Belleau, B., DiTullio, V., and Godin, D. (1969). The mechanism of irreversible adrenergic blockade by *N*-carbethoxydihydroquinolines—Model studies with typical serine hydrolases. *Biochem. Pharmacol*. **18**, 1039–1044.

Biegon, A., and McEwen, B. S. (1982). Modulation by estradiol of serotonin receptors in brain. *J. Neurosci*. **2**, 199–205.

Biegon, A., Bercovitz, H., and Samuel, D. (1980). Serotonin receptor concentration during the estrous cycle of the rat. *Brain Res*., **187**, 221–225.

Biegon, A., Fischette, C. T., Rainbow, T. C., and McEwen, B. S. (1982). Serotonin receptor modulation by estrogen in discrete brain nuclei. *Neuroendocrinology.* **35**, 287–291.

Biegon, A., Reches, A., Snyder, L., and McEwen, B. S. (1983). Serotonergic and noradrenergic receptors in the rat brain: Modulation by chronic exposure to ovarian hormones. *Life Sci*. **32**, 2015–2021.

Biegon, A., Hanau, M., Greenberger, V., and Segal, M. (1989). Aging and brain cholinergic muscarinic receptor subtypes: An autoradiographic study in the rat. *Neurobiol. Aging* **10**, 305–310.

Bliss, T. V. P., and Collingridge, G. L. (1993). A synaptic model of memory: Long-term potentiation in the hippocampus. *Nature (London)*, **361**, 31–39.

Blum, I., Vered, Y., Lifshitz, A., Harel, D., Blum, M., Nordenberg, Y., Harsat, A., Sulkes, J., Gabbay, U., and Graff, E. (1996). The effect of estrogen replacement therapy on plasma serotonin and catecholamines of postmenopausal women. *Isr. J. Med. Sci*. **32**, 1158–1162.

Burnet, P. W. J., Eastwood, S. L., and Harrison, P. J. (1994). Detection and quantitation of 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor mRNAs in human hippocampus using a reverse transcriptase-polymerase chain reaction (RT-PCR) technique and their correlation with binding site densities and age. *Neurosci. Lett*. **178**, 85–89.

Buyukuysal, R. L., Ulus, I. H., and Kiran, B. K. (1998). Age-related alterations in pre-synaptic and receptor-mediated cholinergic functions in rat brain. *Neurochem. Res*. **23**, 719–726.

Charney, D. S., Woods, S. W., Krystal, J. H., and Heninger, G. R. (1990). Serotonin function and human anxiety disorders. *Ann. N.Y. Acad. Sci*. **600**, 558–573.

Clarke, W. P., and Maayani, S. (1990). Estrogen effects on 5-HT$_{1A}$ receptors in hippocampal membranes from ovariectomized rats: Functional and binding studies. *Brain Res*. **518**, 287–291.

Cologer-Clifford, A., Smoluk, S. A., and Simon, N. G. (1996). Effects of serotonergic1A and 1B agonists in androgenic versus estrogenic systems for aggression. *Ann. N. Y. Acad. Sci*. **794**, 339–342.

Cowen, P. J. (1993). Serotonin receptor subtypes in depression: Evidence from studies in neuroendocrine regulation. *Clin. Neuropharmacol*. **16**(Suppl. 3), S6–S18.

Cox, R. F., Meller, E., and Waszczak, B. L. (1993). Electrophysiological evidence for a large receptor reserve for the inhibition of dorsal raphe neuronal firing by 5-HT$_{1A}$ agonists. *Synapse* **14**, 297–304.

Crawford, C. A., Rowlett, J. K., McDougall, S. A., and Bardo, M. T. (1994). Age-dependent differences in the rate of recovery of striatal dopamine D$_1$ and D$_2$ receptors after inactivation with EEDQ. *Eur. J. Pharmacol*. **252**, 225–231.

Crisp, T. C., and D. J. Smith, (1989). A local serotonergic component involved in the spinal antinociceptive action of morphine. *Neuropharmacology* **28**, 1047–1053.

Cross, A., and Rossor, M. (1983). Dopamine D-1 and D-2 receptors in Huntington's disease. *Eur. J. Pharmacol*. **88**, 223–229.

de Castro, R. M., Bolaños-Jiménez, F., Seguin L., Sarhan, H., Drieu, K., and Fillion, G. (1996). Sub-chronic cold stress reduces 5-HT$_{1A}$ receptor responsiveness in the old but not the young rat. *Neurosci. Lett*. **203**, 21–24.

Delgado, P. L., Charney, D. S., Price, L. H., Aghajanian, G. K., Landis, H., and Heninger, G. R. (1990). Serotonin function and the mechanism of antidepressant action. *Arch. Gen. Psychiatry* **47**, 411–418.

Della Vedova, F., Fumagalli, F., Sacchetti, G., Rcagni, G., and Brunello, N. (1992). Age-related variations in relative abundance of alternative spliced D2 receptor mRNAs in brain areas of two rat strains. *Mol. Brain Res*. **12**, 357–359.

Dillon, K. A., Gross-Isseroff, R., Israeli, M., and Biegon, A. (1991). Autoradiographic analysis of serotonin 5-HT$_{1A}$ receptor binding in the human brain postmortem: Effects of age and alcohol. *Brain Res*. **554**, 56–64.

Druse, M. J., Tajuddin, N. F., and Ricken, J. D. (1997). Effects of chronic ethanol consumption and aging on 5-HT2A receptors and 5-HT reuptake sites. *Alcohol.: Clin. Exp. Res*. **21**, 1157–1164.

Duncan, G. E., Knapp, D. J., Breese, G. R., Crews, F. T., and Little, K. Y. (1998). Species differences in regional patterns of ^{3}H-8-OH-DPAT and ^{3}H-Zolpidem binding in the rat and human brain. *Pharmacol. Biochem. Behav.* **60**, 439–448.

Eichenbaum, H., Otto, T., and Cohen, N. J. (1992). The hippocampus—What does it do? *Behav. Neural Biol*. **57**, 2–36.

Fernández-Guasti, A., and López-Rubalcava, C. (1990). Evidence for the involvement of the 5-HT$_{1A}$ receptor in the anxiolytic action of indorenate and ipsapirone. *Psychopharmacology* **101**, 354–358.

Fillit, H., Weinreb, H., Cholst, I., Luine, V., McWean, B., Amador, R., and Zabriskie, J. (1986). Observations in a preliminary open trial of estradiol therapy for senile dementia, Alzheimer's type. *Psychoneuroendocrinology* **11**, 337–345.

Frankfurt, M., McKittrick, C. R., Mendelson, S. D., and McEwen, B. S. (1994). Effect of 5,7-dihydroxytryptamine, ovariectomy and gonadal steroids on serotonin receptor binding in rat brain. *Neuroendocrinology* **59**, 245–250.

Gould, T. J., and Bickford, P. C. (1997). Age-related deficits in the cerebellar beta adrenergic signal transduction cascade in Fischer 344 rats. *J. Pharmacol. Exp. Ther.* **281**, 965–971.

Gozlan, H., El Mestikawy, S., Pichat, L., Glowinski, J., and Hamon, M. (1983). Identification of presynaptic serotonin autoreceptors using a new ligand: ^{3}H-PAT. *Nature (London)* **305**, 140–142.

Gozlan, H., Laporte, A. -M., Thibault S., Schechter, L. E., Bolaños, F., and Hamon, M. (1994). Differential effects of *n*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) on various 5-HT receptor binding sites in the rat brain. *Neuropharmacology.* **33**, 423–431.

Greenberg, L. H., and Wiess, B. (1979). Ability of aged rats to alter beta adrenergic receptors of brain in response to repeated administration of reserpine and desmethylimipramine. *J. Pharmacol. Exp. Ther.* **211**, 309–316.

Greenwood, C. E., Tatton, W. G., Seniuk, N. A., and Biddle, F. G. (1991). Increased dopamine synthesis in aging substantia nigra neurons. *Neurobiol. Aging* **12**, 557–565.

Hammer, R. P., and Jacobson, C. D. (1984). Sex differences in dendritic development of the sexually dimorphic nucleus of the preoptic area in the rat. *Int. J. Dev. Neurosci*. **2**, 77–86.

Han, Z., Kuyatt, B. L., Kochman, K. A., DeSouza, E. B., and Roth, G. S. (1989). Effect of aging on concentrations of D2-receptor-containing neurons in the rat striatum. *Brain Res*. **498**, 299–307.

Henry, J. M., and Roth, G. S. (1984). Effect of aging on recovery of striatal dopamine receptors following *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) blockade. *Life Sci*. **35**, 899–904.

Henry, J. M., Joseph, J. A., Kochman, K., and Roth, G. S. (1987). Effect of aging on striatal dopamine receptor subtype recovery following N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline blockade and relation to motor function in Wistar rats. *Brain Res*. **418**, 334–342

Jacobs, D. M., Tang, M. X., Stern, Y., Sano, M., Marder, K., Bell, K. L., Schofield, P., Dooneief, G., Gurland, B., and Mayeux, R. (1998). Cognitive function in nondemented older women who took estrogen after menopause. *Neurology* **50**, 368–373.

Johnson, H., Ulfhake, B., Dagerlind, Å., Bennett, G. W., Fone, K. C. F., and Hökfelt, T. (1993). The serotonergic bulbospinal system and brainstem-spinal cord content of serotonin-, TRH-, and substance P-like immunoreactivity in the aged rat with special reference to the spinal cord motor nucleus. *Synapse* **15**, 63–89.

Jolas, T., Schreiber, R., Laporte, A. M., Chastanet, M., De Vry, J., Glaser, T., Adrien J., and Hamon, M. (1995). Are postsynaptic 5-HT_{1A} receptors involved in the anxiolytic effects of 5-HT_{1A} receptor agonists and in their inhibitory effects on the firing of serotonergic neurons in the rat? *J. Pharmacol. Exp. Ther.* **272**, 920–929.

Joseph, J. A., Bartus, R. T., Clody, D., Morgan, D., Finch, C., Beer, B., and Sesack, S. (1983). Psychomotor performance in the sensecent rodent: Reduction of deficits via striatal dopamine receptor up-regulation. *Neurobiol. Aging* **4**, 313–319.

Keck, B. J., and Lakoski, J. M. (1996a). Age-related assessment of central 5-HT_{1A} receptors following irreversible inactivation by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). *Brain Res*. **728**, 130–134.

Keck, B. J., and Lakoski, J. M. (1996b). Region-specific serotonin$_{1A}$ receptor turnover following irreversible blockade with EEDQ. *Neuro. Report*. **7**, 2717–2721.

Keck, B. J., and Lakoski, J. M. (1997). N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) administration for studies of 5-HT_{1A} receptor binding site inactivation and turnover. *Brain Res. Prot*. **1**, 364–367.

Keck, B. J., and Lakoski, J. M. (2000). Regional heterogeneity of serotonin$_{1A}$ receptor inactivation and turnover in the aging rat brain following EEDQ. *Neuropharmacology.* (in press).

Kettle, C. J., Cheetham, S. C., Martin, K. F., Prow, M. R., and Heal, D. J. (1999). The effects of the peptide-coupling agent, EEDQ, on 5-HT2A receptor binding and function in rat frontal cortex. *Pharmacology* **38**, 1421–1430.

Kitamura, Y., and Nomura, Y. (1987). Uncoupling of rat cerebral corticalα_2-adrenoceptors from GTP-binding proteins by *N*-ethylmaleimide. *J. Neurochem*. **49**, 1894–1901.

Ko, M. L., King, M. A., Gordon, T. L., and Crisp, T. (1997). The effects of aging on spinal neurochemistry in the rat. *Brain Res. Bull*. **42**, 95–98.

Kula, N. S., George, T., and Baldessarini, R. J. (1992). Rate of recovery of D_1 and D_2 dopaminergic receptors in young vs. adult rat striatal tissue following alkylation with ethoxycarbonyl-ethoxy-dihydroquinoline (EEDQ). *Dev. Brain Res*. **66**, 286–289.

Kung, H. F., Stevenson, D. A., Zhuang, Z.-P., Kung, M.-P., Frederick, D., and Hurt, S. D. (1996). New 5-HT_{1A} receptor antagonist: [^{3}H]*p*-MPPF. *Synapse* **23**, 344–346.

Lakoski, J. M. (1988). Estrogen-induced modulation of serotonin 5-HT_{1A} mediated responses in the dorsal raphe nucleus (DRN). *Pharmacologist* **30**, A126–A128.

Lakoski, J. M. (1989). Cellular electrophysiological approaches to the central regulation of female reproductive aging. *In* ''Neurology and Neurobiology: Neural Control of Reproductive Function'' J. M. Lakoski, J. R. Perez-Polo, and D. K. Rassin, (eds.), pp. 209–220. Alan R. Liss, New York.

Leff, S. E., Gariano, R., and Creese, I. (1984). Dopamine receptor turnover rates in rat striatum are age-dependent. *Proc. Natl. Acad. Sci. U.S.A*. **81**, 3910–3914.

Lippert, T. H., Filshie, M., Mück, A. O., Seeger, H., and Zwirner, M. (1996). Serotonin metabolite excretion after postmenopausal estradiol therapy. *Maturitas* **24**, 37–41.

Maines, L. W., Keck, B. J., Dugar, A., and Lakoski, J. M. (1998). Age-dependent loss of corticosterone modulation of central serotonin 5-HT_{1A} receptor binding sites. *J. Neurosci. Res*. **53**, 86–98.

Maines, L. W., Keck, B. J., Smith, J. E., and Lakoski, J. M. (1999). Corticosterone regulation of serotonin transporter and 5-HT_{1A} receptor expression in the aging brain. *Synapse* **32**, 58–66.

Malyszko, J. S., Malyszko, J., Azzadin, A., Buczko, W., and Mylsliwiec, M. (1995). Conjugated estrogens shorten bleeding time in uraemia: A possible role of serotonin? *Thromb. Haemostasis* **73**, 164–165.

Marder, K., Tang, M. X., Alfaro, B., Mejia, H., Cote, L., Jacobs, D., Stern, Y., Sano, M., and Mayeux, R. (1998). Postmenopausal estrogen use and Parkinson's disease with and without dementia. *Neurology* **50**, 1141–1143.

Martin, P., Beninger, R. J., Hamon, M., and Puech, A. J. (1990). Antidepressant-like action of 8-OH-DPAT, a 5-HT_{1A} agonist, in the learned helplessness paradigm: Evidence for a postsynaptic mechanism. *Behav. Brain Res*. **38**, 135–144.

Marutle, A., Warpman, U., Bogdanovic, N., and Nordberg, A. (1998). Regional distribution of subtypes of nicotinic receptors in human brain and effect of aging studied by (+/−)-[^{3}H]epibatidine. *Brain Res*. **801**, 143–149.

Maswood, N., and Uphouse, L. (1997). Modulation of the behavioral effects of 8-OH-DPAT by estrogen and DOI. *Pharmacol. Biochem. Behav.* **58**, 859–866.

Maswood, S., Stewart, G., and Uphouse, L. (1995). Gender and estrous cycle effects of the 5-HT_{1A} agonist, 8-OH-DPAT, on hypothalamic serotonin. *Pharmacol. Biochem. Behav.* **51**, 807–813.

Matsuda, T., Nakano, Y., Kanda, T., Iwata, H., and Baba, A. (1991). Gonadectomy changes the pituitary-adrenocortical response in mice to 5-HT_{1A} receptor agonists. *Eur. J. Pharmacol*. **200**, 299–304.

McCusker, J., Cole, M., Keller, E., Bellavance, F., and Berard, A. (1998). Effectiveness of treatments of depression in older ambulatory patients. *Arch. Intern. Med*. **158**, 705–712.

McKittrick, C. R., and McEwen, B. S. (1996). Regulation of serotonergic function in the CNS by steroid hormones and stress. *In* ''CNS Neurotransmitters and Neuromodulators: Neuroactive Steroids'' T. W. Stone, (ed.), pp. 37–76. CRC Press, Boca Raton, FL.

Meltzer, C. C., Smith, G., Price, J. C., Reynolds, C. F., 3rd, Mathis, C. A., Greer, P., Lopresti, B., Mintun, M. A., Pollock, B. G., Ben-Eliezer, D., Cantwell, M. N., Kaye, W., and DeKosky, K. T. (1998). Reduced binding of [^{18}F]altanserin to serotonin type 2A receptors in aging: Persistence of effect after partial volume correction. *Brain Res*. **813**, 167–171.

Meltzer, H. Y. (1990). Role of serotonin in depression. *Ann. N.Y. Acad. Sci*. **600**, 486–499.

Mesco, E. R., Joseph, J. A., Blake, M. J., and Roth, G. S. (1991). Loss of D2 receptors during aging is partially due to decreased levels of mRNA. *Brain Res*. **545**, 355–357.

Mesco, E. R., Carlson, S. G., Joseph, J. A., and Roth, G. S. (1993). Decreased striatal D2 dopamine receptor mRNA synthesis during aging. *Mol. Brain Res*. **17**, 160–162.

Middlemiss, D. N., Palmer, A. M., Edel, N., and Bowen, D. M. (1986). Binding of the novel serotonin agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin in normal and Alzheimer brain. *J. Neurochem*. **46**, 993–996.

Miller, J. A., and Zahniser, N. R. (1988). Quantitative autoradiographic analysis of ^{125}I-Pindolol binding in Fischer 344 rat brain: Changes in β-adrenergic receptor density with aging. *Neurobiol. Aging* **9**, 267–272.

Mongeau, R., Welner, S. A., Quirion, R., and Suranyi-Cadotte. B. E. (1992). Further evidence for differential affinity states of the serotonin$_{1A}$ receptor in rat hippocampus. *Brain Res*. **590**, 229–238.

Mueck, A. O., Seeger, H., Kasspohl-Bulz, S., Teichmann, A. T., and Lippert, T. H. (1997). Influence of norethisterone acetate and estradiol on the serotonin metabolism of postmenopausal women. *Horm. Metab. Res*. **29**, 80–83.

Nénonéné, E. K., Radja, F., Carli, M., Grondin, L., and Reader, T. A. (1994). Heterogeneity of cortical and hippocampal 5-HT$_{1A}$ receptors: A reappraisal of homogenate binding with 8-[^{3}H]hydroxydipropylaminotetralin. *J. Neurochem*. **62**, 1822–1834.

Neve, K. A., and Molinoff, P. B. (1986). Turnover of β1- and β2-adrenergic receptors after down-regulation or irreversible blockade. *Mol. Pharmacol*. **40**, 104–111.

Ni, Y. G., Camacho, N., and Miledi, R. (1997). Irreversible antagonism of 5HT2c receptors by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). *Proc. Natl. Acad. Sci. U.S.A*. **94**, 2715–2718.

Nichizuka, N., and Arai, Y. (1981). Sexual dimorphism in synaptic organization in the amygdala and its dependence on neonatal hormone environment. *Brain Res*. **212**, 31–38.

Norman, A. B., and Creese, I. (1986). Effects of in vivo and in vitro treatments with *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline on putative muscarinic receptor subtypes in rat brain. *Mol. Pharmacol*. **30**, 96–103.

Norman, A. B., Battaglia, G., and Creese, I. (1987). Differential recovery rates of D_2 dopamine receptors as a function of aging and chronic reserpine treatment following irreversible modification: A key to receptor regulatory mechanisms. *J. Neurosci*. **7**, 1484–1491.

O'Boyle, K. M., and Waddington, J. L. (1984). Loss of rat striatal dopamine receptors with ageing is selective for D-2 but not D-1 sites: Association with increased non-specific binding of the D-1 ligand [^{3}H]piflutixol. *Eur. J. Pharmacol*. **105**, 171–174.

Palmer, A. M., and DeKosky, S. T. (1993). Monoamine neurons in aging and Alzheimer's disease. *J. Neural Transm*. **91**, 135–159.

Pecins-Thompson, M., Brown, N. A., Kohama, S. G., and Bethea, C. L. (1996). Ovarian steroid regulation of tryptophan hydroxylase mRNA expression in rhesus macaques. *J. Neurosci*. **16**, 7021–7029.

Penev, P. D., Zee, P. C., Wallen, E. P., and Turek, F. W. (1995). Aging alters the phase-resetting properties of a serotonin agonist on hamster circadian rhythmicity. *Am. J. Physiol*. **268**, R293–R298.

Petkov, V. D., Stancheva, S. L., Benignus, V. V., and Alova, L. G. (1987). Age-related changes in brain biogenic monoamines and monoamine oxidase. *Gen. Pharmacol*. **18**, 397–401.

Pinto, W., and Battaglia, G. (1993). In vivo EEDQ dose-dependently inactivates rat brain 5-HT receptors but not 5-HT uptake sites. *NeuroReport* **5**, 61–64.

Pinto, W., and Battaglia, G. (1994). Comparative recovery kinetics of 5-hydroxytryptamine 1A, 1B, and 2A receptor subtypes in rat cortex after receptor inactivation: Evidence for differences in receptor production and degradation. *Mol. Pharmacol*. **46**, 1111–1119.

Pitha, J., Hughes, B. A., Kusiak, J. W., Dax, E. M., and Baker, S. P. (1982). Regeneration of β-adrenergic receptors in senescent rats: A study using an irreversible binding antagonist. *Proc. Natl. Acad. Sci. U.S.A*. **79**, 4424–4427.

Raghupathi, R. K., Brousseau, D. A., and McGonigle, P. (1996). Time-course of recovery of 5-HT$_{1A}$ receptors and changes in 5-HT$_{1A}$ receptor mRNA after irreversible inactivation with EEDQ. *Mol. Brain Res*. **38**, 233–242.

Reader, T. A., Radja, F., Nénonéné, E. K., Afkhami-Dastjerdian, S., and Carli, M. (1995). Alkylation of central 5-HT$_{1A}$ receptors: Further evidences for [^{3}H]8-OH-DPAT binding heterogeneity. *J. Neurochem*. **64**(Suppl.), S106.

Riekkinen, P., Jr., Riekkinen, M., Valjakka, A., Riekkinen, P., and Sirviö, J. (1992). DSP-4, a noradrenergic neurotoxin, produces more sever biochemical and functional deficits in aged than young rats. *Brain Res*. **570**, 293–299.

Rinne, J. O., Laihinen, A., Lonnberg, P., Marjamaki, P., and Rinne, U.K. (1991). A post-mortem study on striatal dopamine receptors in Parkinson's disease. *Brain Res*. **556**, 117–122.

Robson, L., Gower, A. J., Kendall, D. A., and Marsden, C. A. (1993). Age-related behavioural, neurochemical and radioligand binding changes in the central 5-HT system of Sprague-Dawley rats. *Psychopharmacology* **113**, 274–281.

Roth, G. S., and Joseph, J. A. (1994). Cellular and molecular mechanisms of impaired dopminergic function during aging. *Ann. N. Y. Acad. Sci*. **719**, 129–135.

Rubinow, D. R., Schmidt, P. J., and Roca, C. A. (1998). Estrogen-serotonin interactions: Implications for affective regulation. *Biol. Psychiatry* **44**, 839–850.

Salamanca, S., and Uphouse, L. (1992). Estradiol modulation of the hyperphagia induced by the 5-HT$_{1A}$ agonist, 8-OH-DPAT. *Pharmacol., Biochem. Behav*. **43**, 953–955.

Schlicker, E., Betz, R., and Gothert, M. (1989). Investigation into the age-dependence of release of serotonin and noradrenaline in the rat brain cortex and of autoreceptor-mediated modulation of release. *Neuropharmacology*. **28**, 811–815.

Schneider, L. S., Small, G. W., Hamilton, S. H., Bystritsky, A., Nemeroff, C. B., Meyers, B. S., and the Fluoxetine Collaborative Study Group. (1997). Estrogen replacement and response to fluoxetine in a multicenter geriatric depression trial. *Am. J. Gen. Psychiatry* **5**, 97–106.

Stanley, H. F., Borthwick, N. M., and Fink, G. (1986). Brain protein changes during development and sexual differentiation in the rat. *Brain Res*. **370**, 215–222.

Steinbusch, H. W. M., Van Luijtelaar, M. G. P. A., Dijkstra, H., Nijssen, A., and Tonnaer, J. A. D. M. (1990). Aging and regenerative capacity of the rat serotonergic system. *Ann. N. Y. Acad. Sci*. **600**, 385–403.

Stockmeier, C. A., Shapiro, L. A., Dilley, G. E., Kolli, T. N., Friedman, L., and Rajkowska, G., (1998). Increase in serotonin-1A autoreceptors in the midbrain of suicide victims with major depression — postmortem evidence for decreased serotonin activity. *J. Neurosci*. **18**, 7394–7401.

Storga, D., Vrecko, K., Birkmayer, J. G. D., and Reibnegger, G. (1996). Monoaminergic neurotransmitters, their precursors and metabolites in brains of Alzheimer patients. *Neurosci. Lett*. **203**, 29–32.

Sumner, B. E. H., and Fink, G. (1993). Effects of acute estradiol on 5-hydroxytryptamine and dopamine receptor subtype mRNA expression in female rat brain. *Mol. Cell. Neurosci*. **4**, 83–92.

Tajuddin, N. F., and Druse, M. J. (1996). Effects of chronic alcohol consumption and aging on dopamine D2 receptors in Fischer 344 rats. *Alcohol.: Clin. Exp. Res*. **20**, 144–151.

Tanila, H., Taira, T., Piepponen, T. P., and Honkanen, A. (1994). Effect of sex and age on brain monoamines and spatial learning in rats. *Neurobiol. Aging* **15**, 733–741.

Timiras, P. S. (1994). Aging of the nervous system: functional changes. *In* "Physiological Basis of Aging and Geriatrics" (P. S. Timiras, ed.), pp. 103–114. CRC Press, Boca Raton, FL.

Tohgi, H., Utsugisawa, K., Yoshimura, M., Nagane, Y., and Mihara, M. (1998). Age-related changes in nicotinic acetylcholine receptor subunits alpha4 and beta2 messenger RNA expression in post-

mortem human frontal cortex and hippocampus. *Neurosci. Lett*. **245**, 139–142.

Turjanski, N., Weeks, R., Dolan, R., Harding, A. E., and Brooks, D. J. (1995). Striatal D1 and D2 receptor binding in patients with Huntington's disease and other choreas. *Brain* **118**, 689–696.

Uphouse, L., Caldarola-Pastuszka, M., Maswood, S., Andrade, M., and Moore, N. (1994). Estrogen-progesterone and 8-OH-DPAT attenuate the lordosis-inhibiting effects of the 5-HT_{1A} agonist in the VMN. *Brain Res*. **637**, 173–180.

Vannuchi, M. G., Scali, C., Kopf, S. R., Pepeu, G., and Casamenti, F. (1997). Selective muscarinic antagonists differentially affect in vivo acetylcholine release and memory performances of young and aged rats. *Neuroscience* **79**, 837–846.

Wong, D. F., Wagner, H. N., Jr., Dannals, R. F., Links, J. M., Frost, J. J., Ravert, H. T., Wilson, A. A., Rosenbaum, A. E., Gjedde, A., Douglass, K. H., Petronis, J. D., Folstein, M. F., Toung, J. K. T., Burns, H. D., and Kuhar, M. J. (1984). Effect of age on dopamine and serotonin receptors measured by positron tomography in the living human brain. *Science* **226**, 1393–1396.

Wong, D. F., Young, D., Wilson, P. D., Meltzer, C. C., and Gjedde, A. (1997). Quantification of neuroreceptors in the living human brain: III. D2-like dopamine receptors: theory, validation, and changes during normal aging. *J. Cereb. Blood Flow Metab*. **17**, 316–330.

Wong, W. F., Pearlson, G. D., Tune, L. E., Young, L. T., Meltzer, C. C., Dannals, R. F., Ravert, H. T., Reith, J., Kuhar, M. J., and Gjedde, A. (1997). Quantification of neuroreceptors in the libing human brain: IV. Effect of aging and elevations of D2-like receptors in schizophrenia and bipolar illness. *J. Cereb. Blood Flow Metab*. **17**, 331–342.

Yaksh, T. L., and Wilson, P. R. (1979). Spinal serotonin terminal system mediates antinociception. *J. Pharmacol. Exp. Ther*. **208**, 446–453.

Yamaguchi, T., and Yamagata, A. (1991). Serotonergic ligand binding in aging brain of experimental animals. *Neurochem. Res*. **16**, 469–473.

Young, A. H., Dow, R. C., Goodwin, G. M., and Fink, G. (1993). The effects of adrenalectomy and ovariectomy on the behavioral and hypothermic responses of rats to 8-hydroxy-2(di-*n*-propylamino) tetralin (8-OH-DPAT). *Neuropharmacology* **32**, 653–657.

Zawia, N., Arendash, G. W., and Wecker, L. (1992). Basal forebrain cholinergic neurons in aged rat brain are more susceptible to ibotenate-induced degeneration than neurons in young adult brain. *Brain Res*. **589**, 333–337.

Zgombick, J. M., Beck, S. G., Mahle, C. D., Craddock-Royal, B., and Maayani, S. (1989). Pertussis toxin-sensitive guanine nucleotide-binding protein(s) couple adenosine A_1 and 5-hydroxytryptamine$_{1A}$ receptors to the same effector systems in rat hippocampus: Biochemical and electrophysiological studies. *Mol. Pharmacol*. **35**, 484–494.

Zhou, L.-W., Weiss, B., Freilich, J. S., and Greenberg, L. H. (1984). Impaired recovery of alpha$_1$- and alpha$_2$-adrenergic receptors in brain tissue of aged rats. *J. Gerontol*. **39**, 538–546.

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4

Demography and Epidemiology of Age-Associated Neuronal Impairment

With the increasing longevity of the United States population, the clinical challenge is shifting from preventing premature death to improving function and quality of life. Some of the major neurological impairments—stroke, Alzheimer's disease, and Parkinson's disease, to name a few—are strongly associated with age. Thus, increasing longevity means many elderly persons must cope with cognitive, sensory, or motor impairments, some of which may result in fundamental and often permanent declines in quality of life. Although the elderly belong to the fastest growing segment of the population and the numbers of people afflicted with these impairments will likely keep pace, many of the diseases remain poorly defined in epidemiological terms. This chapter reviews what is known of the demography and epidemiological risk factors of the major age-related neurological impairments. © 2001 Academic Press.

I. Introduction

The average 65-year-old person in the United States today can expect another 18 years of life, due to mortality declines over the last 100 years. Some 13 out of every 100 Americans are 65 years old or older, and the numbers of elderly are projected to grow. By 2030, according to Census Bureau estimates, the group will make up 20% of the nation's population. Of the 34 million people 65 years or over today, 4 million of them are in the ''oldest old'' category, 85 years and older. The oldest old comprise the fastest-growing segment of the United States population, and their numbers are expected to more than double in the next few decades, as Fig. 4.1 shows [U.S. Department of Health and Human Services (USDHHS), 1999].

Explanations for the gains in life expectancy are a source of controversy among demographers. In the first half of the 20th century, approximately 20 years were added to the average life expectancy (USDHHS, 1999). Gains made over this time period and prior to it were mainly attributed to public health measures, among them control of infectious diseases through water, food, and sanitation improvements, workplace and motor-vehicle safety improvements, and greater access to family planning (Ten Great Public Health Achievements—United States, 1900–1999). Since 1950, another 8 years have been added to the average life expectancy, which as of 1997 stood at 79.4 years for women and 73.6 years for men. Explanations offered for the increasing longevity of the population have shifted since the 1960s away from public health advances toward a debate over whether improvements in lifestyle or advances in medicine should receive the most credit. On the lifestyle side of the debate, better education, better nutrition, diet, and less tobacco use are factors commonly cited for declines in mortality. However, advances in medicine have turned what were once inevitably fatal diseases—diseases of the heart, malignant neoplasms, and cerebrovascular disease—into survivable ones, by either delaying their onset or transforming them into chronic diseases. Thus the clinical challenge has shifted from preventing premature death to improving function and quality of life.

Better survival of these diseases also means the opportunity for other chronic, nonfatal ailments to cause disability in the population as it ages. Many of the major neurological impairments, such as stroke, Alzheimer's disease, and Parkinson's disease, are age associated. The age of onset of many of these impairments has unfortunately not changed and will therefore affect a larger number of people for a longer amount of time as life expectancy increases. In addition, a distinction should be made wherever possible between neuronal changes that are normal with aging and age associated degenerative neuronal diseases.

A. Epidemiological Caveats

Estimates of the incidence of neurological impairments, i.e., the rate of new cases occurring in a given population over a specific time period, are given to uncertainty by the very nature of the diseases themselves. Because these diseases typically begin and progress slowly, the age of onset may be difficult to pinpoint. For a variety of cultural or socioeconomic reasons, patients may not seek out medical care even when symptoms

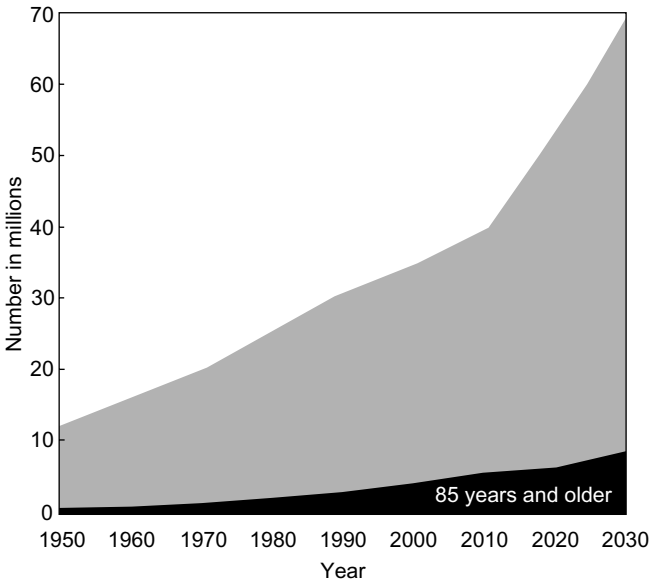

FIG. 4.1. Population 65 years of age and older: United States, 1950–2030. The population 65 years and older is expected to swell in the next few decades, particularly the segment 85 years and older. From U.S. Department of Health and Human Services (1999) (available online: *http://www.cdc.gov/nchs/products/pubs/pubd/hus/charts/hus99f01.pdf*).

do become apparent or may mistake symptoms for changes due to normal aging. Data derived from door-to-door surveys may therefore differ from results of clinic-based studies. When diagnostic criteria also differ between studies, comparisons of incidence data yield little more than a range of estimates.

Estimates of prevalence—the number of cases present in a population at one particular time—are also hampered by these difficulties, as prevalence is a function of incidence as well as of survival. Underreporting of conditions on death certificates adds to the uncertainty of prevalence figures for neurological impairments associated with aging. As few as a quarter of dementia cases may actually be reported on death certificates—typically, pneumonia, or cerebrovascular disease is listed and mention of dementia is neglected as an underlying cause (Ganguli and Rodriguez, 1999). In 1979 the Centers for Disease Control added a classification for Alzheimer's disease in its record keeping. Between 1994 and 1995, Alzheimer's disease was the only one of the 15 leading causes of death the center tracks to show statistically significant increases in age-adjusted death rate, increasing by 8% (Monthly Vital Statistics Report, 1997). Better reporting is probably responsible for this trend rather than true increases in prevalence, and this may be the case for other age-associated neurological impairments as well.

Several other general caveats should be considered when assessing the national scope of a particular type of neurological impairment. Dementia statistics, for example, are often lumped together under the general category of dementia rather than separating out cases by Alzheimer's disease, vascular dementia, Lewy body disease, dementia in Parkinson's disease, or other types. Mixed-type cases of neurological disorders further complicate attempts at classification. Studies also differ on the degree of disease included in data. Some look only at cases of dementia diagnosed as moderate to severe, for example, whereas others also include mild and even questionable cases in their data.

Differential use of medical services among sectors of the population affects the overall quality of data collected in clinic-based studies, whereas changing lifestyles and diets may cause temporal variation in data. Exposure to risk factors is similarly difficult to determine, particularly in retrospectively collected data such as case-controlled studies.

II. Stroke

Stroke is defined as "rapidly developing clinical signs of focal (or global) disturbances of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin" (World Health Organization, 1989). The term cerebral vascular accident (CVA) also refers to stroke, but has fallen out of favor in recent years as various public health organizations began a push for increased awareness of the more preventable causes of stroke. The phrase "brain attack" was introduced in the mid-1990s to make health professionals and the public more aware of the need to recognize stroke symptoms and begin therapeutic intervention immediately. A 1996 NSA/Gallup poll on stroke revealed that the majority of persons surveyed could not recognize even basic symptoms of stroke. Even as recently as the late 1980s, only 37% of victims presented for treatment within the first 24 hr after a stroke, and emergency rooms often gave possible stroke patients low triage priority, believing that post-stroke rehabilitation was the most that could be offered (Heros *et al*., 1997). Introduction of the term "brain attack" reflects changing attitudes toward strokes with the recognition that the window of opportunity for treatment is extremely short and that prompt intervention must begin within that time in order to lessen the burden of the disease in terms of both mortality and disability.

A. Mortality

Approximately 20% of all first-time strokes result in death within a month (Warlow, 1998). Stroke is the third leading cause of death in the United States after coronary heart disease and cancer, and has been since 1938. Although age-adjusted death rates from stroke have declined 70% over the past 50 years in the United States, about 160,000 deaths per year are still directly attributable to strokes (Morbidity and Mortality, 1999). Decreases in stroke death rates are considered a major public health achievement and parallel the decrease in death rates from cardiovascular disease over the last half-century. The early 1990s saw a slight trend toward increasing death rates from stroke, but this trend appears to have reversed: in 1997 the age-adjusted death rate per 100,000 was 25.9, down 1.9% from 1996 (Hoyert *et al*., 1999).

B. Stroke-Related Disability

As the most common source of neurological impairment as well as a primary source of severe long-term disability, the consequences of stroke in the United States are enormous.

Because one of the two main risk factors for stroke is age, the elderly bear most of the burden of resulting disability. Of people who survive 6 months after a first-time stroke, one-third are dependent on others for care. Some four million people are stroke survivors in the United States, according to the National Stroke Association. Of those, one-third are left with moderate poststroke disability, whereas another third live with severe disability after stroke (American Heart Association, 1999). Stroke-related disabilities disproportionately affect the elderly, however. Three quarters of older stroke victims are left with permanent impairments, whereas only one-third of younger stroke victims are (Malmagren *et al*., 1989). Medicare reimbursement costs for inpatient rehabilitation are higher for stroke than for any other condition (Steiner and Neu, 1993).

Cognitive, language, and emotional deficits are common after stroke. Motor impairments, including paralysis, muscle weakness, and difficulties eating and swallowing, may also result. Risk of dementia rises markedly after stroke. Case-control and retrospective studies have found dementia risk at least nine times higher in stroke survivors than in the stroke-free population (Prencipe *et al*., 1997). Chances for a repeat stroke are high, particularly in the days immediately following the first stroke. Cumulative risk of a second stroke rises from 10–18% 1 year after the initial stroke to 20–34% 3 years after (Viitanen *et al*., 1988). In addition, stroke survivors are more prone to other vascular events outside of the cerebrum, including a 3% per annum rate of serious coronary events (Warlow, 1998).

C. Types of Stroke

Most available epidemiological data lump the different types of brain events together under the umbrella term stroke, without differentiating between even the two broadest subtypes: ischemic stroke and hemorrhagic stroke. Some 80% of all strokes are of the ischemic or cerebral infarct type, within which there are four subcategories: lacunes of small arteries, artherostenosis of larger intracranial and extracranial arteries, hypoperfusion, and cerebroembolism of cardiac origin. The elderly are disproportionately affected by strokes of the cerebroembolitic and lacunar type when compared to younger populations (Caplan, 1997). Approximately 70% of people who suffer a severe stroke of the cerebral infarct type require nursing home care, and more than half remain in nursing homes 1 year after the stroke (Brown *et al*., 1999).

The majority of the remaining 20% of strokes are of the hemorrhagic type, of which the two main subcategories are intracerebral hemorrhage and subarachnoid hemorrhage type (Thompson and Furlan, 1997). Subarachnoid hemorrhage accounts for 8 to 10% of all strokes. Intracerebral hemorrhage accounts for 8% of strokes but has been declining in frequency (Caplan, 1997).

D. Incidence

Although there are important regional and temporal variations, the incidence of stroke in the United States is comparable to that in other Western countries. From the early 1950s to the latter half of the 1970s, the average annual age- and sex-adjusted stroke incidence rate for the residents of Rochester, Minnesota declined from 213 per 100,000 people to 115, a 46% decline (Broderick *et al*., 1989). By the early 1980s, however, annual incidence rates rose back up to 145 per 100,000, with increases seen for both sexes and all age groups (Brown *et al*., 1996). Imaging techniques and better diagnosis may play a role in the trend toward increases through better detection of even mild cases of stroke. Because strokes are closely linked to aging, incidence rates for older populations are higher: ischemic strokes resulting in hospitalization among people over 40 in Manhattan occurred at an annual rate of 327 per 100,000 (Sacco *et al*., 1991). This figure is in line with results of a large-scale comparison of incidence rates in Western countries for populations 45 and older, where age-standardized incidence rates ranged from 300 to 500 per 100,000 (Sudlow and Warlow, 1997).

E. Prevalence

The National Stroke Association estimates that four out of five families will be affected by stroke. The most recent estimates for the United States are 730,000 strokes per year, up from previous estimates of half a million strokes per year (Broderick *et al*., 1998). Three-quarters of all strokes occurring are first-time strokes. Second-time strokes make up about 20% of all strokes (Thompson and Furlan, 1997). A number of population-based studies have shown overall prevalence rates for those over the age of 55 to be between 1 and 8% (Prencipe *et al*., 1997).

F. Age

Two-thirds of all strokes occur past the age of 65 (National Institutes of Health, 1998). Stroke mortality rates nearly double with each 5-year age increment (Sacco, 1994). Both incidence and prevalence rates also rise exponentially with age. The Framingham study showed incidence doubling with each decade of life, whereas the Rochester study saw 10% increases in incidence rates per year of life (Sacco, 1994). The Rotterdam study, based on self-reported cases of stroke, found prevalence to be 2.5% for men and 1.6% for women in the 55- to 64-year-old age group. This rose to 5.0 and 3.3% for men and women in the 65–74 group and to 8.9 and 6.7% in the 75–84 age group. For men and women 85 and older, rates were even higher: 11.6 and 10.5%, respectively (Bots *et al*., 1996).

G. Gender

Men are at greater risk for stroke incidence than women. The International Stroke Incidence Collaboration, which analyzed 11 incidence studies from Western countries, found consistently higher incidence rates in men for all age groups (Sudlow and Warlow, 1997). A male:female ratio of 1.3:1 is typical for incidence studies of most types of stroke, although women appear to be at greater risk than men for subarachnoid hemorrhage-type strokes (Sacco, 1994). Despite the fact that women have a lower overall incidence of stroke than men, they account for over 60% of deaths from strokes, most likely due to women's longer life expectancies and higher age at time of stroke (Hoyert *et al*., 1999). The Cardiovascular Health

study noted that stroke prevalence rates, while higher overall for men, are actually higher for women than for men in the very oldest age ranges, above 85 years of age (Mittelmark *et al*., 1993). Other studies, however, including the Rotterdam study, have found that men have higher prevalence rates than women in all age ranges (Bots *et al*., 1996).

H. Race

Major studies on stroke in the United States, such as the Framingham and Rochester studies, are not representative of the racial diversity of the nation. Only 1% of the population in Rochester is black, and most of the population is relatively affluent. A more recent large-scale population-based study, the Greater Cincinnati/Northern Kentucky Stroke Study, covered a diverse community and revealed significantly higher incidence rates for blacks than for whites. The overall incidence of first-time strokes among blacks during the first 6 months of 1993 was 288 per 100,000, far exceeding the first-time stroke rate among the white population of the Rochester study (Broderick *et al*., 1998).

Stroke mortality for blacks is nearly twice that for whites (Gillum, 1999). In 1997, age-adjusted death rates per 100,000 were 48.6 for black men and 37.9 for black women vs 25.7 for white men and 22.5 for white women (Hoyert *et al*., 1999). However, the excess stroke mortality in blacks appears to occur mainly in younger persons; at ages greater than 75 years, whites are more likely than blacks to have strokes (Howard *et al*., 1994). Blacks are more likely to suffer cerebrovascular events of greater severity, have a greater level of disability following a stroke, and require longer stays hospital stays following acute stroke than are members of other racial groups. Blacks are also more likely than whites to die during hospitalization for acute stroke (Horner, 1991; Kuhlemeier and Stiens, 1994). A number of risk factors for stroke, including hypertension, diabetes, obesity, and smoking, are present in undue proportion among the black population and may account at least partially for these trends (National Institutes of Health, 1999).

A multiracial comparison in Manhattan of annual stroke incidence resulting in hospitalization also yielded wide rate discrepancies between race, as Fig. 4.2 shows. For people 85 and over, for example, the average annual age-specific stroke incidence rate per 100,000 was 651 for whites, 1461 for blacks, and 1778 for Hispanics. Stroke rates among Asian Americans are generally similar to those of whites in the United States, although stroke rates among Asians living in the Far East are much higher, suggesting a role for environmental factors (National Institutes of Health, 1999; Sacco *et al*., 1999).

I. Geography

Within the United States, there are marked regional variations in stroke rates. For the past 6 decades the southeastern area of the country has had a higher incidence rate of stroke and a stroke mortality rate 10% higher than the rest of the country. The area has been dubbed the "stroke belt," with the population born in these states, especially South Carolina, North Carolina, and Georgia, at much higher risk of stroke (Lackland *et al*., 1999; National Institutes of Health, 1999). Blacks living in the stroke belt are at greater risk for stroke than whites. In addition to race, suggested factors for the greater stroke risk in the southeast are diet and a proportionally greater number of elderly people living in the region.

The International Stroke Incidence Collaboration revealed wide variation in age-standardized annual incidence rates in Western countries for the age group 45–84. The high was 627 per 100,000 in Novosibirsk, Russia; the low was 238 per

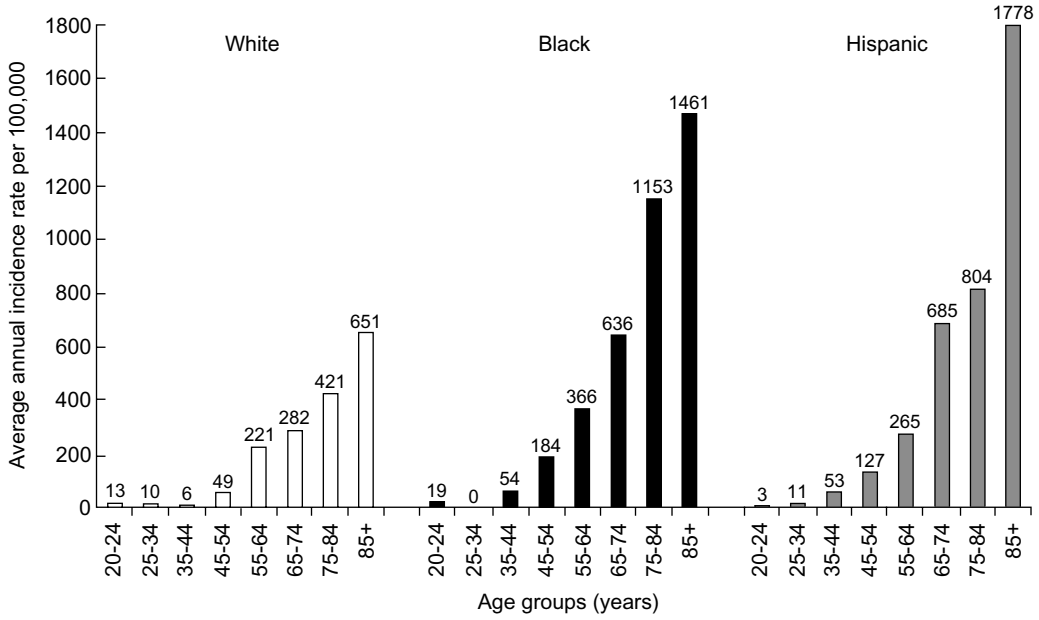

FIG. 4.2. Stroke population at risk: Average annual age-specific incidence rates of stroke (per 100,000 Population) among whites, blacks, and Hispanics aged $\geq$ 20 years in northern Manhattan (1993 to 1996). Risk of stroke increases sharply with age, but also varies widely by race. From Sacco *et al.* (1998).

100,000 in Dijon, France (Sudlow and Warlow, 1997). Prevalence rates also vary widely between countries: prevalence ranges from 500 to 600 per 100,000 in most Western countries to 900 in Eastern countries (Sacco, 1994). Data from less developed nations often suffer from underreporting on death certificates, whereas methodologies and diagnostic criteria for incidence and prevalence studies are not consistent in these nations.

J. Risk Factors: Hypertension

The single greatest modifiable risk factor for stroke is hypertension. Along with age, it is the biggest predictor of stroke. In the Framingham study, even borderline hypertension increased relative risk by as much as 1.5; for definite hypertensives, the relative risk of stroke was 3.1 for men and 2.9 for women compared to those with normal blood pressure. The Rochester study found a 4.0 relative risk for definite hypertensives (Sacco, 1994). Forty to 90% of all stroke victims have hypertension prior to the stroke (National Institutes of Health, 1999). For every 7.5 mm Hg increase in diastolic pressure, the incidence of stroke increases by 46%, according to one meta-analysis of hypertensive drug treatments (Wolf, 1998). The likelihood of hypertension increases with age. Isolated systolic hypertension affects 18% of men and 30% of women over the age of 75, dramatically increasing the risk of stroke in this population [Systolic Hypertension on the Elderly Program (SHEP), Cooperative Research Group, 1991].

K. Other Risk Factors

Atrial fibrillation is present in as many as 15% of all stroke victims, and in those over the age of 80, one in four strokes is directly caused by the condition (National Institutes of Health, 1999). Other forms of cardiac disease, including coronary artery disease, congestive heart failure, and left ventricular hypertrophy, are linked to higher stroke rates, as are vascular malformations. Diabetes mellitus is often accompanied by atherosclerosis and other vascular complications. Estimates of the relative risk for stroke in diabetics range from 1.5 to 3.0 (Sacco, 1994). High plasma cholesterol, fibrinogen, and homocysteine are other suspected risk factors (Warlow, 1998; Bostom *et al*., 1999).

Roughly 15% of strokes are preceded by transient ischemic attacks (TIAs) (Poungvarin, 1998). TIAs are often the first warning of subsequent major stroke. Some 50,000 people per year in the United States have a TIA; of these victims, one-third have an acute stroke at a later time (National Institutes of Health, 1999). The subsequent stroke typically strikes in the same vascular area (Sacco, 1994).

Lifestyle choices strongly influence stroke risk. The major offender is cigarette smoking. A meta-analysis of 32 studies found that tobacco use increased risk by 50% (Wolf, 1998). According to the Framingham study, the relationship was also dose dependent: those who smoked 40 or more cigarettes per day had double the risk of those who smoked 10 cigarettes a day or fewer (Wolf *et al*., 1988). Heavy alcohol consumption and physical inactivity also appear to be offenders. Eating fruit and vegetables—other than legumes and potatoes—may be protective for ischemic stroke risk (Joshipura *et al*., 1999).

III. Dementias: Age-Associated Cognitive Impairment

A. Alzheimer's Disease

1. Prevalence

Prevalence estimates and projections on Alzheimer's disease vary according to study. The General Accounting Office completed a recent report on prevalence to the Secretary of Health and Human Services based on a meta-analysis of 18 studies both in the United States and abroad (U.S. General Accounting Office, 1998). According to the report, at least 1.9 million Americans 65 years and older in 1995 had Alzheimer's disease when all levels of severity of the disease were included, and 1.1 million were afflicted when only moderate and severe forms were included. Overall numbers are probably underestimated, with figures closer to 2.1 million due to the likelihood of missed cases and cases of mixed-type dementia excluded from the count. By 1997, there were an estimated 2.32 million people with Alzheimer's disease, although this figure ranges from 1.09 million at the low end to 4.58 million at the high end (Brookmeyer *et al*., 1998). The Centers for Disease Control National Center for Health Statistics reported 22,475 deaths from Alzheimer's listed on death certificates that year, and an age-adjusted mortality rate of 2.7 (Hoyert *et al*., 1999).

If U.S. Census Bureau projections for the aging of the nation are taken into account, the General Accounting Office report predicts that between 2.9 million and 3.2 million Americans will have some degree of Alzheimer's disease by 2015 (U.S. General Accounting Office, 1998). Other similarly derived projections suggest that 1 in 45 older Americans can expect to have the disease by 2050, a near quadrupling in prevalence from today's figures. A high-end projection shows 14 million patients with the disease by 2040 (Evans, 1990).

2. Incidence

Four studies in the United States establish current incidence rates for Alzheimer's disease, each using standard criteria for diagnosis (Shock *et al*., 1984; Kokmen *et al*., 1988; Bachman *et al*., 1993; Hebert *et al*., 1995). From these studies (Framingham, East Boston, Rochester, MN, and Baltimore) the age-specific incidence rate was found to increase exponentially after the age of 65, doubling every 5 years at least through the age of 90 (Brookmeyer *et al*., 1998). Fig. 4.3 shows age-specific incidence rates derived from these studies on a log scale.

The number of new Alzheimer's cases likely to be diagnosed over the first five decades of the 21st century is determined by multiplying U.S. Census Bureau projections of the U.S. elderly population for that time period by known age-specific incidence rates of the disease. One such estimate predicts a growth in new cases from 360,000 in 1997 to 1.14 million in 2047, even in the absence of new diagnostic tools (Brookmeyer *et al*., 1998). These projections also assume no new treatments or preventions.

3. Age

The largest risk factor for Alzheimer's disease is age. Table 4.1 shows estimates of prevalence derived from 18 major

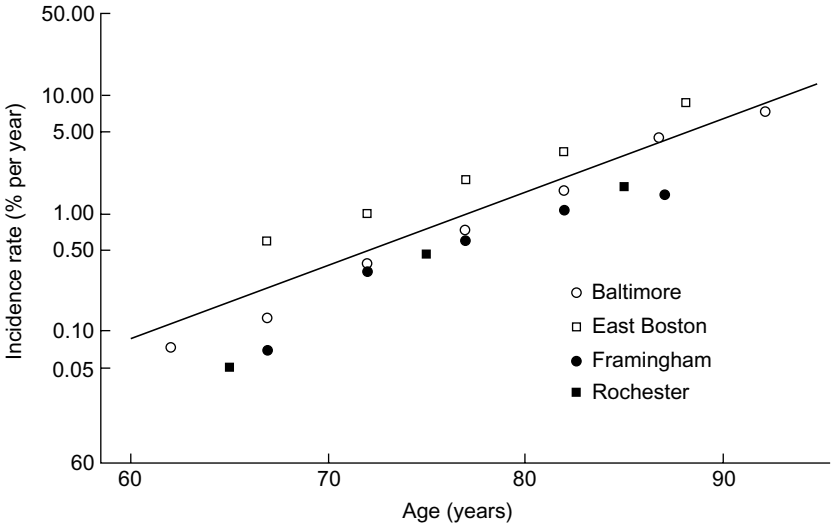

FIG. 4.3. Age-specific incidence rates of Alzheimer's disease on a log scale from 4 U.S. studies: Framingham, East Boston, Rochester, and Baltimore. Incidence of Alzheimer's disease rises exponentially after age 65, doubling every 5 years. From Brookmeyer *et al.* (1998).

studies. When the U.S. elderly population is broken down into 5-year incremental age groups beginning at age 65, the majority of Alzheimer's cases occur within the three groups encompassing ages 75 to 89. These combined age groups claim 1.1 million out of the 1.9 million total Alzheimer's patients among the elderly and create a crucible for the elderly: out of the 13.5 million people alive between the ages of 75 and 89 in 1995, Alzheimer's disease afflicted 8.4% of them (U.S. General Accounting Office, 1998; U.S. Census Bureau). Taking the number of cases among a particular age group as a percentage of the total population alive of that same age, the age group with the smallest percentage of cases—1.1%—is the youngest group beginning at age 65. Unsurprisingly for a disease whose major risk factor is age, the percentages increase steadily by higher age group until a full 50% of the population aged 95 and over are afflicted.

TABLE 4.1 Estimates of Alzheimer's Disease (AD) Prevalence by Age[a]

	Any AD		Moderate or severe AD	
Age	Number	Percent	Number	Percent
65–69	104,785	1.1	61,815	0.6
70–74	194,716	2.2	111,111	1.3
75–79	304,399	4.6	169,549	2.5
80–84	411,363	9.2	227,757	5.1
85–89	412,764	17.8	232,726	10.0
90–94	312,509	31.5	185,516	18.7
95+	166,287	52.5	110,595	34.9
Total	**1,906,822**	**5.7**	**1,099,069**	**3.3**

[a]The integration of 18 major studies shows Alzheimer's disease prevalence also increases by age, with at least 1.1 million people between the ages of 75 to 89 years old afflicted. Alzheimer's disease: estimates of prevalence in the United States. Report to the Secretary of Health and Human Services. United States General Accounting Office, January 1998. GAO/HEHS-98-16 (available online: http://frwebgate.access.gpo.gov/cgi-bin/useftp.cgi? IP address=162.140.64.88; filename: he98016.pdf; directory: /diskb/wais/data/gao).

Unfortunately, both prevalence and incidence estimates for this oldest population are sketchier than for other age ranges due to small sample size in all of the studies analyzed. Some literature suggests that the incidence rate may level off or decline past the age of 95, but recent studies have found the opposite trend. A very high rate of incidence for this group is shown from extrapolations of the four main U.S. studies and a Swedish study (Fratiglioni *et al*., 1997). In a recent study of nonagenarians, the prevalence of general dementia appeared to increase from 13% among 77 to 84 year olds to 48% in those 95 years old and over, and to as high as 61% when questionable cases of dementia were included (von Strauss *et al*., 1999). The Canadian Study of Health and Aging found similar results (Ebly *et al*., 1994). The prevalence of general dementia rose from 23% in the 85- to 89-year old population to 40% in the 90- to 94-year old group. For those 95 years and over, the prevalence of dementia reached 58%, with three-quarters of the cases of dementia thought to be due to Alzheimer's disease.

Even less data exist on the prevalence and incidence of Alzheimer's disease among centenarians. A 1991 study of all Finnish centenarians revealed that 26.8% of the 179 subjects had clinically diagnosed Alzheimer's (Sobel *et al*., 1995). Of a smaller Japanese study of 49 centenarians, 70.2% had some form of dementia, with three-quarters of the dementia attributed to Alzheimer's disease (Asada *et al*., 1996). Ebly *et al.* (1994) of the Canadian Study on Health and Aging suggested that dementia is nearly unavoidable in those living past 100, finding that 84.6% of those people 100 years or older are affected, the majority by Alzheimer's disease. In contrast, early findings of the New England Centenarian Study show fewer than expected cases of Alzheimer's disease at that age, with at least one-third of the subjects free of all types of dementia (Perls *et al*., 1999).

4. Family History

Another confirmed risk factor for Alzheimer's disease is family history. Specific genetic risk factors will be discussed in a subsequent chapter; however, a general association exists between positive family history of Alzheimer's disease and incidence risk in first-degree relatives. The association is statistically significant for a family history of early-onset Alzheimer's disease. The association for late-onset disease, while still positive, is thought to be nonsignificant (Thal *et al*., 1988).

A family history of Down syndrome may be a risk factor for Alzheimer's disease, although the low incidence of Down syndrome in the general population affects the reliability of data (Jorm, 1990). One study showed an increased risk of dementia in mothers, not fathers, of Down patients (Schupf *et al*., 1994). Alzheimer's neuropathology is known to be nearly ubiquitous in elderly Down syndrome patients at autopsy; however, actual symptoms of Alzheimer's disease are expressed in only a minority of them (Wisniewski *et al*., 1985).

Less clear-cut than age and family history risks are risks with gender, ethnicity or cultural differences, and education level. For these topics, what data do exist are often conflicting, and sample sizes are often less than adequate.

5. Gender

There is mounting evidence that Alzheimer's disease prevalence rates are higher in women than in men (U.S. General Accounting Office, 1998). Neuropathological studies consistently confirm this (Nishihara and Ishii, 1986; Ojeda *et al*., 1986; Wade *et al*., 1987). Among nonagenerians in particular, women are at significantly greater risk of having Alzheimer's disease than men (von Strauss *et al*., 1999). Higher prevalence rates among women may in part reflect better survival with the disease, as one study showed significantly worse survival for males than females (Beard *et al*., 1994).

Women also appear to be at greater incidence risk for Alzheimer's disease than men, although the evidence is not conclusive. A meta-analysis by Gao *et al*. (1998) of eight studies revealed a higher incidence rate in women than in men without respect to age, but at least five other studies did not (Nilsson, 1984; Copeland *et al*., 1992; Bachman *et al*., 1993; Kokmen *et al*., 1993; Letenneur *et al*., 1994). A greater incidence risk for women may be seen only in those over the age of 80; below that age incidence risk may actually be greater in men (Letenneur *et al*., 1999).

6. Race/Ethnicity

A major criticism leveled at national prevalence and incidence data on Alzheimer's disease is the largely Caucasian populations the data are derived from, calling into question the relevance of the results to the diverse population of the United States. The 18 studies from the United States and Europe that were integrated for the General Accounting Office analysis include very few nonwhite elderly subjects. The racial and ethnic composition of the United States elderly is expected to change dramatically in the next 50 years, and lack of data on Alzheimer's disease among minority groups renders national projections of the disease for this time period less reliable.

Although a 3-year comparison of general dementia prevalence rates and incidence in elderly blacks and whites showed no statistically significant differences in risk for race (Fillenbaum *et al*., 1998), other evidence shows a higher prevalence of dementia among certain ethnic groups, particularly among blacks (Schoenberg *et al*., 1985; Heyman *et al*., 1991). However, studies specifically comparing Alzheimer's disease prevalence among various racial groups, rather than that of general dementia, are scarce. Tang *et al*. (1998) suggested that blacks and Hispanics of a certain genotype may be at higher risk of developing Alzheimer's disease than whites. When members of all three groups who lacked the susceptibility allele APOE $\epsilon 4$ were compared, blacks had a four times higher cumulative risk for developing late-onset Alzheimer's disease than whites, and Caribbean Hispanics had a risk two times as great as whites. For those subjects who do possess the APOE $\epsilon 4$ allele, risk appears elevated for Caucasians but not other ethnic groups.

There is also some evidence that Alzheimer's disease patients of different racial backgrounds may experience differences in disease progression. One study shows a racial difference in neuropsychiatric symptoms without separating by type of dementing illness: blacks have a significantly greater likelihood of psychotic symptoms with dementia whereas whites have a greater likelihood of depression with the disease (Cohen and Magai, 1999).

Cultural or environmental factors may influence risk. Japanese males who emmigrate to the island of Hawaii are more likely to have Alzheimer's disease than Japanese men of the same age living in Japan, suggesting influences other than race. The prevalence rate of Alzheimer's disease among Hawaiian Japanese-American men approaches that seen for North Americans in general, while far exceeding rates typically seen in Japan (White *et al*., 1996). African Americans living in Indianapolis have a significantly higher age-adjusted prevalence rate of Alzheimer's disease, as well as other types of dementia, than members of a Nigerian African population living in Ibadan, Nigeria, despite similar racial origins (Hendrie *et al*., 1995). In a study based on hospital discharge records, Israeli Jews of American or European origin had double the Alzheimer's disease incidence rates of Israeli Jews of Asian or African origin. This study did not rule out cultural differences in hospital service use, however (Treves *et al*., 1986). One other example of possible environmental or cultural effect is a study showing higher prevalence rates of Alzheimer's disease in New York than in London (Copeland *et al*., 1987).

7. Other Risk Factors

A positive history of head trauma became a suspected risk factor in Alzheimer's disease after the discovery of Alzheimer's-like neuropathology in boxers afflicted with dementia pugilistica, as well as in 30% of nonboxers dying after one severe head injury (Graham *et al*., 1996). While there is consensus that a history of head injury in subjects with the apolipoprotein E-$\epsilon 4$ genotype increases risk for Alzheimer's disease, studies looking for a more general relationship with head injury without respect to genetic factors are not in agreement. Head injury with loss of consciousness has been shown to be a significant predisposing factor in Alzheimer's disease in

several recent case-control studies but not in others (van Duijin, 1996). Recall bias is one possible flaw in case-controlled studies: retrospectively collected information on head injury reveals a positive association with Alzheimer's disease, whereas prospectively collected information does not support such a link (Chandra *et al*., 1989). One longitudinal incidence study did show an increased risk for Alzheimer's disease in subjects with a history of head injuries involving loss of consciousness greater than 5 min and also for subjects whose injuries occurred within the past 30 years (Schofield *et al*., 1997).

Lack of education may be a risk factor. Studies showing lower education as a risk factor of developing Alzheimer's disease slightly outweigh in number those studies that fail to find any link between education and risk (Beard *et al*., 1992). The possibility that people with more education or higher premorbid intelligence may better mask evidence of decline on cognitive function tests cannot be excluded. Other confounding factors, such as occupational or early life exposures, are also difficult to rule out.

The presence of aluminum in neuritic plaques of Alzheimer's disease patients at autopsy spurred investigation into a link between exposure to the metal and development of the disease. Aluminum in drinking water and in mines has been linked to increased risk for Alzheimer's disease (Rifat *et al*., 1990; Doll, 1993; Jacquim *et al*., 1994). Risk from other sources of aluminum, such as medications and antiperspirants, has not been established conclusively (van Duijin, 1996). Interestingly, no difference has been found in serum and bone aluminum levels between control patients and Alzheimer's disease patients (O'Mahony *et al*., 1995; Zapatero *et al*., 1995). Other possible risk factors for Alzheimer's disease, such as depression, smoking, and exposure to glues, fertilizers, and pesticides, have been investigated. Here again, conclusive results are lacking.

8. Possible Protective Factors

Estrogen replacement therapy is a possible but not conclusively proven protective factor against developing Alzheimer's disease. Women in one study who took estrogen for longer than 1 year after menopause had an 80% lower incidence risk for Alzheimer's disease when compared to women who did not take the therapy, whereas the Baltimore Longitudinal Study on Aging found Alzheimer's risk reduced by half in women who had a postmenopausal history of estrogen replacement therapy (National Institute on Aging, 1998). A review of 10 randomized studies and 9 observational studies on estrogen use and Alzheimer's disease found some support for use of the therapy, but not enough to conclusively endorse it for the prevention of Alzheimer's disease (Haskell *et al*., 1997).

Arthritis sufferers appear to be at lower risk of Alzheimer's disease. This has been linked to regular use of nonsteroidal inflammatory drugs other than aspirin (Breitner *et al*., 1994). Regular use of nonsteroidal inflammatory such as ibuprofen, naproxen sodium, and indomethacin for 2 years led to a 60% reduction in the risk of developing Alzheimer's disease among subjects in the Baltimore Longitudinal Study on Aging (National Institute on Aging, 1998). Use of aspirin lowered risk only slightly, whereas acetominophen use was not associated with any risk reduction.

B. Vascular Dementia

Vascular dementia is thought to be the second leading cause of dementia after Alzheimer's disease in the United States and Europe (Jorm, 1990). Whereas Alzheimer's disease accounts for two-thirds or more of all cases of dementia, vascular dementia is believed to cause between 10 and 20% of the cases (Small *et al*., 1997; Nyenhuis and Gorelick, 1998). There is debate as to whether the category vascular dementia is a clinically useful one at all, however. The term encompasses several subtypes of pathology, including multi-infarct dementia, Binswanger-type dementia, and dementia of the hemodynamic type. Prevalence and incidence data on vascular dementia typically do not discriminate between subtype.

Estimates on the prevalence and incidence of the disease may be influenced by bias in diagnostic criteria. Diagnosis requires the presence of both cerebrovascular disease and dementia, even though a direct causal relationship remains to be found; thus areas with a high prevalence of cerebrovascular disease may be prone to overestimation of vascular dementia rates at the expense of other diagnoses (van Duijin, 1996; Brust, 1988). Nolan *et al*. (1998) found at autopsy that cerebrovascular disease alone was the cause of none of 87 cases of dementia, although cerebrovascular disease was seen to coexist with Alzheimer's disease pathology.

In Japan, China, and Russia, where stroke rates are high, estimates of vascular dementia rates range from moderately high to very high. Some studies show it to be as common or more common than Alzheimer's disease (Jorm, 1991; Li *et al*., 1991; Udea *et al*., 1992; Yoshitake *et al*., 1995). At least three studies suggest that vascular dementia is overdiagnosed in these regions and that rates are in fact much more similar to those seen in the United States, i.e., trailing Alzheimer's disease as the underlying cause of most dementia (Lin *et al*., 1998; Nolan *et al*., 1998; Yamada *et al*., 1999).

Age, gender, and race are risk factors for vascular dementia. Like Alzheimer's disease, rates increase exponentially with age, although the rise appears to be steeper for vascular dementia than for Alzheimer's disease (Jorm *et al*., 1987). The balance of both prevalence and incidence evidence shows males more likely to have vascular dementia (Jorm, 1990; van Duijin, 1996). Blacks appear to suffer from the disease in greater proportion than members of other races (Gorelick, 1997; Lindsay *et al*., 1997). Stroke risk factors are closely linked to, but not necessarily identical to, vascular dementia risk factors (Leys *et al*., 1998). Age, gender, and race are also risk factors for stroke. Other factors include arterial hypertension, diabetes mellitus, and low HDL cholesterol levels.

C. Dementia with Lewy Body Disease

Pathological studies suggest that Lewy body disease may vie with vascular dementia—or even exceed it—as the second leading cause of dementia. Seventeen to 36% of all cases of dementia appear to be caused by the disease (Gomez-Tortosa *et al*., 1998). An autopsy series found that the disease accounted for 25% of dementia cases (McKeith *et al*., 1996).

When mixed-type dementia cases are included, Lewy body disease may be far more widespread than was first believed. As many as 30% of elderly patients diagnosed clinically and

pathologically with Alzheimer's disease also showed significant Lewy body pathology at autopsy. In addition, at least some Lewy body pathology is nearly ubiquitous at autopsy of Parkinson's disease patients (Hughes, 1997). Because clinical and pathological aspects of the disease overlap with both Alzheimer's disease and Parkinson's disease, prevalence and incidence rates of dementia with Lewy body disease are not yet established (Holmes *et al*., 1999).

As with Alzheimer's disease and vascular dementia, the largest risk factor for incidence of the disease is age in that above the age of 50, incidence rates rise steadily. Family history is also implicated. Alzheimer's disease in a first-degree relative increases the risk of developing dementia with Lewy body disease greatly. Presence of the apolipoprotein E ϵ4 allele is a risk factor, but somewhat less so for Lewy body disease than for Alzheimer's disease. Gender appears to be a risk factor as well: men are twice as likely as women to have dementia with Lewy body disease (Papka *et al*., 1998). Data on other demographic and epidemiological aspects of the disease are lacking.

IV. Age-Associated Sensory–Motor Impairments

A. Visual Impairment

Visual function changes and ocular disease often accompany the aging process. Decreases in acuity, accommodation, and dark adaptation are common among the elderly. Among Americans 65 and older, 7 million people (or 21%) have some degree of visual impairment. Of those, 3.5 million people report severe vision loss. Based on these estimates, visual impairments will affect 15 million elderly people, 7.6 million of them severely, by the year 2030 (Lighthouse, Inc., 1995). Figure 4.4 shows U.S. Department of Health estimates of visual impairment among the noninstitutionalized elderly. Age-related macular degeneration, primary glaucoma, and diabetic retinopathy are the main neurological impairments affecting vision in the elderly.

1. Age-Related Macular Degeneration

Retinal degeneration affects the elderly in two forms, one of which, peripheral retinal degeneration, occurs in as much as half of the elderly population but results in little change in vision and is not treated (Ernest, 1997). The other form, age-related macular degeneration (AMD), is far more serious. Also referred to as age-related maculopathy, it is the leading cause of new cases of legal blindness in the United States as well as the leading cause of visual impairment among those 70 years and older.

AMD itself has two types, the ''dry'' or nonexudative type, also called the atrophic form, and the ''wet'' or neovascular/exudative type. The latter form results in more severe vision loss. The dry type affects more people, however, accounting for 80% of all AMD patients, some of whose condition may then deteriorate into the wet form (Ferris *et al*., 1984).

An estimated 4.5 million people had AMD in the United States in 1992 (Hyman, 1992). When longer life expectancies and current prevalence data are taken into account, as many as 7.5 million people are likely to have AMD by the year 2020. Incidence data on the disease are lacking, however. This affects the accuracy of projections, particularly since several studies have reported an increase in the rate of AMD-related blindness registrations in the past few decades. It is not known if the increases are genuine or an artifact of better diagnosis and reporting (Hyman, 1992).

Prevalence rate estimates for AMD vary from study to study, particularly since no uniform diagnostic criteria have been established for the disease. In the United States, two major population-based prevalence studies, one national in scope and one in Framingham, Massachusetts, showed overall prevalence rates of 5.8 and 8.8%, respectively (Kahn *et al*., 1977a; Klein and Klein, 1982). A more recent study suggests that 15% of Caucasians 40 years and older (13.2 million people) show some signs of the disease (Prevent Blindness America, 1994).

The disease is strongly age related. Prevalence rates among people under the age of 55 approach zero (O'Shea, 1998). The Framingham study found that prevalence more than doubled

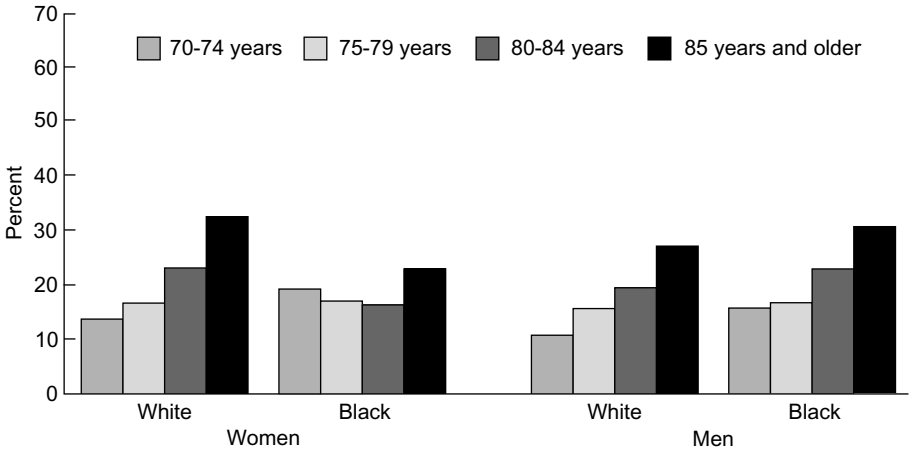

FIG. 4.4. Prevalence of vision impairment by age, sex, and race in 1995. Visual impairment becomes more common with age, although there are underlying gender and racial differences in prevalence. From U.S. Department of Health and Human Services (1999) (available online: *http://www.cdc.gov/nchs/products/pubs/pubd/hus/charts/hus99f12.pdf*).

between the age groups 65–74 and 75–85, rising from 11 to 28% (Kahn *et al*., 1977a,b; Klein and Klein, 1982). Other research indicates that more than one-third of the population 75 and over in the United States is affected by AMD, 3% of them severely so (Prevent Blindness America, 1994).

Whether there is an association between gender and AMD remains under debate. Some studies show a slight female preponderance among AMD sufferers. In one Scandinavian study on the incidence of blindness caused by AMD, female cases outnumbered male cases by as much as 2.8:1 (Rosenberg and Klie, 1996). In that study, the age-specific incidence of AMD blindness was 140:100,000 for females and 66:100,000 for males in the age group 60 and over. A national study in the United States, however, found no clear-cut gender differences in prevalence rates, but did not assess incidence rates (Klein and Klein, 1982).

Race may be a risk factor in AMD, although this also has not been determined conclusively. It should be noted first that African Americans have a greater proportion of visual impairments overall than do whites, which may be due to diminished access to eye care (Schmeidler and Halfmann, 1998). For AMD, however, blacks are thought to have a lower risk of the disease than whites. Theories on this topic focus on the possible protective effects of darker pigmentation. Signs of the disease are common in both groups, but more severe, late-stage forms of the disease appear more commonly in elderly whites than blacks in the United States (Friedman *et al*., 1999). At least four studies have shown higher overall rates of AMD among whites than blacks (Hyman, 1992), and another study has shown whites to have a higher frequency of early stage, age-related macular degeneration than blacks (Schachat *et al*., 1995). Rates among whites may exceed those found among Asians as well. The National Health and Nutrition Examination Survey, however, showed no significant differences by race in prevalence of AMD (Goldberg *et al.*, 1988).

Other factors that have been examined for positive risk association with AMD include high blood pressure, cardiovascular disease, exposure to sunlight, altitude, light-colored iris pigmentation, smoking, heredity, and lack of dietary antioxidants. Some evidence suggesting elevated risk for these factors has been demonstrated but all remain under debate. Income and education are not risk factors for AMD (Klein *et al*., 1994).

2. Primary Glaucoma

Of existing, not incident, cases of legal blindness in the United States, glaucoma is the leading cause (National Society to Prevent Blindness, 1980). There are several forms of glaucoma, all involving optic nerve atrophy and defects of the visual field (Ernest, 1997). Two of the forms, open angle and angle closure, are together known as primary glaucoma. They are most closely associated with aging and cause visual impairment in as many as 6% of Americans over the age of 65 (Glaucoma Foundation, Inc., 1997). Worldwide, an estimated 67 million people live with glaucoma (Flanagan, 1998).

Angle closure glaucoma is the rarer of the two forms. It is an acute disease, with onset late in life. Open angle glaucoma occurs five times as commonly and is a chronic, slow-progressing form of the disease (Ernest, 1997). Unlike acute angle closure glaucoma, which typically causes pain at onset and causes patients to seek medical care, open angle glaucoma may go undetected for years. Incidence and prevalence estimates may thus be too low. Researchers in Sweden themselves uncovered nearly half of the cases of open angle glaucoma in one population-based survey (Ekström, 1996). Among an Australian population 49 and older, 3.0% were affected, and half of the cases had remained undetected until the study (Mitchell *et al*., 1996).

Estimates for 1997 showed four million Americans overall with open angle glaucoma, more than half of whom are middle-aged or older (Glaucoma Foundation, Inc., 1997). The Beaver Dam Eye Study found an overall prevalence of open angle glaucoma of 2.1%. It also found a strong association with age: prevalence was 0.9% among 43 to 54 year olds, but increased to 4.7% among those 75 years and older (Klein *et al*., 1992). Among those aged 80 to 89 years, the disease affects as many as 9.7% of the population (Wensor *et al*., 1998). Incidence of this form is thought to rise from 0.2% among 50 year olds to 2% among 70 year olds (Ernest, 1997).

Older African Americans are at as much as three times higher risk of having primary glaucoma than whites. Approximately half a million, or 6%, of older African Americans have the disease compared to 2%, or nearly 1.5 million, of older Caucasians (Prevent Blindness America, 1994). The likelihood of visual impairment due to the disease is even greater among older African Americans. Risk of impairment may be as much as 6% greater among blacks than whites (Tielsch *et al*., 1990). The major accepted risk factor for the disease is abnormally high intraocular pressure (Flanagan, 1998). Sleep apnea may also increase risk (Mojon *et al*., 1999).

3. Diabetic Retinopathy

The risk of diabetic retinopathy increases as the length of time living with diabetes increases. Risk, therefore, is also strongly linked to aging, as the majority of diabetes cases are diagnosed after the age of 40. The incidence of diabetic retinopathy is greatest during the years 45 to 75. Nearly 2% of people living 15 years with diabetes are blind, and 10% have some form of visual impairment from the disease. In a Rochester, Minnesota study, a cumulative blindness incidence of 8.2% was demonstrated in patients living 20 years with diabetes (Sanchez-Thorin, 1998). Diabetic retinopathy caused 14.4% of legal blindness among 65 to 74 year olds in the United States in 1978, the only data inclusive of all races and types of blindness (National Society to Prevent Blindness, 1980).

Race is a risk factor. African Americans and Hispanic Americans are more likely to have diabetes and diabetic retinopathy than Caucasian Americans (Prevent Blindness America, 1994). Black women have the greatest risk of blindness with the disease, although a study has shown slower disease progression in African Americans than in whites (Kahn and Hiller, 1974; Arkfen *et al*., 1994). Native Americans also have a greater risk of diabetic retinopathy compared to whites (Lee *et al*., 1992a,b). White men appear to have the lowest overall incidence of the disease (Kahn and Hiller, 1974).

Risk for diabetic retinopathy is also elevated with hyperglycemia. High blood pressure and gross proteinuria are other predisposing factors for diabetic retinopathy among type I

diabetics. Type I diabetics have a greater risk for blindness with the disease than type II diabetics.

B. Hearing Impairment

Hearing loss associated with aging has numerous causes and is regarded as the most common medical problem affecting the elderly (Mhoon, 1997). Figure 4.5 shows the prevalence of hearing impairment among the noninstitutionalized elderly. Most studies on hearing loss show prevalence rates ranging from 27 and 47% among the elderly (Moscicki *et al*., 1985; Ries, 1985; Adams and Benson, 1990). A recent population-based study of 4541 people aged 48 to 92 found prevalence of all degrees of hearing loss to be 45.9% among those 65 and older, with moderate and severe hearing loss accounting for 41.9% of the cases (Cruickshanks *et al*., 1998). In an audiologic survey of people 70 years and older living at home, 60% were affected by significant losses in the speech frequency range (Herbst and Humphrey, 1980). Even after adjusting for noise exposure, occupation, and education, a statistically significant preponderance of hearing loss was seen in males (Cruickshanks *et al*., 1998). Among nursing home residents, hearing losses affect as much as 80 to 90% of the population (Hull, 1995).

Otologic changes may occur with aging in all parts of the ear, but those involving sensorineural impairment are the most common. Sensorineural loss can be divided into sensory loss, involving the cochlea or organ of Corti, and neural loss, involving peripheral nerves, ganglion cells, the auditory nerve, or central auditory pathways.

Forms of sensorineural impairment, in addition to traumas and hereditary or congenital defects, include ototoxicity, Ménière's disease, and hearing loss due to metabolic, autoimmune, and demyelinating disorders. Infection, neoplasms, and brain stem ischemias also cause sensorineural hearing deficits. These disorders are not necessarily age related, but they do commonly affect the elderly. Noise, smoking, cardiovascular disease, hyperlipoproteinemia, and low dietary antioxidant intake are all thought to be factors contributing to sensory impairment in hearing. Exposure to these factors increases with age. Tinnitus is an example of this—increases in prevalence rates are associated with both age and level of noise exposure (Coles, *et al*., 1987).

1. Presbycusis

By far, however, the hearing loss associated most commonly with aging is a progressive, bilaterally symmetric hearing impairment of unknown cause, affecting primarily the ability to hear high tones. Onset rarely occurs before middle age. The general term presbycusis is used to describe this disease.

Four types of presbycusis have been described: sensory, neural, strial, and cochlear conductive types (Kennedy and Clemis, 1990). The four types are discriminated by area of sensorineural degeneration and atrophy in the inner ear. The elderly are often affected by more than one type. However, one-quarter of the cases of presbycusis fit into none of the four categories (Mhoon, 1997).

Because presbycusis is used as an umbrella term for a variety of underlying pathologies, demographic and epidemiological studies on it are not readily available. However, prevalence and incidence data are available for general hearing losses among the elderly and, as mentioned earlier, presbycusis accounts for the vast majority of all hearing loss in this population. Although the disease is accepted to be strongly age related, conflicting evidence exists on whether presbycusis incidence and prevalence rates continue to rise steadily among the oldest old segment of the population. A recent longitudinal study of presbycusis in a Swedish population suggests that annual declines in hearing threshold level off in the ninth decade of life (Jonsson and Rosenhall, 1998).

2. Ototoxicity

Medication effects can also cause sensorineural impairment in the inner ear. Known as ototoxicity, this form of hearing loss affects the elderly in greater proportion than other segments of the population because of the greater likelihood of polyphar-

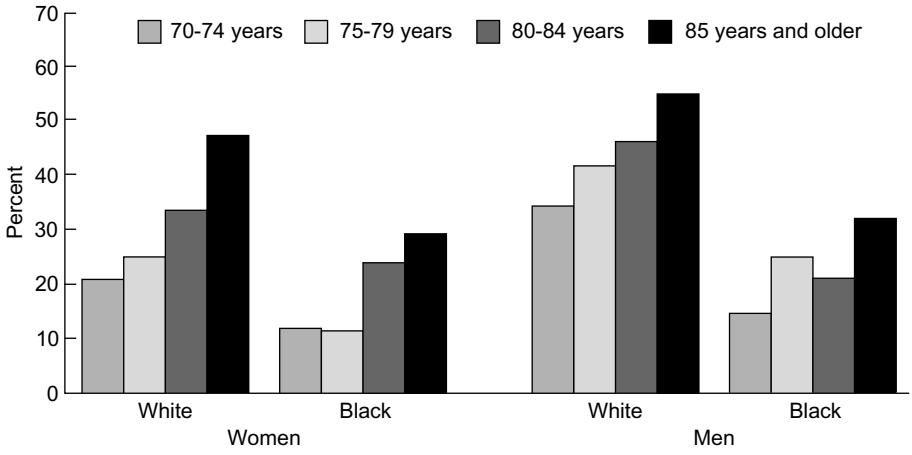

FIG. 4.5. Prevalence of hearing impairment by age, sex, and race in 1995. Hearing impairments may affect as many as half the population 85 years and older, although overall prevalence rates vary significantly by race and gender. From U.S. Department of Health and Human Services (1999). (available online: *http://www.cdc.gov/nchs/products/pubs/pubd/hus/charts/hus99f13.pdf*)

macy among the elderly and the greater sensitivity of the elderly to side effects. Temporary ototoxicity can be caused by ibuprofen and aspirin, drugs commonly used by older patients with arthritis. Other drugs with ototoxic effects are aminoglycoside antibiotics, quinine, ethacrynic acid, and furosemide (Mhoon, 1997).

C. Gait Impairment and Postural Instability

Postural stability and gait depend on the integration of a host of sensory, neural, and musculoskeletal factors, all of which are susceptible to changes with aging. Nutt (1997) classifies balance and gait disorders by a broad area of dysfunction: afferent or sensory disturbances, central or integrative disturbances, and efferent or motor disturbances.

Afferent dysfunctions, such as age-related vestibular and proprioceptive changes and the aforementioned age-related vision losses, contribute to spatial disorientation and distortion of environmental signals. Because these senses normally provide redundant and overlapping information, loss of one is not in itself enough to cause significant instability. In the elderly, however, more than one of the senses are often compromised or provide conflicting information, affecting balance and gait. Central nervous system disorders affect stability in the elderly, especially through diseases that affect the frontal lobes, basal ganglia, and brain stem. Cognition-related judgement decline is a common cause of gait impairment and falls. Efferent declines with age typically include changes in muscle strength and tone, but may also involve motor neuropathies, corticospinal pathways, and diseases of the cerebellum. These declines are accompanied by an inability to coordinate motor function and also result in gait disturbances.

Gait disorders affect as many as 57% of women and 42% of men over the age of 75 (Waite *et al*., 1997). Spastic gait and ataxic gait are examples commonly afflicting the elderly. These disorders are caused by a specific area of decline (afferent, central, or efferent) discussed in detail later. However, the most common gait disturbance in the elderly, ''cautious'' gait, is not described by a particular area of decline. Cautious gait, also known as senile gait, is a general term for unsteadiness with aging. One theory as to cause is normal-pressure hydrocephalus; another is the cumulative effects of many minor neurologic defects and progressive losses that can accompany neuronal aging in the elderly (Jenkyn and Reeves, 1981; Fisher, 1982; Horak *et al*., 1989).

1. Afferent Impairment

Dysfunction of the peripheral vestibular system most commonly causes a weaving or ''drunken'' gait, whereas central disorders of the vestibular system may produce an ataxic gait or weaving. Prioprioceptive declines, either of the peripheral system or of spinal pathways, cause ataxic gait disorders and loss of position sense.

a. Vestibular Changes. Disorders of the vestibular system, manifest chiefly as complaints of dizziness and/or vertigo, are extremely common in the elderly. At least half of home-dwelling subjects over the age of 62 had some form of vertigo, and the numbers are thought to be significantly higher among nursing home and hospital residents (Babin and Harker 1982). Sloane *et al*. (1989) found that dizziness or loss of balance affected 65% of community-dwelling individuals over the age of 60, many of them on a daily basis. As many as 69% of elderly individuals demonstrate impairment on vestibular function tests (Lord *et al*., 1991).

Age-related changes in vestibular function can be due to peripheral, central, or systemic disorders. Neural involvement is limited to the former two, although causes other than neural degeneration, such as motion sickness, tumors, and vascular anomalies, can also account for peripheral and central vestibular dysfunction. Peripheral vestibular disorders typically cause severe symptoms of vertigo (the sensation of individual or environment rotating), whereas central disorders more often result in milder symptoms of dizziness (general sensations of dysequilibrium and spatial disorientation including light-headedness, swaying, and unsteadiness) (Nutt, 1997).

Disorders of the peripheral vestibular system are not well classified. Degenerative losses with aging may be seen in the sensory epithelium, otoconia of the saccule, hair cells, or peripheral nerve. Belal and Glorig (1986) used the general term presbyastasis to describe disequilibrium due to peripheral vestibular changes with aging and found the condition prevalent in 79% of elderly patients presenting with dizziness. Histopathologic evidence in the vestibular labyrinth necessary to consider presbyastasis a distinct disease entity is not well established, however.

Another general term for peripheral vestibular dysfunction, this one associated with vertigo when the head is placed in certain positions, is benign paroxysmal positional vertigo. Whereas symptoms of this condition are well established, etiology is not. General degenerative processes in the labyrinth are thought to cause the majority of cases, but vestibular neuronitis, surgical trauma, head injury, and medication effects can also be predisposing factors. Benign paroxysmal positional vertigo was the most prevalent diagnosis of 1194 patients in a Montreal dizziness clinic, affecting nearly 40% of patients (Katsarkas, 1994).

b. Proprioceptive Changes. Proprioceptive changes with aging can result from losses in the sensory nerve terminals of muscles, tendons, and joints or in the multiple central nervous system connections. Peripheral sensation is vital to postural stability, walking on uneven ground, and spatial orientation during position changes, particularly when other senses are compromised. It accounts for roughly 56% of the maintenance of static posture, whereas vestibular and visual senses are secondary, accounting for roughly 22 and 21%, respectively (Lord *et al*., 1991).

Loss of sensory input from lower limbs is particularly prevalent among the elderly. Among nursing home residents in Australia, nearly 36% of elderly subjects received abnormal ratings on static balance tests, whereas on dynamic balance tests, over 73% of subjects demonstrated impairment (Lord *et al*., 1991). Poor scores on both types of tests were significantly associated with reduced tactile sense as well as reduced joint and vibration sense.

Proprioceptive losses are nearly unavoidable with age. In a comparison of healthy 80 year olds with healthy 20 year olds, ankle vibration sense declined 86% with age. One-leg standing

with eyes open declined 32%, whereas with eyes closed it declined 100% (Potvin *et al*., 1980). Bohannon *et al*. (1984) found that no subjects in their eighth decade of life were able to balance on one leg with eyes closed for 30 sec, whereas three-quarters of subjects in their third decade of life were able to do so.

2. Central Nervous System Impairment

Impairments of higher-level central integrative functions in the elderly affect postural stability and gait in a number of ways. Input from sensory systems can be compromised by neurological losses in central pathways. In the vestibular system, for example, central vestibular nuclei and pathways are vulnerable to atherosclerosis and chronic hypertension (Belal and Glorig, 1986). Ischemias and infarcts, neuromas, and demyelinating diseases in the brain stem, basal ganglia, cerebellum, and thalamus compromise central pathways of all types of sensory functions. Walking may become impossible due to the resulting severity of disequilibrium (Nutt, 1997).

The elderly are at greater risk for a number of diseases affecting cerebral blood flow, such as postural hypotension, stroke, cardiac dysrhythmias, and systemic diseases such as diabetes. These may cause central neurologic deficits in deep white or gray matter as well as in the frontal lobes. These types of dysfunction are associated with loss of balance, freezing, or shortened steps, although central gait disorders are still somewhat controversial and not generally well classified.

Loss of cognitive function due to dementing illnesses affects stability through impaired judgement and gait. Medications commonly taken by the elderly, particularly certain psychotropic, cardiac, and analgesic drugs, are known to compromise central functioning and thus stability through a variety of mechanisms.

3. Efferent Impairment

Diminished effector component function of the motor system affects the ability of the elderly to maintain postural stability, producing a wide variety of gait disturbances. Distal and proximal nerve declines such as motor neuropathy result in a waddling or slapping gait. Corticospinal pathway dysfunction commonly causes ''spastic'' gait. Parkinsonism or dysfunction in the basal ganglia results in tremor, rigidity, and bradykinesia, whereas choreic diseases produce distinctly irregular movements. Cerebellar changes cause ataxic gait and balance incoordination (Nutt, 1997).

4. Falls

Postural instability is of primary importance in day-to-day function in the elderly in that its presence is the major predictor of falls. Among those over the age of 65, the sixth leading cause of death is unintentional injury, mainly from complications of falls (Sattin, 1992). Falls affect at least a third of community dwellers over the age of 65 (Tinetti *et al*., 1988). Risk of not being able to get up after a fall is as high as 50% in the elderly. Falling increases the likelihood of nursing home placement; unfortunately, however, falls in nursing homes are even more prevalent, affecting half of all residents annually and directly accounting for 1800 fatalities each year (Tinetti, 1997). The incidence rate of falls among residents of nursing homes is as high as 1.5 falls per bed per year (Rubenstein *et al*., 1994).

The model that best describes falls is a multifactorial one, taking into account environmental, activity, and host factors. Because postural stability in the host requires sensory, central integrative, and effector contributions, impairments in any of these systems can compromise balance and equilibrium. The age-related visual, vestibular, proprioceptive, and central nervous system losses described earlier therefore strongly predispose the elderly to falling. Nonneuronal host factors such as arthritis, joint and muscle weakness, systemic diseases such as electrolyte and sugar imbalances, postural hypotension, and acute illnesses also contribute to the high rate of falls among the elderly. The heightened sensitivity to the effects of medications among the elderly is another very common factor in falls.

D. Parkinson's Disease and Parkinsonism

Parkinson's disease in the most common disease of the cluster of chronic, progressive, and age-related motor system disorders. Also known as idiopathic Parkinson's disease or primary parkinsonism, it predominates this group of movement disorders by as much as 68% and is of still unknown etiology (de Rijk *et al*., 1997). The term parkinsonism is a general one, covering related disorders with most, but not necessarily all, of the same symptoms as Parkinson's disease, and may also include symptoms distinct from those of Parkinson's disease. In some literature the term includes idiopathic Parkinson's disease.

Non-Parkinson's disease forms of parkinsonism are relatively uncommon and are of both known and unknown cause. The second most common form of parkinsonism after Parkinson's disease is progressive supranuclear palsy (Litvan and Hutton, 1998). Mayeux *et al*. (1995) found the disease to be 12 times less frequent than Parkinson's disease in terms of crude annual incidence. Progressive supranuclear palsy increases in incidence rate with age, rising from 1.7 for 50- to 59-year olds to 14.7 for 80- to 99-year olds (Bower *et al*., 1997). Other non-Parkinson's forms of parkinsonism include postencephalitic parkinsonism, drug-induced parkinsonism, striatonigral parkinsonism, arteriosclerotic parkinsonism, toxin-induced parkinsonism, the parkinsonism–dementia complex of Guam, and parkinsonism accompanying distinct neurological disorders (National Institutes of Health, 1994). The Europarkinson collaborative study separated the various forms of parkinsonism by frequency of occurrence and found that after Parkinson's disease, unspecified parkinsonism accounted for 14% of all types in Europe (de Rijk *et al*., 1997).

1. Prevalence

Prevalence estimates range widely by study and are subject to the usual caveats: differing diagnostic criteria and methodology by study, underestimates caused by delays in diagnosis due to the progressive nature of the disease, and overestimates

caused by symptom overlap with other neurological diseases. In five community studies in Europe, the survey investigators themselves uncovered 24% of the total cases of Parkinson's disease in the population, suggesting that many patients do not seek medical attention for symptoms and thus go undiagnosed (de Rijk *et al*., 1997). However, as many as 25% of expertly diagnosed cases of Parkinson's disease were found at postmortem to be otherwise, according to data from the Parkinson's Disease Society Brain Bank of the United Kingdom (Hughes *et al*., 1992). Up to 40% of false-positive diagnoses may be due to essential tremor (Tanner and Goldman, 1996).

Parkinson's disease affects some 500,000 Americans at this time (National Institutes of Health, 1994.). It accounted for 4.6% of all visits to neurologists in the United States in 1991–1992, but among those 65 and older, it accounted for 16.9% of visits to neurologists (Schappert, 1995). The prevalence in North America is thought to be 150 per 100,000 people (Checkoway and Nelson, 1999). For a disease with possible environmental causes, however, regional variations in prevalence are important and are masked by large-scale national estimates.

Within America there are notable geographic variations in Parkinson's disease mortality, suggesting the possibility of an accompanying uneven distribution by region and perhaps also by race in prevalence. Lanska (1997) found a decreasing gradient from north to south for underlying cause and all-cause mortality rates among whites, but a weaker gradient and lower overall mortality rates for blacks. A twofold increased risk of death is associated with parkinsonism (Bennett *et al*., 1996).

Variations in prevalence estimates have been found at the international level. In an analysis of age-adjusted prevalence rates from 27 regions of the world, there was a 13-fold difference between the highest and the lowest end rates (234 per 100,000 in Uruguay and 18 per 100,000 in China, respectively) (Zhang and Roman, 1993). If data from Chinese provinces are excluded, however, only a fourfold difference is detected worldwide (Wang, 1990). Low prevalence rates in China have been reported consistently. Other data from door-to-door surveys conducted by WHO investigators found the lowest worldwide age-adjusted prevalence rates among blacks in Nigeria, followed by China (Zhang and Roman, 1993). High-end estimates include a crude prevalence of 244.4 per 100,000 in a door-to-door survey in Alberta, Canada (Svenson *et al*., 1993).

2. Incidence

The latency period between the beginning of dopaminergic neurodegeneration and the actual onset of symptoms has yet to be determined, nor are there definitive antemortem diagnostic tests. Approximately 50,000 new cases of Parkinson's disease are diagnosed each year in the United States (National Institutes of Health, 1994). A population-based longitudinal study in Olmsted County, Minnesota revealed an increase in annual incidence rates over the period from 1935 to 1984 of 9.2 per 100,000 to 16.3 per 100,000 (Tanner *et al*., 1992). Other studies have shown no changes in incidence in the past half century, suggesting little temporal variation (Tanner and Goldman, 1996).

Internationally, annual incidence figures as low as 4.5 per 100,000 were determined in Libya and as high as 16.1 per 100,000 in Iceland (Tanner and Goldman, 1996). Incidence rates by region and nation vary widely and include discrepancies as to what forms of parkinsonism are included in data. Overall rates for the United States typically fall at the high end of international incidence comparisons.

3. Age

As with dementia, Parkinson's disease is strongly related to aging. Young-onset forms (defined as below the age of 40) are known and thought to be increasing in prevalence, but the typical age of onset is 60 years of age. Both incidence and prevalence of the disease rise steeply above the age of 50 worldwide, regardless of overall prevalence variations by geographic region. Older literature reported that prevalence and incidence rates drop among the oldest-old. More recent data suggest this trend is most likely due to missed diagnoses and predict a continued exponential rise with age (Ben-Shlomo, 1996).

Advancing age brings with it an increase in prevalence of nearly all types of parkinsonian clinical signs. The exception appears to be resting tremor, which shows a decrease in prevalence with age (see Table 4.2). Bennett *et al* (1996) assessed the prevalence of parkinsonian signs: bradykinesia, gait disturbance, rigidity, and tremor. With Parkinson's disease patients included, 52.4% of those 85 and older had at least two signs. For elderly aged 65–74 and 75–84, 14.9 and 29.5% had at least two parkinsonian signs, respectively.

4. Gender

Men outnumber women in Parkinson's disease. Most of the evidence collected shows age-adjusted prevalence rates slightly higher in men than in women, and male cases appear to outnumber female cases by 20 to 30% (Checkoway and Nelson, 1999). Mayeux *et al.* (1995) found a lower cumulative incidence rate among women regardless of racial background.

5. Race/Ethnicity

The wide discrepancies in prevalence and incidence rates between different regions of the world may reflect underlying differences in racial susceptibility to the disease, or may be due to other factors entirely. Zhang and Roman (1993) found that regardless of region, door-to-door surveys yielded consistently higher rates than other methods and suggested that regional variations may be due to case-ascertainment differences rather than true variation in Parkinson's disease between populations. Several studies report reduced rates of Parkinson's disease among black Africans and also among blacks in other regions of the world in clinic-based studies (Richards and Chaudhuri, 1996). In Copiah County, Mississippi, lower age-adjusted rates were determined among blacks than whites in door-to-door surveys; identical survey methods in Nigeria, however, revealed a fivefold lower prevalence among Nigerian blacks than American blacks (Osuntokum *et al*., 1987; Schoenberg *et al*., 1998).

Mayeux *et al*. (1995) found lower age-adjusted prevalence rates of Parkinson's disease among blacks than other racial groups in a Manhattan community registry study, but impor-

TABLE 4.2 Age-Specific Estimates of the Prevalence of Parkinsonian Signs[a]

Parkinsonian sign	No. in sample	Prevalence according to age %			*P* value for trend (age)
		65–74 years	75–84 years	≥85 years	
Bradykinesia					
Paucity of movements of the extremities	98	9.3±2.0	16.6±2.0	30.1±3.8	<0.001
Paucity of movements of the face	116	12.6±2.4	21.2±2.2	35.5±4.0	<0.001
Slow finger taps	197	22.8±3.3	40.0±2.7	62.5±3.9	<0.001
Gait disturbance					
Reduced arm swing	210	24.4±3.3	41.8±2.7	64.8±3.9	<0.001
Shufling gait	83	6.4±1.6	13.8±1.8	29.7±3.8	<0.001
Prolonged turning	153	15.7±2.7	29.6±2.5	50.8±4.1	<0.001
Rigidity					
Right leg	141	16.7±2.8	27.6±2.4	43.3±4.1	<0.001
Left leg	154	16.9±2.8	28.6±2.4	46.3±4.1	<0.001
Right arm	62	8.7±2.1	12.0±1.7	17.2±3.1	0.046
Left arm	69	9.8±2.3	14.4±1.9	20.7±3.4	0.009
Tremor					
Postural	99	16.5±2.9	20.7±2.2	25.6±3.5	0.02
Resting	24	6.1±2.0	5.6±1.3	5.0±1.8	0.681

[a]Parkinsonian signs are increasingly prevalent with age. From Bennett *et al.* (1996).

tantly, highest incidence rates and mortality rates from the disease among black men. Richards and Chaudhuri (1996) proposed the explanation that blacks, with higher stroke and cardiovascular disease risk, may be more susceptible to vascular parkinsonism, poorer survival, and therefore appear to have lower prevalence rates of Parkinson's disease than whites.

6. Risk Factors

Symptoms of Parkinson's disease have been described as long ago as 1000 B.C. but the disorder continues to be an etiological enigma. Theories as to cause shifted dramatically over the course of the 20th century. In the first half of the century the major risk factor was thought to be exposure to infectious agents, after a form of parkinsonism was linked to pandemic encephalitis lethargica in the early decades of the 20th century. The disease caused by this agent is now recognized as postencephalitic parkinsonism and is considered distinct from Parkinson's disease. Research for other infectious agents led to the discovery of rare forms of parkinsonism caused by Western and Eastern equine encephalomyelitis and Japanese B encephalitis (National Institutes of Health, 1994).

Toward the latter part of the 20th century research shifted from viral agents toward environmental toxins after the identification of drug-induced and other chemical injury forms of parkinsonism. Research intensified following the discovery in the mid-1980s of parkinsonism among heroin users who had been exposed to the street drug contaminant 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP). Although onset of this form of parkinsonism was sudden rather than progressive, it otherwise closely mimicked pathologically and clinically Parkinson's disease, whereas other known chemical toxins caused more general neurological injury with some parkinsonian signs. The finding that the common herbicide paraquat is structurally similar to MPTP led to speculation on exposure to agricultural chemicals as major risk factors for Parkinson's disease.

Results from numerous studies on pesticide and herbicide exposure have yielded contradictory results as to risk. A high prevalence of Parkinson's disease has been found in rural areas, in areas with vegetable farming, and in areas with steel and wood pulp mills, according to data extracted from sales of antiparkinsonian drugs (Aquilonius and Hartvig., 1986; Barbeau *et al*., 1987; Rybicki *et al*., 1993). Studies of varying population size and methodology have shown rural living, well water, and farming associated with an increased risk of Parkinson's disease. When these associations were examined by Hubble *et al.* (1993) using multiple logistic regression, they were each found to be a function of pesticide exposure. Increasing years of exposure to organophosphates and certain herbicides are linked to increasing risk of Parkinson's disease in two studies (Seidler *et al*., 1996; Gorell *et al*., 1998). However, many other case-controlled studies have found no significant risk from exposure to pesticides or rural living, and chemical toxins remain far from being conclusively established risk factors (Checkoway and Nelson, 1999). Metals such as mercury and manganese have also been examined for their role in Parkinson's disease, with similarly inconclusive results.

Family studies in the 1990s have produced evidence for genetic explanations (Payami and Zareparsi, 1998). A positive family history of parkinsonian signs is associated with increased risk of Parkinson's disease, but it is still unclear

whether this is due to genetic or environmental influences (Tanner, 1994; Elbaz *et al*., 1999). Although nearly one-third of Parkinson's disease cases are familial, no clear inheritance patterns have been found other than in a very small subset of the population (Payami and Zareparsi, 1998). Autosomal dominant inheritance of Parkinson's disease appears to be rare and is particularly associated with young-onset disease. Twin studies also have not shown strict Mendelian inheritance.

More recently, theories on risk factors have centered on multifactorial explanations that include both environmental and endogenous toxins in concert with genetics. Susceptibility is thought to be heightened in individuals with impaired mitochondrial DNA or damaged antioxidative mechanisms, and current research focuses on identifying these genetic polymorphisms.

7. Possible Protective Factors

Both case-controlled and prospectively collected data consistently reveal an inverse association between risk of Parkinson's disease and smoking. Risk may be reduced by as much as 50% in smokers compared to nonsmokers (Checkoway and Nelson, 1999). Antioxidant vitamin use and tocopherol intake have also been examined in case-controlled studies for possible protective effects against Parkinson's disease; early results have shown they may decrease risk, but the studies are small (Tanner and Goldman, 1996).

V. Conclusion

Degenerative neurological disorders affecting cognition, sensory function, and motor ability are more common with advancing age. The resulting functional impairment causes impaired quality of life in older years and dependence, leading to the need for help with basic personal care. These disorders are highly associated with the need for professional long-term care services, either in the home or in an institutional setting. In the goal of making increasing life expectancy a story of successful aging more than increasing disability, research into the causes and treatments of age-related neurological disorders is of paramount importance.

References

Adams, P. F., and Benson, V. (1990). ''Current Estimates from the National Health Interview Survey, 1989.'' Vital and Health Statistics, Ser. 10: Data from the National Health Survey, No. 176, DHHS Publ. No. (PHS) 90–1504. National Center for Health Statistics, Hyattsville, MD.

U.S. General Accounting Office. (1998). ''Alzheimer's Disease: Estimates of Prevalence in the United States.'' Report to the Secretary of Health and Human Services, GAO/HEHS-98-16. U.S. Government Printing Office, Washington, DC.

American Heart Association. (1999). ''Heart and Stroke Statistical Update.'' American Heart Association, Dallas, TX.

Aquilonius, S. M., and Hartvig, P. (1986). A Swedish county with unexpectedly high ultilization of anti-parkinsonian drugs. *Acta Neurol. Scand.* **74**, 379–382.

Arfken, C. L., Salicrup, A. E., Meuer, S. M., Del Priore, L. V., Klein, R., McGill, J. B., Rucker, C. S., White, N. H., and Santiago, J. V. (1994). Development and progression of retinopathy in African Americans and whites with insulin-dependent diabetes. *Arch. Intern. Med.* **154**, 2597–2602.

Asada, J., Yamagata, Z., Kinoshita, T., Kinoshita, A., Kariya, T., Asaka, A., and Kakuma, T. (1996). Prevalence of dementia and distribution of ApoE alleles in Japanese centenarians: An almost-complete survey in Yamanashi Prefecture, Japan. *J. Am. Geriatr. Soc.* **44**, 151–155.

Babin, R. W., and Harker, L. A. (1982). The vestibular system in the elderly. *Otolaryngol. Clin. North Am.* **15**, 387–393.

Bachman, D. L., Wolf, P. A., Linn, R. T., Knoefel, J. E., Cobb, J. L., Bélanger, A. J., White, L. R., and D'Agostino, R. B. (1993). Incidence of dementia and probable Alzheimer's disease in a general population: The Framingham Study. *Neurology* **43**, 515–519.

Barbeau, A., Roy, M., Bernier, G., Camanella, G., and Paris, S. (1987). Ecogenetics of Parkinson's disease: Prevalence and environmental aspects in rural areas. *Can. J. Neurol. Sci.* **14**, 36–41.

Beard, C. M., Kokmen, E., Offord, K. P., and Kurland, L. T. (1992). Lack of association between Alzheimer's disease and education, occupation, marital status, or living arrangement. *Neurology* **42**, 2063–2068.

Beard, C. M., Kokmen, E., O'Brien, P. C., and Kurland, L. T. (1994). Are patients with Alzheimer's disease surviving longer in recent years? *Neurology* **44**, 1869–1871.

Belal, A., and Glorig, A. (1986). Dysequilibrium of aging (presbyastasis). *J. Laryngol. Otol.* **100**, 1037–1041.

Bennett, D. A., Beckett, L. A., and Murray, A. M. (1996). Prevalence of parkinsonian signs and associated mortality in a community population of older people. *N. Engl. J. Med.* **334**, 71–76.

Ben-Shlomo, Y. (1996). How far are we in understanding the cause of Parkinson's disease? *J. Neurol. Neurosurg. Psychiatry* **61**, 4–16.

Bohannon, R. W., Larkin, P. A, Cook, A. C., Gear, J., and Singer, J. (1984). Decrease in timed balance test scores with aging. *Phys. Ther.* **64**, 1067.

Bostom, A. G., Rosenberg, I. H., Silbershatz, H., Jacques, P. F., Selhub, J., D'Agostino, R. B., Wilson, P. W., and Wolf, P.A. (1999). Nonfasting plasma total homocysteine levels and stroke incidence in elderly persons: The Framingham study. *Ann. Intern. Med.* **131**, 352–355.

Bots, M. L., Looman, S. J., Koudstaal, P. J., Hofman, A., Hoes, A. W., and Grobbee, D. E. (1996). Prevalence of stroke in the general population. The Rotterdam study. *Stroke* **27**, 1499–1501.

Bower, J. H., Maraganore, D. M., McDonnell, S. K., and Rocca, W. A. (1997). Incidence of progressive supranuclear palsy and multiple system atrophy in Olmstead County, Minnesota, 1976–1990. *Neurology* **49**, 1284–1288.

Breitner, J. C. S., Gau, B. A., Welsh, K. A., Plassman, B. L., McDonald, W. M., Helms, M. J., and Anthony, J. C. (1994). Inverse association of anti-inflammatory treatments and Alzheimer's disease: Initial results of a co-twin control study. *Neurology* **44**, 227–232.

Broderick, J., Brott, T., Kothari, R., Miller, R., Khoury, J., Pancioli, A., Gebel, J., Mills, D., Minneci, L., and Shukla, R. (1998). The greater Cincinnati/northern Kentucky Stroke Study: Preliminary first-ever and total incidence rates of stroke among blacks. *Stroke* **29**, 415–421.

Broderick, J. P., Phillips, S. J., Whisnant, J. P., O'Fallon, W. M., and Bergstralh, E. J. (1989). Incidence rates of stroke in the eighties: The end of the decline of stroke? *Stroke* **20**, 577–582.

Brookmeyer, R., Gray, S., and Kawas, C. (1998). Projections of Alzheimer's disease in the United States and the public health impact of delaying disease onset. *Am. J. Public Health* **88**, 1337–1342.

Brown, R. D., Whisnant, J. P., Sicks, J. D., O'Fallon, W. M, and Wiebers, D. O. (1996). Stroke incidence, prevalence, and survival: Secular trends in Rochester, Minnesota, through 1989. *Stroke* **27**, 373–380.

Brown, R. D., Ransom, J., and Hass, S. (1999). Use of nursing home after stroke and dependence on stroke severity. *Stroke* **30**, 924–929.

Brust, J. C. (1988). Vascular dementia is overdiagnosed. *Arch. Neurol. (Chicago)* **45**, 799–801.

Caplan, L. (1997). Cerebrovascular disease and stroke. *In* ''Geriatric Medicine'' (C. K. Cassel *et al*., eds.), 3rd ed., p. 923–938. Springer-Verlag New York.

Chandra, V., Kokmen, E., Schoenberg, B., and Beard, C. M. (1989). Head trauma with loss of consciousness as a risk factor for Alzheimer's disease. *Neurology* **39**, 1576–1578.

Checkoway, H., and Nelson, L. M. (1999). Epidemiological approaches to the study of Parkinson's disease. *Epidemiology* **10**, 327–336.

Cohen, C. I., and Magai, C. (1999). Racial differences in neuropsychiatric symptoms among dementia outpatients. *Am. J. Geriatr. Psychiatry* **7**, 57–63.

Coles, R. R. A., Davis, A. C., and Haggard, M. P. (1987). Epidemiology of tinnitus. *In* "Tinnitus" (J. W. Hazell, ed.), pp. 46–70. Churchill-Livingstone, Edinburgh.

Copeland, J. R., Gurland, B. J., Dewey, M. E., Kelleher, M. J., Smith, A. M., and Davidson, I. A. (1987). Is there more dementia, depression, and neurosis in New York? A comparative study of the elderly in New York and London using the computer diagnosis AGECAT. *Br. J. Psychiatry* **151**, 466–473.

Copeland, J. R. M., Davidson, I. A., Dewey, M. E., Gilmore, C., Larkin, B. A., McWilliam, C., Saunders, P. A., Scott, A., Sharma, V., and Sullivan, C. (1992). Alzheimer's disease, other dementia, depression and pseudo-dementia: Prevalence, incidence, and three-year outcome in Liverpool. *Br. J. Psychiatry* **161**, 230–239.

Cruickshanks, K. J., Wiley, T. L., Tweed, T. S., Klein, B. E., Klein, R., Mares-Perlman, J. A., and Nondahl, D. M. (1998). Prevalence of hearing loss in older adults in Beaver Dam, Wisconsin: The Epidemiology of Hearing Loss Study. *Am. J. Epidemiol.* **148**, 879–886.

de Rijk, M. C., Tzourio, C., Breteler, M. M., Dartigues, L., Amaducci, L., Lopez-Pouza, S., Manubens-Bertran, J. M., Alperovitch, A., and Rocca, W. A. (1997). Prevalence of parkinsonism and Parkinson's disease in Europe: The Europarkinson collaborative study. *J. Neurol. Neurosurg. Psychiatry* **62**, 10–15.

Doll, R. (1993). Review: Alzheimer's disease and environmental survival bias. *Age Ageing* **22**, 138–153.

Ebly, E. M., Parhad, I. M., Hogan, D. B., and Fung, T. S. (1994). Prevalence and types of dementia in the very old: Results from the Canadian Study on Health and Aging. *Neurology* **44**, 1593–1600.

Ekström, C. (1996). Prevalence of open-angle glaucoma in central Sweden. The Tierp Glaucoma Survey. *Acta Ophthalmol. Scand.* **74**, 107–112.

Elbaz, A., Grigoletto, F., Baldereschi, M., Breteler, M. M., Manubens-Bertran, J. M., Lopez-Pousa, S., Dartigues, J. F., Alperovitch, A., Tzourio, C., and Rocca, W.A. (1999). Familial aggregation of Parkinson's disease: A population-based case-control study in Europe. Europarkinson Study Group. *Neurology* **52**, 1876–1882.

Ernest, J. T. (1997). Changes and diseases of the aging eye. *In* "Geriatric Medicine" (C. K. Cassel *et al.,* eds.), 3rd ed., pp. 923–938. Springer-Verlag New York.

Evans, D. A. (1990). Estimated prevalence of Alzheimer's disease in the US. *Milbank Q.* **68**, 267–289.

Ferris, F. L. III, Fine, S. L., and Hyman, L. G. (1984). Age-related macular degeneration and blindness due to neovascular maculopathy. *Arch. Ophthalmol. (Chicago)* **102**, 1640–1642.

Fillenbaum, G. C., Heyman, A., Huber, M. S., Woodbury, M. A., Leiss, J., Schmader, K. E., Bohannon, A., and Trapp-Moen, B. (1998). The prevalence and 3-year incidence of dementia in older black and white community residents. *J. Clin. Epidemiol.* **51**, 587–595.

Fisher, C. M. (1982). Hypdrocephalus as a cause of disturbances of gait in the elderly. *Neurology* **32**, 1358–1363.

Flanagan, J. G. (1998). Glaucoma update: Epidemiology and new approaches to medical managment. *Ophthalmic Physiol. Opt.* **18**, 126–132.

Fratiglioni, L., Viitanen, M., von Strauss, E., Tontodonati, V., Herlitz, A., and Winblad, B. (1997). Very old women at highest risk of dementia and Alzheimer's disease: Incidence data from the Kungsholmen Project, Stockholm. *Neurology* **48**, 132–138.

Friedman, D. S., Katz, J., Bressler, N. M., Rahmani, B., and Tielsch, J. M. (1999). Racial differences in the prevalence of age-related macular degeneration: The Baltimore Eye Survey. *Ophthalmology* **106**, 1049–1055.

Ganguli, M., and Rodriguez, E. G. (1999). Reporting of dementia on death certificates: A community study. *J. Am. Geriatr. Soc.* **47**, 842–849.

Gao, S., Hendrie, H. C., Hall, K.S., and Hui, S. (1998). The relationships between age, sex, and the incidence of dementia and Alzheimer's disease: A meta-analysis. *Arch. Gen. Psychiatry* **55**, 809–815.

Gillum, R. F. (1999). Stroke mortality in blacks. Disturbing trends. *Stroke* **30**, 1711–1715.

Glaucoma Foundation, Inc. (1997). About glaucoma. [Online]. Available: http://www.glaucomafoundation.org/info [1998, December].

Goldberg, J., Flowerdew, G., Smith, E., Brody, J. A., and Tso, M. O. (1988). Factors associated with age-related macular degeneration. *Am. J. Epidemiol.* **128**, 700–710.

Gomez-Tortosa, E., Ingraham, A. O., Irizarry, M. C., and Hyman, B. T. (1998). Dementia with Lewy body disease. *J. Am. Geriatr. Soc.* **46**, 1449–1458.

Gorelick, P. B. (1997). Status of risk factors for dementia associated with stroke. *Stroke* **28**, 459–463.

Gorell, J. M., Johnson, C. C., Rybicki, B. A. Peterson, E. L., and Richardson, R. J. (1998). The risk of Parkinson's disease with exposure to pesticides, farming, well water, and rural living. *Neurology* **50**, 1346–1350.

Graham, D. I., Gentleman, S. M., Nicoll, J. A., Royston, M. C., McKenzie, J. E., Roberts, G. W., and Griffin, W. S. (1996). Altered beta-APP metabolism after head injury and its relationship to the aetiology of Alzheimer's disease. *Acta Neurochir., Suppl.* **66**, 96–102.

Haskell, S. G, Richardson, E. D, and Horwitz, R. I. (1997). The effect of estrogen replacement therapy on cognitive function in women: A critical review of the literature. *J. Clin. Epidemiol.* **50**, 1249–1264.

Hebert, L., Scherr, P. A., Beckett, L. A., Albert, M. S., Pilgrim, D. M., Chown, M. J., Funkenstein, H. H., and Evans, D. A. (1995). Age-specific incidence of Alzheimer's disease in a community population. *JAMA, J. Am. Med. Assoc.* **273**, 1343–1359.

Hendrie, H. C., Osuntokun, B. O., Hall, K. S., Ogunniyi, A. O., Hui, S. L., Unverzagt, F. W., Gureje, O., Rodenberg, C. A., Baiyewu, O., and Musick, B. S. (1995). Prevalence of Alzheimer's disease and dementia in two communities: Nigerian Africans and African Americans. *Am. J. Psychiatry* **152**, 1485–1492.

Herbst, K. G., and Humphrey, C. (1980). Hearing impairment and mental state in the elderly living at home. *Br. Med. J.* **281**, 903.

Heros, R. C., Camarata, P. J., and Latchaw, R. E. (1997). Brain attack. Introduction. *Neurosurg Clin. North Am.* **8**, 135–144.

Heyman, A., Fillenbaum, G., Prosnitz, B., Raiford, K., Burchett, B., and Clark, C. (1991). Estimated prevalence of dementia among elderly black and white community residents. *Arch. Neurol. (Chicago)* **48**, 594–598.

Holmes, C., Cairns, N., Lantos, P., and Mann, A. (1999). Validity of current clinical criteria for Alzheimer's disease, vascular dementia, and dementia with Lewy bodies. *Br. J. Psychiatry* **174**, 45–50.

Horak, F. B., Shupert, C. L., and Mirka, A. (1989). Components of postural dyscontrol in the elderly: A review. *Neurobiol. Aging* **10**, 727–738.

Horner, R. (1991). Racial variations in ischemic stroke-related physical and functional impairments. *Stroke* **22**, 1497–1501.

Howard, G., Anderson, R., Sorlie, P., Andrews, V., Backlund, E., and Burke, G. L. (1994). Ethnic differences in stroke mortality between non-Hispanic whites, Hispanic whites, and blacks. *Stroke* **25**, 2120–2125.

Hoyert, D. L., Kochanek, K. D., and Murphy, S. L. (1999). "Deaths: Final Data for 1997," National Vital Statistics Report, Vol. 47(19). National Center for Health Statistics, Washington, DC.

Hubble, J. P., Cao, T., Hassanein, R. E., Neuberger, J. S., and Koller, W. C. (1993). Risk factors for Parkinson's disease. *Neurology* **43**, 1693–1697.

Hughes, A. J. (1997). Clinicopathological aspects of Parkinson's disease. *Eur. Neurol.* **38**(Suppl. 2), 13–20.

Hughes, A. J., Daniel, S. E., Kilford, L., and Lees, A. J. (1992). Accuracy of clinical diagnosis of idiopathic Parkinson's disease: A clinico-

pathological study of 100 cases. *J. Neurol. Neurosurg. Psychiatry* **55**, 181–184.

Hull, R. H. (1995) "Hearing in Aging." Singular Publishing, San Diego, CA.

Hyman, L. (1992). Epidemiology of age-related macular degeneration. *In* "Age Related Macular Degeneration: Principles and Practice" (G. R. Hampton and P. T. Nelson, eds.), pp. 3–35. Raven Press, New York.

Jacquim, H., Commenges, D., Letanneur, L., Barberger-Gateau, P., and Dartigues, J. F. (1994). Components of drinking water and risk of cognitive impairment in the elderly. *Am. J. Epidemiol.* **139**, 48–57.

Jenkyn, L. R., and Reeves, A. G. (1981). Neurologic signs in uncomplicated aging (senescence). *Semin. Neurol.* **1**, 21–30.

Jonsson, R., and Rosenhall, U. (1998). Hearing in advanced age: A study of presbyacusis in 85-, 88- and 90-year old people. *Audiology* **37**, 207–218.

Jorm, A. F. (1990). "The Epidemiology of Alzheimer's Disease and Related Disorders." Chapman & Hall, London.

Jorm, A. F. (1991). Cross-national comparison of the occurrence of Alzheimer's disease and vascular dementia. *Eur. Arch. Psychiatry Clin. Neurosci.* **240**, 218–222.

Jorm, A. F., Korten, A. E., and Henderson, A. S. (1987). The prevalence of dementia: A quantitative integration of the literature. *Acta Psychiatr. Scand.* **76**, 465–479.

Joshipura, K. J., Ascherio, A., Manson, J. E., Stampfer, M. J., Rimm, E. B., Speizer, F. E., Hennekens, C. H., Spiegelman, D., and Willett, W. C. (1999). Fruit and veg intake in relation to risk of ischemic stroke. *JAMA J. Am. Med. Assoc.* **282**, 1233–1239.

Kahn, H., and Hiller, R. (1974). Blindness caused by diabetic retinopathy. *Am. J. Ophthalmol.* **78**, 58–67.

Kahn, H. A., Leibowitz, H. M., Ganley, J. P., Kini, M. M., Colton, T., Nickerson, R. S., and Dawber, T. R. (1977a). The Framingham Eye Study. I. Outline and major prevalence findings. *Am. J. Epidemiol.* **106**, 17–32.

Kahn, H. A., Leibowitz, H. M., Ganley, J. P., Kini, M. M., Colton, T., Nickerson, R. S., and Dawber, T. R. (1977b). The Framingham Eye Study. II. Association of ophthalmic pathology with single variables previously measured in the Framingham Heart Study. *Am. J. Epidemiol.* **106**, 33–41.

Katsarkas, A. (1994). Dizziness in aging: A retrospective study of 1194 cases. *Otolaryngol. Head Neck Surg.* **110**, 296–301.

Kennedy, R., and Clemis, J. D. (1990). The geriatric auditory and vestibular systems. *Otolaryngol. Clin. North Am.* **23**, 1075–1078.

Klein, B. E., and Klein, R. (1982). Cataracts and macular degeneration in older Americans. *Arch. Ophthalmol. (Chicago)* **100**, 571–573.

Klein, B. E., Klein, R., Sponsel, W. E., Franke, T., Cantor, L. B., Martone, J., and Menage, M. J. (1992). Prevalence of glaucoma. The Beaver Dam Eye Study. *Ophthalmology* **99**, 1499–1504.

Klein, R., Klein, B. E., Jensen, S. C., Moss, S. E., and Cruickshanks, K. J. (1994). The relation of socioeconomic factors to age-related cataract, maculopathy, and impaired vision. The Beaver Dam Eye Study. *Ophthalmology* **101**, 1969–1979.

Kokmen, E., Chandra, V., and Schoenberg, B. S. (1988). Trends in incidence of dementing illness in Rochester, Minnesota in three quinquennial periods. *Neurology* **38**, 975–980.

Kokmen, E., Beard, C. M., O'Brien, P. C., Offord, K. P., and Kurland, L. T. (1993). Is the incidence of dementing illness changing? A 25-year time trend study in Rochester, Minnesota (1960–1984). *Neurology* **43**, 1887–1892.

Kuhlemeier, K. V., and Stiens, S. A. (1994). Racial disparities in severity of cerebrovascular events. *Stroke* **25**, 2126–2131.

Lackland, D. T., Egan, B. M., and Jones, P. J. (1999). Impact of nativity and race on stroke belt mortality. *Hypertension* **34**, 57–62.

Lanska, D. J. (1997). The geographic distribution of Parkinson's disease mortality in the United States. *J. Neurol. Sci.* **150**, 63–70.

Lee, E. T., Lee, V. S., Lu, M., and Russell, D. (1992a). Development of proliferative diabetic retinopathy in NIDDM: A follow-up study of American Indians in Oklahoma. *Diabetes* **41**, 359–367.

Lee, E. T., Lee, V. S., Kingsley, R. M., Lu, M., Russell, D., Asal, N. R., Wilkinson, C. P., and Bradford, R. H., Jr. (1992b). Diabetic retinopathy in Oklahoma Indians with NIDDM: Incidence and risk factors. *Diabetes Care* **15**, 1620–1627.

Letenneur, L., Commenges, R. D., Dartigues, J. F., and Barberger-Gateau, P. (1994). Incidence of dementia and Alzheimer's disease in elderly community residents of south-western France. *Int. J. Epidemiol.* **23**, 1256–1261.

Letenneur, L., Gilleron, V., and Commenges, D. (1999). Are sex and education level independent predictors of dementia and Alzheimer's disease? Incidence data from the PAQUID project. *J. Neurol., Neurosurg. Psychiatry* **66**, 177–183.

Leys, D., Pasquier, F., and Parnetti, L. (1998). Epidemiology of vascular dementia. *Haemostasis* **28**, 134–150.

Li, G., Shen, Y. C., Chen, C. H., Zhau, Y. W., Li, S. R., and Lu, M. (1991). A three-year follow-up study of age-related dementia in an urban area of Beijing. *Acta Psychiatr. Scand.* **83**, 99–104.

Lighthouse, Inc. (1995). "The Lighthouse National Survey on Vision Loss: The Experience, Attitudes, and Knowledge of Middle-aged and Older Americans." Lighthouse, Inc., New York.

Lin, R. T., Lai, C. T., Liu, C. K., Yen, Y. Y., and Howng, S. L. (1998). Prevalence and subtypes of dementia in southern Taiwan: Impact of age, sex, education, and urbanization. *J. Neurol. Sci.* **160**, 67–75.

Lindsay, J., Herbert, R., and Rockwood, K. (1997). The Canadian Study of Health and Aging: Risk factors for vascular dementia. *Stroke* **28**, 526–530.

Litvan, I., and Hutton, M. (1998). Clinical and genetic aspects of progressive supranuclear palsy. *J. Geriatr. Psychiatry Neurol.* **11**, 107–114.

Lord, S. R., Russell, D. C., and Webster, I. W. (1991). Postural stability and associated physiological factors in a population of aged persons. *J. Gerontol.* **46**, M69–M76.

Malmagren, R., Bamford, J., Warlow, C., Snadercock, P., and Slattery, J. (1989). Projecting the number of patients with first-ever strokes and patients newly handicapped by stroke in England and Wales. *Br. Med. J.* **298**, 656–660.

Mayeux, R., Marker, K., Cote, L. J., Denaro, J., Heminegildo, N., Mejia, H., Tang, M. X., Lantigua, R., Wilder, D., Gurland, B., *et al*. (1995). The frequency of idiopathic Parkinson's disease by age, ethnic group, and sex in Northern Manhattan, 1988–1993. *Am. J. Epidemiol.* **142**, 820–827.

McKeith, L. G., Galasko, D., Kosaka, K., Perry, E. K., Dickson, D. W., Hansen, L. A., Salmon, D. P., Lowe, J., Mirra, S. S., Byrne, E. J., Lennox, G., Quinn, N. P., Edwardson, J. A., Ince, P. G., Bergeron, C., Burns, A., Miller, B. L., Lovestone, S., Collerton, D., Jansen, E. N., Ballard, C., de Vos, R. A., Wilcock, G. K., Jellinger, K. A., and Perry, R. H. (1996). Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): Report of the consortium on DLB international workshop. *Neurology* **47**, 1113–1124.

Mhoon, E. E. (1997). Otologic changes and disorders. *In* "Geriatric Medicine" (C. K. Cassel *et al*., eds.), 3rd ed., pp. 923–938. Springer-Verlag New York.

Mitchell, P., Smith, W., Attebo, K., and Healey, P. R. (1996). Prevalence of open-angle glaucoma in Australia. The Blue Mountain Eye Study. *Opthalmology* **103**, 1661–1669.

Mittelmark, M. B., Psaty, B. M., Rautaharju, P. M., Fried, L. P., Borhani, N. O., Tracy, R. P., Gardin, J. M., and O'Leary, D. H. (1993). Prevalence of cardiovascular disease among older adults: The Cardiovascular Health Study. *Am. J. Epidemiol.* **137**, 311–317.

Mojon, D. S., Hess, C. W., Goldblum, D., Fleischhauer, J., Koerner, F., Bassetti, C., and Mathis, J. (1999). High prevalence of glaucoma in patients with sleep apnea syndrome. *Ophthalmology* **106**, 1009–1012.

Monthly Vital Statistics Report. (1997). "Report of Final Monthly Statistics, 1995," Vol. 45, No. 11(S)2. National Center for Health Statistics, Washington, DC.

Morbidity and Mortality. (1999). Decline in deaths from heart disease and stroke—United States, 1900–1999 *Morbid. Mortal. Wkly. Rep.* **48**, 649–656.

Moscicki, E. K., Elkins, E. F., Baum, H. M., and McNamara, P. M. (1985). Hearing loss in the elderly: An epidemiologic study of the Framingham Heart Study cohort. *Ear Hear* **6**, 184–190.

National Institute on Aging. (1998). ''Progress Report on Alzheimer's Disease,'' NIH Publ. No. 99-3616. National Institute on Aging, Washington, DC.

National Institutes of Health. (1994). ''Parkinson's Disease: Hope through Research.'' National Institute of Neurological Disorders and Stroke, Bethesda, MD.

National Institutes of Health. (1998). ''Chartbook on Cardiovascular, Lung and Blood Diseases.'' NHLBI, Washington, DC.

National Institutes of Health (NIH). (1999). ''Stroke: Hope through Research,'' NIH Publ. No. 99-2222. National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD.

National Society to Prevent Blindness. (1980). ''Vision Problems in the U.S. Data Analysis, Definitions, Data Sources, Detailed Data Tables, Analysis, Interpretation.'' National Society to Prevent Blindness, New York.

National Stroke Association. (1999). ''Stroke/ Brain Attack Briefing,'' p. 5. National Stroke Association, New York.

Nilsson, L. V. (1984). Incidence of severe dementia in an urban sample followed from 70–79 years of age. *Acta Psychiatr. Scand.* **70**, 478–486.

Nishihara, Y., and Ishii, N. (1986). Pathological study of demented old people in Japan. *Kyushu Neuro-psychiatry* **31**, 41–45.

Nolan, K. A., Lino, M. M., Seligmann, A. W., and Blass, J. P. (1998). Absence of vascular dementia in an autopsy series from a dementia clinic. *J. Am. Geriatr. Soc.* **46**, 597–604.

Nutt, J. G. (1997). Abnormalities of posture and movement. *In* ''Geriatric Medicine'' (C. K. Cassel *et al*., eds.), 3rd ed., pp. 923–938. Springer-Verlag New York.

Nyenhuis, D. L., and Gorelick, P. B. (1998). Vascular dementia: A contemporary review of epidemiology, diagnosis, prevention, and treatment. *J. Am. Geriatr. Soc.* **46**, 1437–1448.

Ojeda, V., Mastaglia, F. L., and Kakulas, B. A. (1986). Causes of organic dementia: A necropsy survey of 60 cases. *Med. J. Aust.* **145**, 69–71.

O'Mahony, D., Denton, J., Templar, J., O'Hara, M., Day, J. P., Murphy, S., Walsh, J. B., and Coakley, D. (1995). Bone aluminum content in Alzheimer's disease. *Dementia* **6**, 69–72.

O'Shea, J. G. (1998). Age-related macular degeneration. *Postgrad. Med. J.* **74**, 203–207.

Osuntokum, B. O., Adeuja, A. O., Schoenberg, B. S., Bademosi, O., Nottidge, V. A., Ollumide, A. O., Ige, O., Yaria, F., and Bolis, C. L. (1987). Neurological disorders in Nigerian Africans: A community-based study. *Acta Neurol. Scand.* **75**, 13–21.

Papka, M., Rubio, A., and Schiffer, R. B. (1998). A review of Lewy body disease, an emerging concept of cortical dementia. *J. Neuropsychiatry Clin. Neurosci.* **10**, 267–279.

Payami, H., and Zareparsi, S. (1998). Genetic epidemiology of Parkinson's disease. *J. Geriatr. Psychiatry Neurol.* **11**, 98–106.

Perls, T. T., Silver, M. H., and Lauerman, J. F. (1999). ''Living to 100.'' Basic Books, New York.

Potvin, A. R., Syndulko, K., Tourtellotte, W. W., Lemmon, J. A., and Potvin, J. H. (1980). Human neurologic function and the aging process. *J. Am. Geriatr. Soc*. **28**, 1.

Poungvarin, N. (1998). Stroke in the developing world. *Lancet* **352**(Suppl. III), 19–22.

Prencipe, M., Ferretti, C., and Casini, A. (1997). Stroke, disability, dementia: Results of a population survey. *Stroke* **28**, 531–536.

Prevent Blindness America. (1994). ''Vision Problems in the United States.'' Prevent Blindness America, Schaumburg, IL.

Richards, M., and Chaudhuri, K. R. (1996). Parkinson's disease in populations of African origin: A review. *Neuroepidemiology* **15**, 214–221.

Ries, P. W. (1985). Demography of hearing loss. *In* ''Adjustment to Adult Hearing Loss'' (H. Orlans, ed.), CA. San Diego, Vol. 6, pp. 184–90. College Hill Press.

Rifat, S. L., Eastwood, M. R., McLachlan, D. R., and Corey, P. N. (1990). Effect of exposure of miners to aluminum powder. *Lancet* **336**, 1162–1165.

Rosenberg, T., and Klie, F. (1996). The incidence of registered blindness caused by age-related macular degeneration. *Acta Ophthalmol. Scand.* **74**, 399–402.

Rubenstein, L. Z., Josephson, K. R., and Robbins, A. S. (1994). Falls in the nursing home. *Ann. Intern. Med.* **121**, 442–451.

Rybicki, B. A., Johnson, C. C., Uman, J., and Gorell, J. M. (1993). Parkinson's disease mortality and the industrial use of heavy metals in Michingan. *Movement Disord.* **8**, 87–92.

Sacco, R. L. (1994). Ischemic stroke. *In* ''Handbook of Neuroepidemiology'' (P. B. Gorelick and M. Alter, eds.), pp. 77–121. Dekker, New York.

Sacco, R. L., Boden-Albala, B., Gan, R., Chen, X., Kargman, D.E., Shea, S., Paik, M. C., Hauser, W. A., and the Northern Manhattan Stroke Collaborators. (1998). Stroke Incidence among White, Black, and Hispanic Residents of an Urban Community. *Am. J. Epidemiol.* **147**, 259–268.

Sacco, R. L., Hauser, W. A., and Mohr, J. P. (1991). Hospitalized stroke in blacks and Hispanics in northern Manhattan. *Stroke* **22**, 1491–1496.

Sacco, R. L., Wolf, P. A., and Gorelick, P. B. (1999). Risk factors and their management for stroke prevention: Outlook for 1999 and beyond. *Neurology* **53**(Suppl.14), S15–S24.

Sanchez-Thorin, J. C. (1998). The epidemiology of diabetes mellitus and diabetic retinopathy. *In* ''Ophthalmic Complications of Diabetes Mellitus'' (J. C. Sanchez-Thorin, ed.), Int. Ophthalmol. Clin., Vol. 38 Lippincott-Raven, Philadelphia.

Sattin, R. W. (1992). Falls among older persons: A public health perspective. *Annu. Rev. Public Health* **13**, 489–508.

Schachat, A. P., Hyman, L., Leske, M. C., Connell, A. M., and Wu, S. Y. (1995). Features of age-related macular degeneration in a black population. The Barbados Eye Study Group. *Arch. Ophthalmol. (Chicago)* **113**, 728–735.

Schappert, S. M. (1995). ''Office Visits to Neurologists: United States, 1991–92,'' No. 267. Advance Data, Vital and Health Statistics of the Centers for Disease Control and Prevention/National Center for Health Statistics, U.S. Department of Health and Human Services; Washington, DC.

Schmeidler, E., and Halfmann, D. (1998). Race and ethnicity of persons with visual impairments; education of children with disabilities; and living arrangements of older Americans. *J. Visual Impairment Blindness* **92**, 539–540.

Schoenberg, B. S., Anderson, D. W., and Haerer, A. F. (1985). Severe dementia: Prevalence and clinical features in a biracial US population. *Arch. Neurol. (Chicago)* **42**, 740–743.

Schoenberg, B. S., Osuntokun, B. O., Adeuja, A. O., Bademosi, O., Nottidge, V., Anderson, D. W., and Haerer, A. F. (1998). Comparison of the prevalence of Parkinson's disease in black populations in the rural United States and in rural Nigeria: Door-to-door community studies. *Neurology* **38**, 645–646.

Schofield, P. W., Tang, M., Marder, K., Bell, K., Dooneief, G., Chun, M., Sano, M., Stern, Y., and Mayeux, R. (1997). Alzheimer's disease after remote head injury: An incidence study. *J. Neurol., Neurosurg. Psychiatry* **62**, 119–124.

Schupf, N., Kapell, D., Lee, J. H., Ottman, R., and Mayeux, R. (1994). Increased risk of Alzheimer's disease in mothers of adults with Down's syndrome. *Lancet* **344**, 353–356.

Seidler, A., Hellenbrand, W., Robra, B. P., Vieregge, P., Nischan, P., Joerg, J., Oertel, W. H., Ulm, G., and Schneider, E. (1996). Possible environmental, occupational, and other etiologic factors for Parkinson's disease: A case-control study in Germany. *Neurology* **46**, 1275–1284.

Shock, N. W., Greulich, R. C, Andres, R., Arenberg, D., Costa, P. T., Jr., Lakotta, E. G., and Tobin, J. D. (1984). *"Normal Human Aging: The*

Baltimore Longitudinal Study of Aging," NIH Publ. 84-2450. U.S. Government Printing Office, Washington, DC.

Sloane, P., Blazer, D., and George, L.K. (1989). Dizziness in a community elderly population. *J. Am. Geriatr. Soc.* **37**, 101–108.

Small, G. W., Rabins, P. V., Barry, P. B., Buckholtz N. S., DeKosky, S. T., Ferris, S. H., Finkel, S. I., Gwyther, L. P., Khachaturian, Z. S., Lebowitz, B. D., McRae, T. D., Morris, J. C., Oakley, F., Schneider, L. S., Streim, J. E., Sunderland, T., Teri, L. A., and Tune, L. E. (1997). Diagnosis and treatment of Alzheimer disease and related disorders: Consensus statement of the American Association for Geriatric Psychiatry, the Alzheimer's Association, and the American Geriatrics Society. *JAMA, J. Am. Med. Assoc.* **278**, 1363–1371.

Sobel, E., Louhija, J., Sulkava, R., Davanipour, Z., Kontala, K., Miettinen, H., Tikkanen, M., Kainulainen, K., and Tilvis, R. (1995). Lack of association of apolipoprotein E allele epsilon 4 with late-onset Alzheimer's disease among Finnish centenarians. *Neurology* **45**, 903–907.

Steiner, A., and Neu, C. R. (1993). "Monitoring the Changes in Use of Medicare Post-hospital Services." UCLA-Harvard Center for Health Care Financing Policy Research, Santa Monica, CA.

Sudlow, C. L., and Warlow, C. P. (1997). Comparable studies of the incidence of stroke and its pathological types: Results from an international collaboration. *Stroke* **28**, 491–499.

Svenson, L. W., Platt, G. H., and Woodhead, S. E. (1993). Geographic variations in the prevalence rates of Parkinson's disease in Alberta. *Can. J. Neurol. Sci.* **20**, 307–311.

Systolic Hypertension in the Elderly Program (SHEP) Cooperative Research Group. (1991). Prevention of stroke by antihypertensive drug treatment in older persons with isolated systolic hypertension: Final results of the Systolic Hypertension in the Elderly Program (SHEP). *JAMA, J. Am. Med. Assoc.* **265**, 3255–3264.

Tang, M. X., Stern, Y., and Marder, K. (1998). The APOE-e4 allele and the risk of Alzheimer's disease among African Americans, Whites, and Hispanics. *JAMA, J. Am. Med. Assoc.* **279**, 751–755.

Tanner, C. M. (1994). Parkinson's disease. *In* "Handbook of Neuro-epidemiology" (P. B. Gorelick and M. Alter, eds.), pp.253–280. Dekker, New York.

Tanner, C. M., and Goldman, S. M. (1996). Epidemiology of Parkinson's disease. *Neurol. Clin.* **14**, 317–335.

Tanner, C. M., Thelen, J. A., Offord, K .P., Rademacher, D., Goetz, C. G., and Kurland, L. T. (1992). Parkinson's disease incidence in Olmsted County, MN: 1935–1988. *Neurology* **42**(Suppl. 3), 194.

Ten Great Public Health Achievements—United States (1900–1999). *Morbid Mortal Wkly. Rep.* **48**, 241–243.

Thal, L. J., Grundman, M., and Klauber, M. R. (1988). Dementia: Characteristics of a referral population and factors associated with progression. *Neurology* **38**, 1083–1090.

Thompson, D. W., and Furlan, A. J. (1997). Clinical epidemiology of stroke. *Neurosurg. Clin. North. Am.* **8**, 265–269.

Tielsch, J. M., Sommer, A., Witt, K., Katz, J., and Royall, R. M. (1990). Blindness and visual impairment in an American urban population: The Baltimore Eye Survey. *Arch. Ophthalmol. (Chicago)* **108**, 286–290.

Tinetti, M. E. (1997). Falls. *In* "Geriatric Medicine" (C. K. Cassel *et al*., eds.), 3rd ed., pp. 923–938. Springer-Verlag, New York.

Tinetti, M. E., Speechley M, and Ginter, S. F. (1988). Risk factors for falls among elderly persons living in the community. *N. Engl. J. Med.* **319**, 1701–1707.

Treves, T., Korczyn, A. D., Zilber, N., Kahana, E., Leibowitz, Y., Alter, M., and Schoenberg, B. S. (1986). Presenile dementia in Israel. *Arch. Neurol. (Chicago)* **43**, 26–29.

Udea, K., Hasuo, Y., and Fujishima, M. (1992). Prevalence and etiology of dementia in a Japanese community. *Stroke* **23**, 798–803.

U.S. Census Bureau. Population Estimates Program, Population Division. U.S. Census Bureau, Washington, DC. [Online.] Available:*http://www.census.gov*

U.S. Department of Health and Human Services, (USDHHS). (1999). "Health, United States, 1999. Health and Aging Chartbook," (PHS) 99-1232-1. Centers for Disease Control and Prevention/National Center for Health Statistics, USDHHS, Washington, DC.

van Duijin, C. M. (1996). Epidemiology of the dementias: Recent developments and new approaches. *J. Neurol., Neurosurg. Psychiatry* **60**, 478–488.

Viitanen, M., Eriksson, S., and Asplund, K. (1988). Risk of recurrent stroke, myocardial infarction and epilepsy during long-term follow-up after stroke. *Eur. Neurol.* **28**, 227–231.

von Strauss, E., Viitanen, M., De Ronchi, D., Winblad, B., and Fratiglioni, L. (1999). Aging and the occurrence of dementia: Findings from a population-based cohort with a large sample of nonagenarians. *Arch. Neurol. (Chicago)* **56**, 587–592.

Wade, A. M., Mirsen, T. R., Hachinski, V. C., Fisman, M., Lau, C., and Merskey, H. (1987). The clinical diagnosis of Alzheimer's disease. *Arch. Neurol. (Chicago)* **44**, 24–29.

Waite, L. M., Broe, G. A., Creasey, H., Grayson, D. A., Cullen, J. S., O'Toole, B., Edelbrock, D., and Dobson, M. (1997). Neurodegenerative and other chronic disorders among people aged 75 and over in the community. *Med. J. Aust.* **167**, 429–432.

Wang, Y. (1991). The incidence and prevalence of Parkinson's disease in the People's Republic of China. *Chung Hua Liu Hsing Ping Hsueh Tsa Chi* **12**, 363–365.

Warlow, C. P. (1998). Epidemiology of stroke. *Lancet* **352**(Suppl. III), 1–4.

Wensor, M. D., McCarty, C. A., Stanislavsky, Y. L., Livingston, P. M., and Taylor, H. R. (1998). The prevalence of glaucoma in the Melbourne Visual Impairment Project. *Ophthalmology* **105**, 733–739.

White, L., Petrovitch, H., Ross, G. W., Masaki, K. H., Abbott, R. D., Teng, E. L., Rodriguez, B. L., Blanchette, P. L., Havlik, R. J., Wergowske, G., Chiu, D., Foley, D. J., Murdaugh, C., and Curb, J. D. (1996). Prevalence of dementia in older Japanese-American men in Hawaii. The Honolulu-Asia Aging Study. *JAMA, J. Am Med. Assoc.* **276**, 955–960.

Wisniewski, K. E., Wisniewski, H. M., and Wen, G. Y. (1985). Occurrence of neuropathological changes and dementia of Alzheimer's disease in Down's syndrome. *Ann. Neurol.* **17**, 278–282.

Wolf, P. A. (1998). Prevention of stroke. *Lancet* **352**(Suppl. III), 15–18.

Wolf, P. A., D'Agostino, R. B., Kannel, W. B., Bonita, R., and Bélanger, A. J. (1988). Cigarette smoking as a risk factor for stroke: The Framingham Study. *JAMA, J. Am. Med. Assoc.* **259**, 1025–1029.

World Health Organization. (1989). Recommendations on stroke prevention, diagnosis, and therapy: Report of the WHO Task Force on stroke and other cerebrovascular disorders. *Stroke* **20**, 1407–1431.

Yamada, M., Sasaki, H., Mimori, Y., Kasagi, F., Sudoh, S., Ikeda, J., Hosoda, Y., Nakamura, S., and Kodama, K. (1999). Prevalence and risks of dementia in the Japanese population: RERF's adult health study Hiroshima subjects. Radiation Effects Research Foundation. *J. Am. Geriatr. Soc.* **47**, 189–195.

Yoshitake, T., Kiyohara, Y., Kato, I., Ohmura, T., Iwamoto, H., Nakayama, K., Ohmori, S., Nomiyama, K., Kawano, H., and Ueda, K. (1995). Incidence and risk factors of vascular dementia and Alzheimer's disease in a defined elderly Japanese population. The Hisayama study. *Neurology* **45**, 1161–1168.

Zapatero, M. D., Garcia de Jalon, A., Pascual, F., Calvo, M. L., Escanero, J., and Marro, A. (1995). Serum aluminum levels in Alzheimer's disease and senile dementias. *Biol. Trace Elem. Res.* **47**, 235–240.

Zhang, Z. X., and Roman, G. C. (1993). Worldwide occurrence of Parkinson's disease. *Neuroepidemiology* **12**, 195–208.

SECTION II

Memory: Neocortical and Hippocampal Functions

A. Neuropsychology of Human Aging
(CHAPTER 5)

B. Histology of Age-Related Cortical Changes in Humans
(CHAPTERS 6–8)

C. Alzheimer's Disease
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D. Non-Alzheimer Age-Associated Dementing Disorders
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E. I*n* V*ivo* Imaging of Aging Brain
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F. Biochemical Correlates of Memory Impairments
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Philip D. Harvey and Richard C. Mohs

5

Memory Changes with Aging and Dementia

I. The Concept of Different Memory Functions

Human memory is quite complex, serving many functions and with component processes that have been separated into several different, although related, types. Some of the distinctions made about memory refer to the way that information is initially acquired and others refer to the specific content of the information (i.e., semantic meanings or autobiographical information). Some of the different memory systems that have been identified interact with each other almost constantly and others appear to operate relatively independently. This chapter provides a general overview of the human memory system and describes how the various aspects of memory change during the course of human aging. These changes associated with normal aging are then compared with the much more devastating changes that occur in patients with dementing illness. Themes that run through the entire chapter are that different aspects of human memory are more or less vulnerable to the effects of aging and disease and that these changes must be understood in terms of neurobiologic changes that occur over periods of years.

II. Aging and Cognition

Many aspects of cognitive functioning change with age and, although many cognitive functions decline with age, the extent and pattern of the decline vary by both the individual and the type of function. Some aspects of cognitive functioning are uniformly performed at lower levels in individuals when they are older, relative to their own level of performance at younger ages. Other cognitive functions change very little across the life span, on average, and some functions even appear to be enhanced at later periods of life. There are considerable individual differences in the extent to which cognition changes with aging. Some individuals age ''successfully'' so that many cognitive functions remain unimpaired during aging and some may even improve (Rowe and Kahn, 1987). Other individuals may follow a more typical course of aging where some cognitive functions remain intact, such as memory for material learned in school or complex motor acts such as driving a car, while performance in other cognitive domains declines. Examples of functions that typically show age-related decline are learning new information with practice and the speed of performance of motor acts (Botwinick and Storandt, 1974; West and Crook, 1990). Some individuals manifest general declines in functioning, relative to population-based expectations, with performance in many different aspects of cognitive functioning appearing to be deteriorated relative to their previous levels of functioning. Finally, some individuals develop age-related conditions that grossly deteriorate their cognitive functioning, including the progressive dementia such as Alzheimer's disease. The pattern of age-related change described in this chapter is those that occur in most people.

Many characteristics of individuals are thought to predict cognitive functioning in late life. The potential predictors of late life cognitive functioning include socioeconomic factors, lifelong patterns of activities, educational history, current and previous psychiatric conditions such as depression or substance abuse, and medical factors. Some factors exert a transient influence on performance, such as suppression of attention or memory during episodes of dysphoria, and others, such as educational background, have more lasting effects on performance.

III. Primary and Secondary Memory

Primary memory, also referred to as ''immediate memory,'' has several important features that distinguish it from secondary (i.e., long-term) memory. Primary memory is a limited capacity memory system used to hold small amounts of information for short periods of time, often while various cognitive operations are performed on that information. Because information in primary memory is available to be manipulated,

this entire memory system is also referred to as "working memory." A variety of evidence has been collected to demonstrate that working memory has different characteristics in the visuospatial and verbal modalities (Baddeley, 1986). Working memory is utilized to hold information "on-line" or "in mind" while it used. A well-known example of working memory involves remembering a telephone number long enough to dial it. Furthermore, primary memory involves a component of adaptive forgetting. For example, if a person were unable to forget information needed only for a brief period of time, then the immediate memory buffer would be full to overflowing with old information. Thus, adaptive forgetting, which is thought to occur because new information displaces old in this limited capacity system, is a component of working memory (Baddeley, 1986).

Secondary memory is the system used to hold memory for long periods of time and is thought to be essentially permanent. Information enters the secondary memory system as a result of exposure and practice. Storage of information in secondary memory enables one to retain it over a period of delay even if attention and cognitive effort are directed elsewhere. For instance, learning a long list of words presented over several exposure trials and then recalling it after a period of delay is an example of secondary memory. Storage of information into secondary memory is influenced by a variety of processes, most notably practice or repetition. Access or retrieval of information from secondary memory can be difficult, and successful retrieval often depends on the availability of appropriate retrieval cues.

Aging is associated with several changes in performance on tasks involving primary and secondary memory. Generally, primary memory capacity remains constant with age, as shown through studies measuring immediate memory span. The capacity of immediate memory is measured with a task such as digit span in which a person is required to listen and then repeat back a list of digits that increase in number from trial to trial. Younger subjects usually have a memory span averaging about 6.5 to 6.7 digits, whereas older subjects average 6.0 to 6.5, for a difference of less than 10% (Botwinick and Storandt, 1974). However, when the task is modified from the usual laboratory version of verbally repeating the digits to a more real world task that includes dialing a phone number, a greater age difference is found (Craik, 1977). The difference between these two cognitive tasks is the move from auditory memory that simply requires the subject to repeat back the number to one involving not only verbal memory but the coordination of memory with a more complicated motor response. It is possible that the additional attention demanded by the motor component leads to a loss of digits prior to recall.

Numerous age-related differences are found with both encoding and retrieval of information in secondary memory. Significant differences are found between young and old subjects on free recall tasks that require both storage and retrieval of information. Tasks that minimize the difficulty of correct retrieval such as recognition and cued-recall tasks show considerably smaller age differences (Botwinick and Storandt, 1974; Hultch, 1975). On tasks that tax the ability to store and retrieve information in secondary memory (e.g., immediate and delayed recall of paired associates), there are significant decrements associated with increasing age (Kausler and Lair, 1966). These decrements indicate that older adults are less able to learn quickly information that exceeds short-term memory capacity and even less able to remember this information after a delay of 20 min or more. However, if older subjects were given greater practice time or allowed to pace themselves during learning rather than following the pace of the experimenter, they can perform as well as their younger counterparts (Canestrari, 1963; Cohen and Faulkner, 1983). The fact that self-paced learning or learning at a relatively slow rate does not show much age-related decline suggests that slower processing speed is at least partly responsible for much age-related decline in the learning rate (Cohen and Falkner, 1983). If the degree of initial learning is equated for young and old subjects, then the rate at which forgetting occurs over time is nearly equal for young and older persons (Albert *et al*., 1987). These latter data confirm that much of the memory deficit observed in older adults is due to learning failures (i.e., failures to store information into secondary memory). With aging the process of storing information in long-term memory slows down and this results in more memory failures, particularly for events that occurred recently.

It could be that studies of list learning and paired-associate acquisition are not the most relevant measures to employ with normal elderly. With independent living as the primary goal of most elderly individuals, there have been many studies of practical or everyday memory designed to assess the relationship of cognitive functioning and activities of daily living (ADL) skills (Fillenbaum, 1985). A study of 151 healthy subjects in three age groups (20–39 years; 40–59 years; older than 60 year) assessed practical memory (i.e., shopping lists, telephone numbers) (Cavanaugh *et al*., 1983). The main problem for the older group was remembering names and numbers or what the investigators referred to as arbitrary facts, but the ability to recall meaningful information (i.e., gist of conversations, newspaper articles) remained intact across the age groups. When old and young subjects were asked to keep daily dairies of their memory failures and use of memory aids, older subjects reported more failures (Poon *et al*., 1979). Most of the memory failures reported were forgetting of names, faces, objects, appointments, locations, addresses, and phone numbers. These tasks for which memory failures increase with age appear to depend heavily on the ability to learn new and relatively arbitrary facts, whereas memory tasks that are less subject to age-related decline involve the learning of meaningful information. The range of strategies that can be employed in learning meaningful information is somewhat greater and these tasks allow the learner to utilize information about meaning and relationships that have previously been stored in memory. Memory failures reported by older persons also tended to occur primarily when the older subjects were out of their normal routine or required to remember information that they had not used recently. By way of contrast, younger subjects tended to forget more during times of stress primarily because they experienced more stress than the older subjects.

As might be expected, the functional capacity of memory is correlated significantly both with physical health ($r=0.54$ to 0.55) and with mental health ($r=0.54$ to 0.60) (Fillenbaum, 1985). These correlations are particularly important in an elderly population where deficits in both physical and mental health are more common and hence are more likely to be

important contributors to individual differences in memory performance.

IV. Implicit and Explicit Memory

Implicit and explicit refer to the means by which information is stored and retrieved from the memory system. In explicit memory tasks, the individual stores and retrieves information from memory on a volitional, controlled access basis. An example of explicit memory is when a subject learns a set of paired words in a learning format and then is asked to recall them later. The conscious attempt to recall the information is referred to as ''explicit memory.'' In contrast, in implicit memory, conscious awareness of the attempt to store and/or recall information is not required, but a change in behavior provides evidence that learning has taken place (Graf and Schachter, 1985). An example of an implicit memory task could be that a subject is asked to learn a list of words, including the word ''three.'' Later, they are shown word stems (e.g., thr_ _) and asked to tell the experimenter the first word that comes to mind. Subjects in this situation are more likely to respond ''three'' than are subjects without prior exposure to the word three; this change in behavior is evidence of implicit memory.

As noted earlier, explicit memory functions change with normal aging, although evidence shows that implicit memory functions are less likely to deteriorate with age (Lupien *et al*., 1997). This finding may suggest that the processes of voluntary or conscious storage and retrieval from long-term storage are a major contributor to changes in memory performance with age.

V. Episodic and Semantic Memory

Episodic memory refers to the process of remembering events. The information stored about these events can be verbal, such as the content of conversations, or visuospatial, such as the recollections of faces and locations. In addition, episodic memory content includes activities and current events. Thus, both information regarding content and context are included in episodic memory. Storage of new information into episodic memory does become more difficult with aging, as this information is stored in secondary memory. The total fund of information stored in episodic memory, however, tends to hold up quite well and may actually increase with age.

Semantic memory refers to stored information about language and the meanings of words. Semantic memory is not contextually dated and this information is generally shared with others who have the same cultural background. While acquisition of new semantic knowledge may become more difficult with normal aging, the total amount of stored semantic information appears to decline little or not at all in normally aged persons (Psychological Corporation, 1997). There are many different ways to assess the intactness of semantic memory. As examples, confrontation naming refers to the ability to name objects presented to the subject, whereas fluency tests examine the ability of the subject to produce verbal output when given either a categorical (e.g., animals) or a phonological (e.g., words starting with F) rule for generating instances. One of the most common complaints of older adults is that they cannot remember the names of things, even those that they know very well. In one study, young (age range 17–23 years) and old (age range 65–85 years) healthy subjects were measured on a word retrieval task (Bowles and Poon, 1985). The stimulus was a definition of a word and the subjects were asked to name the word that had been defined. The younger subjects were superior to the older subjects on both word retrieval and response latency. Older individuals, even those who do not suffer from dementia, are often less able to name objects as compared to younger individuals. Older persons generate more circumlocutions and multiword responses than younger subjects when asked to provide a single word for a picture presented (i.e., confrontation naming; Albert *et al*., 1987; Randolph *et al*., 1993). Circumlocutions indicate that the individuals recognize the item but cannot specifically retrieve its name. These same studies demonstrate that the specificity of the age-related change to retrieval by the finding that the ability to correctly choose which of several words is the correct name for an object is unaffected by aging.

There are also age-related differences in the ability to continuously access semantic storage. Semantic access is typicallymeasured through ''verbal fluency'' procedures. In these assessments, subjects are asked to continuously generate words that either start with the same letter (phonological fluency) or are from the same conceptual category (category fluency). It is routinely found that older individuals produce fewer words than younger individuals in discrete time periods (Rosen, 1980). Older individuals do not make more errors than younger ones, indicating that they are not having problems in adhering to the task demands. One possible explanation for the reduced verbal output of older persons on tasks such as this is that it is simply a reflection of a reduction in general speed of cognitive processing. In summary, modest aging-related declines in the ability to spontaneously access semantic information are routinely found. These declines are apparently not due to an actual decrease in the total amount of information available in the semantic storage system at later ages, as recognition procedures and prompting make the performance of older individuals the same as younger ones.

VI. Declarative versus Procedural Memory

A variety of studies involving patients with specific types of brain damage indicate that there are different neurobiologic systems involved in laying down memory for facts vs laying down memory for procedures or rules for accomplishing tasks (Squire and Cohen, 1982). A review of all the evidence supporting this distinction is beyond the scope of this chapter, but the essence of it is that patients with damage to medial temporal regions of the brain have severe impairments in the ability to learn new facts (e.g., a list of words or a person's name) but have only minor impairments in the ability to learn new skills (e.g., how to read words backwards, do tracing of forms presented in a mirror). Because of the evidence that different neurobiologic systems are involved in these two kinds of learning and memory, a number of studies have looked at how these two kinds of memory are affected by aging.

Memory for facts is referred to as declarative memory. These facts can be recently learned or they can be acquired a long time ago. In the case of information learned long ago, this can be referred to as ''remote memory.'' As described previously, the rate at which new facts can be stored in memory clearly slows down with normal aging. However, the total amount of information available in remote memory (sometimes referred to as tertiary) can be relatively intact throughout the life span. Evaluation of remote memory can be conducted through recognition and/or recall of public events throughout history. Older subjects often perform better than their younger counterparts recalling events over a long period and maintain consistent levels of memory across history (Warrington and Sanders, 1971).

In contrast, procedural memory is the system used to learn and retain the ability to perform skills. An example of procedural learning would be learning the skills required to perform a new motor skill, such as tracing in a mirror. An example of procedural memory would be recalling how to ride a bicycle or to swim, if the individual had not performed either of these acts for a period of time. Other types of procedures stored in remote memory include skills learned long ago, such as the ability to read and spell. It is normal for elderly individuals to perform as well as younger individuals on these tests. A further finding of importance in this area is that the performance of elderly individuals on these tests is generally consistent with or higher than their level of previous academic achievement, i.e., older persons usually are able to read and spell at a level that is equal to a younger person with a similar educational background (Jastak, 1984). This finding indicates that some memory skills are stable over time within individuals and may even improve with exposure to new information over the life span.

VII. Other Age-Related Changes in Cognition

A. Language

A superficial assessment of the language performance of older individuals would indicate that language is well preserved throughout the life span, at least until the late 80s, because performance on the vocabulary subtest on the Wechsler Adult Intelligence Scale has been found to be consistent over time (Owen, 1953). However, there are clear findings suggesting notable age-related declines in the spontaneous production of language (Kemper, 1987). There are some additional changes in verbal skills with aging, most of which were described previously in the section on semantic memory. Lexical functioning refers to the structure of meaning and its representation in words. Intact lexical functions include the ability to access and recognize words on demand. A study of subjects in two age groups (17–23 and 65–85 years) measured performance on a ''lexical decision task'' where they were asked to determine if a briefly presented string of letters created a word (Bowles and Poon, 1985). There was no significant difference across age on either accuracy or response latency. Older and younger individuals perform equally well on tasks of word recognition reading (Jastak, 1984).

Phonologic knowledge refers to the sound-based rules of language. This skill appears to be well preserved across age (Bayles and Kazniak, 1987) except in individuals who experience stroke or other identifiable brain changes. The ability to meaningfully combine words using grammatical rules is referred to as the syntactic component of language and it is also preserved across age (Obler *et al*., 1985) unless other factors intervene.

B. Visuospatial Functioning

Visuospatial functioning requires the ability to perceive and subsequently manipulate visual information. This ability is often measured through either the production or the recognition of figures. Some decline occurs as individuals advance in age. A cross-sectional analysis of 1800 community residents age 65 and older found a decrease in visuospatial ability and speed of execution as age increased (Mazaux *et al*., 1995). There was also poorer performance among female subjects and those with lower education. Age-related declines in the ability to reproduce complex figures have been reported in other studies as well (Plude *et al*., 1986). One potential contributor to the age-related decline in performance on these tasks is that they require manipulation of items in a novel way and often with a speeded component.

While there is a decrease in visuospatial performance as an individual ages, the decline is significantly less than with demented older adults. Comparisons between healthy elderly subjects and those with mild cognitive impairment found that objective psychological measurements, such as those testing visuospatial ability, exhibited high sensitivity and specificity in distinguishing between subjects with and without a decline in cognitive functioning (Flicker *et al*., 1991).

C. Psychomotor Functions

Psychomotor functions involve the combination of precise motor responses, attention, and cognitive problem-solving abilities. Reduced motor speed as age increases has been demonstrated both in laboratory tasks and in real world environments. Older commercial drivers were found to have more accidents than younger drivers because of slower reaction times (Barrett *et al*., 1977). Even though the accident rates of pilots ages 40 to 60 are lower than those of younger pilots, the accidents older pilots had were attributable to slower response times (Birren, 1964). Response times can be improved with training in older individuals, ages 60–80, by having them play video games 2 hr a week for 7 weeks (Clark *et al*., 1987). In general, older typists perform more slowly than their younger counterparts. When highly skilled older typists exhibited performance speed that was equal to that of less skilled younger typists, the older typists made more errors. Thus, reaction time can be reduced if the skill is sufficiently practiced, but there is a possible cost of an increase in errors made due to keystroke errors (Bosman, 1993). Greater distractibility among older typists has been offered as a reason for the decline in typing skills. For example, when older and younger typists were compared after removing all distracting stimuli, there were no age-related differences (Salthouse, 1984). However, attention studies have shown that there are few age-related differences in distractibility when perceptual

difficulties such as vision or hearing are controlled (Denney and Palmer, 1981; Rabbitt, 1985). Even those who age successfully exhibit some decline in psychomotor abilities past the seventh decade of life. Those who age normally have a progressive decline on psychomotor tasks from their mid-60s. Like visuospatial abilities, psychomotor abilities decline significantly and sharply among older adults with dementia.

D. Executive Functions

Executive functions involve an individual's capacity to conceptualize, to think independently, and to utilize self-control, self-direction, and flexibility. Studies of patients with specific types of brain injury and studies using brain imaging provide evidence that the prefrontal areas of the neocortex are involved in cognitive activities of this type (Reitan and Wolfson, 1994). Describing executive functions has proved to be difficult, but the essence of these functions appears to be that they involve a sequence of planning and execution. The first step is that individuals must determine what they need or want to do and develop a global plan. The next step is to identify and organize the steps and elements needed to carry out the intention. Another element of executive functioning is the translation of intention or plan into productive activity. During this stage, there is a continuous process of evaluation of the plan, with shifts following errors in execution. Because executive functioning is thought to involve frontal lobe functioning, measures that have been proven sensitive to dysfunction of the frontal lobe are often the types of tests that are referred to as tests of executive functioning. One of the standard measures of executive functioning has been the Wisconsin Card Sorting Test (WCST; Heaton *et al*., 1993). The WCST is a multidimensional problem-solving test, where individuals are required to identify concepts, respond accordingly, and modify their concepts in response to external feedback. Because the concepts in the test change, the test also requires cognitive flexibility for successful completion. One major barrier to effective performance in executive functioning tests could be the inability to adopt a flexible approach to problem solving. Mental inflexibility is difficult to measure and is often assessed through tests of abstraction that emphasize shifts in concept formulation. A study comparing healthy subjects in the 50s, 60s, and 70s found no statistically significant differences among groups on the WCST and three other frontal lobe measures, suggesting that there is little decline on executive functioning with age (Boone *et al*., 1990). In this same domain, one more specific type of performance has been examined closely, the tendency to perseverate when asked to shift concepts. Research in this area has found that the level of perseveration found in elderly individuals was quite dependent on the tests employed (Boone *et al*., 1993). Age effects were found in 45–65-year-old subjects who had more perseveration than younger subjects (20–35 years) in planning tasks (Ardila and Rosselli, 1987; Daigenault *et al*., 1992). There have been indications that normal aging includes an increase of perseverative behaviors prior to age 65 (Nelson, 1976), and other research has suggested that the increase does not occur until after age 70 or 80 (Haaland *et al*., 1987). The more meaningful and concrete the presentation of a conceptual problem, the more older individuals will succeed (Botwinick, 1978). As a result, it appears as though there is more individual variation in executive functioning and hence more variability in age effects than in simpler aspects of cognitive functioning. This may not be surprising because of the fact that executive functioning tests also require intactness of multiple lower-level skills, including perception, attention, memory, and motor skills. Based on the variability in performance across individuals in the area of executive functioning, it is important to make clear distinctions between healthy elderly individuals and those with any early stages of brain disease. Normal aging begins to show some decline in the late 70s to early 80s, and demented individuals have a very sharp decline on executive abilities (Zec, 1993).

VIII. Cognitive Changes in Dementia

Dementia is a major public health problem, with an estimated 6 million cases in America at this time and more cases expected as the population ages. Neuropsychological assessment is a crucial component of any dementia evaluation. Both American (McKhann *et al*., 1984) and European community (CPMP Working Party, 1992) standards require neuropsychological assessment as a component of the diagnostic workup. Virtually all dementing illnesses are progressive, degenerative conditions and neuropsychological assessment is also crucial for the evaluation of progression (Katzman, 1986).

A. Definition of Dementia

Dementia is defined similarly in several different diagnostic systems (e.g., World Health Organization, 1992; American Psychiatric Association, 1994) as a condition marked by the loss of memory functions and at least one other aspect of cognitive function that is sufficiently severe to interfere with the person's ability to perform ordinary activities of daily living. To differentiate dementia from preexisting conditions such as mental retardation, the impairments must represent a decline from the patients previous level of functioning. Additional cognitive deficits, which may indicate a dementing illness include aphasia (loss of language function), apraxia (loss of the ability to perform a previously learned motor activity), agnosia (inability to recognize familiar objects), and loss of executive functions (i.e., conceptual skills, planning, and control over component skill areas). The definition of dementia also differentiates this condition from others where the loss of cognitive function is limited to a single cognitive function, such as amnesia or aphasia where loss of memory or loss of language competence is the principal change in cognitive functioning. Dementia has multiple etiologies and, whenever possible, the etiological factor is coded during the diagnostic process. In this chapter, our discussion of dementia is limited in scope and describes the key aspects of two different overall categories of dementia, cortical dementia and subcortical dementia. In addition, the typical neuropsychological profiles of the most commonly seen examples of the two types of dementia are compared to each other.

B. Cortical versus Subcortical Dementia

The constellation of impairments seen in Parkinson's disease (PD) and Huntington's disease (HD), combined with the

neuropathology of these disorders, which is found primarily in subcortical regions, has lead to an ongoing distinction between cortical and subcortical dementia (Cummings, 1986; Cummings and Benson, 1990; Rebok and Folstein, 1993). In this conception, dementias such as Alzheimer's disease (AD), which affect the cortex and, eventually, all of the cognitive functions dependent on the intact cortex, can be discriminated from dementing conditions, which impact principally on the subcortical regions. Studies have suggested that subcortical (HD) and cortical (AD) dementias can be discriminated even at the later stages of illness (Paulsen *et al*., 1995a). While there is some controversy about this distinction, in line with findings of certain types of language and other ''cortical'' abnormalities in PD (see Rebok and Folstein, 1993, for a discussion), most dementias that affect subcortical regions (e.g., HIV, HD, and PD) have similar features. The question of the validity of this distinction will continue to be debated, but there are some clear differences between the group of ''subcortical dementias'' and AD in the breadth and magnitude of impairments, especially in terms of early impairments in attention in subcortical dementia, global slowing processes in subcortical dementias even in their early stages, very salient signs of depression, and sparing of recognition memory. Accordingly, we organize our discussion of these conditions in line with this distinction.

C. Alzheimer's Disease

This disease, first reported by Alzheimer at the outset of this century, is the most common of the dementing conditions. At least half of all patients with dementia who are over the age of 65 will be found to meet criteria for AD at a postmortem assessment (Arriagada *et al*., 1992), with the proportion of cases with AD compared to other dementias increasing as the age of the patients increase (Rebok and Folstein, 1993). It has an age of onset ranging from the late 30s (rarely) to the end of life and a prevalence of as much as 6% of the current living population (Terry and Katzman, 1992). These figures are greatly increased in old age, with as much as 50% of the population over the age of 85 meeting criteria for AD (Evans *et al*., 1989). Most studies note that the course of the illness is around 10 years from the first identifiable symptom, unless the patient does not survive this period (Katzman, 1986). Risk factors for the illness are age, family history of AD, reduced educational attainment, Down syndrome, head trauma, and female gender (Cummings and Benson, 1983). The neuropathological signature of the illness includes amyloid plaques and neurofibrillary tangles, localized initially in the medial temporal cortex and hippocampus and found later in the illness in the more lateral structures of the temporal lobe, parietal cortex, and perisylvian region (Khachaturian, 1985; Huff *et al*., 1987). Plaques are irregularly shaped deposits of amyloid, whereas tangles are neurofibrillary masses that are distributed irregularly in the same general regions as plaques. The presence of these neuropathological stigmata is required for the postmortem diagnosis of AD according to all current criteria.

The clinical hallmark of AD is its progressive course. When measured with a global clinical rating scale, such as the Mini-Mental State Examination (MMSE; Folstein *et al*., 1975) or the Alzheimer's Disease Assessment Scale (ADAS; Rosen *et al*., 1984), there is an average deterioration of about 10% per year (Berg *et al*., 1987; Huff *et al*., 1987; Salmon *et al*., 1990). Thus, the expected decline in the MMSE is about 3 points per year on the average. This decline is not, however, linear. In the early and later stages of the illness the annual decline is considerably less than in the middle, leading to a curvilinear course (Morris *et al*., 1993). As a result, the expected annual loss of functioning measured globally depends considerably on the severity of illness at the time of first assessment.

There are multiple aspects of cognitive impairment seen in AD, but the presence and severity of impairment in each cognitive domain depend on the stage of illness. In fact, as the illness progresses, every cognitive function of the cerebral cortex becomes impaired eventually. Some of the cognitive impairments are seen very early on in the course of the illness and others appear later (Welsh *et al*., 1992). Of these cognitive impairments, some of them become progressively worse over time in the illness and others appear to be static after their appearance (Morris *et al*., 1993). The first measurable cognitive sign of AD is a profound deficit in secondary memory, specifically in learning and delayed recall of facts, either with or without intervening distracting information. This empirical finding is not surprising because the first subjective sign of the illness is forgetfulness and problems in learning new information. This deficit is substantial, in that patients with mild AD (MMSE > 23) have been found to learn as little as three words over the course of three exposures to a 10-item serial word list, whereas nondemented persons matched for age and education to these patients learned eight words. At delayed recall, the AD patients recalled less than one word on the average, whereas normals retained seven out of eight words that they learned (Welsh *et al*., 1991). Deficits in delayed recall do not progress with continued overall worsening of the illness, whereas new learning appears to worsen steadily with increases in overall severity of impairment (Welsh *et al*., 1992).

Following the impairments in learning and memory that appear at the earliest stages of the illness, verbal skills such as confrontation naming ability and verbal fluency appear to worsen next and progress with a roughly linear course. The next aspect of functioning to deteriorate is praxis and spatial-perceptual operations. These declines appear to be linear as well (Welsh *et al*., 1992; Morris *et al*., 1993). Thus, the curvilinear course of AD, as measured by global scales such as the MMSE, may be determined by the scaling properties of the assessment instruments as well as by the actual course of the illness. The ''accelerated'' pace of cognitive decline in the middle of the illness may be a function of the fact that more of the cognitive functions measured by the instrument are in decline in the middle of the illness than at the very early stages (where only delayed recall and verbal learning are impaired) and the late stages (where many cognitive tests are now manifesting floor effects). It must be noted that there is heterogeneity in AD across patients and these descriptions are based on average statements about large samples of patients.

There are many other aspects of cognitive assessment in AD that merit attention. Executive functioning impairment appears early in the illness. It is possible that deficits in executive functioning are exacerbated, or possibly even caused, by deficits in the cognitive components controlled by executive functions.

For instance, a profound deficit in working memory would make adequate performance on an executive functioning test such as the WCST essentially impossible. As in many amnestic conditions, procedural learning (i.e., learning of motor skills) appears to be more intact than declarative learning. Similarly, implicit memory functions (i.e., memory aided by prompts or cues) appear more intact than explicit memory, although still impaired (Zec, 1993). Recognition memory (i.e., the ability to identify previously presented information) is not spared, in contrast to findings in specific frontal lobe damage (Freedman, 1990) or HD (see later). Finally, a number of studies have suggested that interventions designed to augment memory functioning, including provision of practice and alteration of encodability of information, do not benefit patients with AD to the same extent as age-matched controls, patients with affective disorders, or patients with other dementing conditions (Weingartner *et al*., 1993).

Other significant deficits contribute to the poor performance of patients with AD on many cognitive tasks. Both motor speed and visuomotor performance are impaired quite early in the course of the illness, with some evidence that this is also a progressive deficit (Nebes and Madden, 1988; Nebes and Brady, 1992). The issue of attentional impairment in AD is a complex one. As a general statement, attentional impairment is a less salient feature of AD than deficits in learning and memory. That said, there is still considerable evidence that concentration impairment is present in the illness, especially with continued progression (Kaszniak *et al*., 1986). In the area of deficient verbal skills, there is some evidence that letter fluency is less impaired than category fluency until the very late stages of the illness, possibly because of the greater dependence of category fluency on the intactness of the temporal and parietal cortices (Randolph *et al*., 1993).

As AD progresses, function is lost to the point that by the time of death, performance on all tests is so poor that all scores are essentially zero (see Zec, 1993, for a comprehensive review of this issue). This is in contrast to some other cortical and subcortical dementias where there is preservation of many functions up until the very latest stages.

Behavioral disturbances in AD, including delusions, hallucinations, agitation, and depression, can also interfere with the assessment of cognitive functions in the illness and special care must be taken in order to ensure that low scores are based on cognitive impairments and not on behavioral abnormalities (Teri *et al*., 1992). AD is one of the dementing conditions where a low baseline level of intellectual functioning is a risk factor for the illness. Several different studies have indicated that using an estimate of premorbid functions, such as a reading level obtained from the Wide-Range Achievement Test or the National Adult Reading Test, is valid up until the severe stages of AD (e.g., O'Carroll *et al*., 1987). Thus, relative decline can be assessed against a reasonable measure of premorbid functioning.

D. Cognitive Assessment of Alzheimer's Disease

Studies of cognitive changes in AD use a variety of different assessment tools, and the aim of this section is to describe those tools briefly. Most neuropsychological assessment batteries for AD include a word list learning test with recall after a delay to assess storage and retrieval into secondary memory, measures of language, including fluency and naming, tests of constructional praxis, assessment of motor speed, and some estimate of premorbid functioning. Global, all-purpose assessment tools, such as the MMSE or ADAS described later, assess most of these functions succinctly in order to gauge the general level of the patient's cognitive intactness. A neuropsychological assessment alone is inadequate for the differential diagnosis of AD because of the very large overlap between the cognitive impairments seen in AD and other dementing conditions, such as vascular dementia. Determining the rate of cognitive decline requires reexamination after 6 to 12 months in order to document a progressive course. For patients with very mild impairments, a documented decline over 6–12 months provides convincing evidence of the prototypical cognitive impairments and decline required to correctly diagnose AD. Because delayed recall deficits are not progressive, evidence of progression in this type of memory function is clearly not to be expected. Furthermore, because praxic deficits are often absent in mild AD, a finding of no impairment in this area does not rule out the presence of the illness. Cognitive assessment is a crucial component of treatment trials in AD because cognitive enhancing drugs should have a detectable effect on many different aspects of the illness. A full discussion of this issue is contained in Mohs (1995).

E. Structured Rating Scales for Alzheimer's Disease

An alternative approach to the use of a neuropsychological battery to assess the severity of AD is the use of a structured rating scale. Several of these instruments are available and in common use, with all demonstrating high reliability. The main difference between these scales is their level of comprehensiveness.

1. Alzheimer's Disease Assessment Scale

The ADAS (Rosen *et al*., 1984) is a 21 item scale designed to assess the severity of cognitive and behavioral impairments in AD. The cognitive component includes both a short neuropsychological assessment and items rated by the interviewer on the basis of interaction with the patient and caregiver. Scores on the cognitive subscale range from 0 to 70, with scores on the noncognitive subscale ranging from 0 to 50.

2. Mini-Mental State Examination

The MMSE (Folstein *et al*., 1975) is a widely used assessment instrument designed to screen the cognitive impairments seen in a variety of dementing conditions, although the content areas focus on those associated with AD. There are 21 different items in 11 different tests, with scores ranging from 0 to a perfect score of 30. Scores of 23 or less are typically seen as reflecting dementia and meriting more detailed assessment. In patients with high levels of premorbid functioning, this cutoff should be raised to any score less than 30. It should be noted that the MMSE may be insensitive to subcortical dementia, in that one study (Rothlind and Brandt, 1993) found that patients with confirmed PD and HD were indistinguishable from normals on the MMSE.

3. Clinical Dementia Rating (CDR)

The CDR (Berg, 1988) is a global summary measure designed to identify the overall severity of dementia. Six different content areas are rated individually (memory, orientation, judgement and problem solving, community affairs, home and hobbies, and personal care). Ratings are assigned on a 0–5 point scale, (0 = absent; 0.5 = questionable; 1 = present, but mild; 2 = moderate; 3 = severe; 4 = profound; 5 = terminal). A global summary score is obtained, leading to the use of the CDR for grouping patients on severity of dementia.

4. Mattis Dementia Rating Scale (DRS)

The DRS (Mattis, 1976) is more comprehensive and longer than the MMSE and the CDR, but is also more informative. Similar to the ADAS, the DRS examines a number of cognitive functions associated with dementia. Scores range from 0 to 144, with the cutoff for normal performance at 140. The cutoff for severe dementia is a score of less than 100.

All of these instruments have a role, particularly in research where large-scale screening is required. For purposes of research-oriented assessment, the use of the ADAS or the DRS is likely to be more efficient than a full neuropsychological battery.

F. Huntington's Dementia

Huntington's disease is a neuropsychiatric disorder marked by choreoathetosis, atrophy of the caudate nucleus, and an autosomal dominant inheritance. Equally common in men and women, HD affects 3 to 10 per 100,000 people (Martin, 1984). The age of onset is typically in the early 40s, although this is quite variable. The typical course is 13–17 years with death usually resulting from pneumonia, choking, nutritional deficiencies, skin ulcers, and suicide or other self-destructive behavior (Lanska *et al*., 1988).

An initial diagnosis of HD is usually based on identification of choreoathetosis, the hallmark movement disorder associated with the disease. Choreoathetosis is a combination of jerky movements and slower, twisting movements that often result in dystonic posturing. These movements, early in the course of the disease, occur on initiation of action and include ''piano-playing'' movements of the fingers, ulnar deviation of the hands, and facial tics. Later in the course, choreoathetosis includes a virtually constant stream of movement, including severe grimacing, head bobbing and rolling, and a ''dancing gait.'' These movements, even when severe, cease during sleep. With time, choreoathetosis decreases and dystonia, with akinesis and rigidity, becomes more salient.

HD is now known to result from a gene on the terminal short arm of chromosome 4 (Mendez, 1994). The HD gene causes delayed atrophy and gliosis of the caudate nuclei, with dendritic abnormalities in small-to-medium spiny cells. The basal ganglia have decreased concentrations of the inhibitory neurotransmitter GABA and the enzymes required for GABA synthesis. Other neurotransmitters are relatively preserved. There is also early cell loss in the putamen and in the globus pallidus and the prefrontal cortex later on. Unlike AD, HD does not involve significant loss of neurons from the nucleus basalis of Meynert or loss of choline acetyltransferase activity in the cortex. As HD progresses, however, neuropathological changes in the caudate nuclei show up consistently in neuroimaging studies.

Huntington's dementia is a prototypical subcortical dementia. Cognitive impairments include slowed motor speed, decreased selective and sustained attention, decreased behavioral initiation, spontaneity and engagement, decreased performance IQ with verbal-performance discrepancy, executive deficits, faulty encoding with poor storage, faulty retrieval strategies, in the context of unimpaired recognition, deficient memory requiring effortful processing, decreased motor skill and procedural learning, decreased verbal fluency and output, abnormal egocentric spatial orientation, and abnormal visuomotor integration (Mendez, 1994). Unlike AD patients, HD patients have insight into their neuropsychiatric disorder, despite suffering personality changes.

About 50% of HD patients suffer from psychiatric disorders unrelated to dementia, with a range of about 35 to 73% (Saugstad and Odegard, 1986; Folstein, 1989; Morris, 1991). Included in these are depression, personality changes, or anxiety disorders that often precede the onset of choreoathetosis by as much as 10 years or more (Dewhurst *et al*., 1970). HD patients complain early in their illness of difficulty with attention and concentration. They do particularly poorly on the WAIS-R subtests that tap attentional capacity, as well as on the visuomotor skills, such as the Trail Making Test. HD patients have been shown to have greater attentional and concentration problems than patients with AD, PD, and progressive supranuclear palsy (Pillon *et al*., 1993; Rothlind *et al*., 1993).

There are significant executive functioning deficits in HD, with dysfunction in the areas of maintaining and changing a cognitive set, abstraction, judgment, and reasoning, as well as difficulty with planning and organization and impaired mental flexibility (Brandt and Butters, 1986; Folstein, 1989; Rothlind *et al*., 1993; Paulsen *et al*., 1995a). Patients with HD usually have verbal and visual memory deficits early in their disease. Although the deficit is not as severe as that found in patients with AD (Paulsen *et al*., 1995b) or amnesia, memory problems in HD are well documented. HD patients have flawed encoding strategies that result in poor storage (Lundervold *et al*., 1994a,b). There is a diminished ability to benefit from cuing in recall (Lyle and Gottesman, 1977), a difficulty with learning of new material (Pillon *et al*., 1993), and an impaired ability to learn items in a sequence (Caine *et al*., 1977; Massman *et al*., 1990). However, unlike AD patients, HD patients can benefit from interventions targeting their encoding strategies (Lyle and Gottesman, 1977; Bylsma *et al*., 1990), as well as from priming or cues based on prior exposure (Heindel *et al*., 1990). The importance of testing focused specifically on discriminating the ability of a patient with HD to benefit from the modification of input strategies, as opposed to simply establishing that a memory impairment exists, has been noted in the literature (Paulsen *et al*., 1995a).

Language in HD is relatively intact insofar as the ability to complete verbally mediated tasks. Confrontation naming is a strength, relative to AD patients. Whereas aphasia is not part of the cognitive picture in HD, early on in the disease there is some impairment of verbal fluency. Fluency changes include more single word or short phrase responses, as well as more

pauses in conversation, with the absolute number of words in speech sample shown to be less than that of normal controls (Gordon and Illes, 1987). The ability to understand verbal intonation (prosody) is also impaired (Speedie *et al*., 1990), as is written expression (Podoll *et al*., 1988). Impairments in visuospatial functioning have been noted in terms of lowered scores on constructional tasks, both building and drawing (Fedio *et al*., 1979; Mohr *et al*., 1991), as well as in spatial orientation (Bylsma *et al*., 1992).

References

Albert, M. S., and Moss, M. B. (1988). "Geriatric Neuropsychology." Guilford Press, New York.

Albert, M. S., Duffy, F. H., and Naeser, M. (1987). Nonlinear changes in cognition with age and their neuropsychologic correlates. *Can. J. Psychol.* **41**, 141–148.

American Psychiatric Association (1994). "Diagnostic and Statistical Manual of Mental Disorders," 4th ed. American Psychiatric Association, Washington, DC.

Ardila, A., and Rosselli, M. (1987). Neuropsychological characteristics of normal aging. *Dev. Neuropsychol.* **5**, 307–321.

Arriagada, P. V., Marzloff, K., and Hyman, B. T. (1992). Distribution of Alzheimer-type pathologic changes in nondemented elderly individuals matches the pattern in Alzheimer s disease. *Neurology* **42**, 1681–1688.

Baddeley, A. D. (1986). "Working Memory." Oxford University Press. Oxford.

Berg, G., Edwards, D. F., Danzinger, W. L., and Berg, L. (1987). Longitudinal change in three brief assessments of SDAT. *J. Am. Geriatr. Soc.* **35**, 205–212.

Birren, J. E. (1964). "The Psychology of Aging." Prentice-Hall, Englewood Cliffs, NJ.

Boone, K. B., Miller, B. L., Lesser, I. M., Hill, E., and D'Elia, L. (1990). Performance on frontal lobe tests in healthy older individuals. *Dev. Neuropsychol.* **6**, 215.

Boone, K. B., Miller, B. L., and Lesser, I. M. (1993). Frontal lobe cognitive functions in aging: Methodologic considerations. *Dementia* **4**, 232.

Bosman, E. A. (1993). Age-related differences in the motoric aspects of transcription typing skills. *Psychol. Aging* **8**, 80.

Botwinick, J., and Storandt, M. (1974). "Memory Related Functions and Age." Thomas, Springfield, IL.

Bowles, N. L., and Poon, L. W. (1985). Aging and retrieval of words in semantic memory. *J. Gerontol.* **40**, 71.

Brandt, J., and Butters, N. (1986). The neuropsychology of Huntington's Disease. *Trends Neurosci.* **9**, 118–120.

Bylsma, F. W., Brandt, J., and Strauss, M. E. (1990). Aspects of procedural memory are differentially impaired in Huntington's Disease. *Arch. Clin. Neuropsychol.* **5**, 287–297.

Bylsma, F. W., Brandt, J., and Strauss, M. E. (1992). Personal and extrapersonal orientation in Huntington's Disease patients and those at risk. *Cortex* **28**, 113–122,

Caine, E. D., Ebert, M. H., and Weingartner, H. (1977). An outline for the analysis of dementia: The memory disorder of Huntington's Disease. *Neurology* **27**, 1087–1092.

Cavanaugh, J. C., Grady, J. G., and Perlmuter, M. P. (1983). Forgetting and the use of memory aids in 20 and 70 year olds' daily life. *Int. J. Aging Hum. Dev.* **17**, 113–122.

Clark, J. E., Lanphear, A. K., and Riddick, C. C. (1987). The effects of videogame play on the response selection processing of elderly adults. *J. Gerontol.* **42**, 82–85.

Cohen, G., and Faulkner, D. (1983). Age differences in performance on two information-processing tasks: strategy selection and processing efficiency. *J. Gerontol.* **38**, 447–454.

CPMP Working Party (1992). "Antidementia Medicinal Products. CPMP Working Party on Efficacy of Medicinal Products, Brussels.

Craik, F. I. M. (1977). Age differences in human memory. *In* "Handbook of Psychology of Aging" (J. E. Birren and K. W. Schaie, eds.), pp. 384–416. Van Nostrand-Reinhold, New York.

Cummings, J. L. (1986). Subcortical dementia: Neuropsychology, neuropsychiatry, and pathophysiology. *Br. J. Psychiatry.* **149**, 682–697.

Cummings, J. L., and Benson, D. F. (1983). "Dementia: A Clinical Approach," 2nd ed. Butterworth-Heinemann, Boston.

Cummings, J. L., and Benson, D. F. (1990). Subcortical mechanisms and human thought. *In* "Subcortical Dementia" (J. L. Cummings, ed.), pp. 251–259. Oxford University Press, New York.

Daigenault, S., Braun, C. M. J., and Whitaker, H. A. (1992). Early effects of normal aging on perseverative and non-perseverative prefrontal measures. *Dev. Neuropsychol.* **8**, 99–107.

Denney, N. W. and Palmer, P. (1981). Adult age differences in traditional and practical problem solving. *J. Gerontol.* **36**, 323–331.

Dewhurst, K., Oliver, J. E., and McKnight, A. L. (1970). Sociopsychiatric consequences of Huntington's Disease. *Br. J. Psychiatry* **116**, 255–258.

Evans, D. A., Funkenstein, H. H., Albert, M. S., Scherr, P. A., Cook, N. R., Chown, M. J., Herbert, L. E., Hennekens, C. H., and Taylor, J. O. (1989). Prevalence of Alzheimer's disease in a community population of older persons. *JAMA, J. Am. Med. Assoc.* **262**, 2551–2556.

Fedio, P., Cox, C. S., Neophytides, A., Canal-Frederick, G., and Chase, T. N. (1979). Neuropsychological profile of Huntington's Disease. *Adv. Neurol.* **23**, 239–255.

Fillenbaum, G. (1985). Screening the elderly: A brief instrumental activities of daily living measure. *J. Am. Geriatr. Soc.* **33**, 698–706.

Flicker, C., Ferris, S. H. and Reisberg, B. (1991). Mild cognitive impairment in the elderly: Predictors of dementia. *Neurology* **41**, 1006–1009.

Folstein, M. F., Folstein, and S. E., McHugh, P. R. (1975). Mini-mental State: A practical method for grading the cognitive state of patients for the clinician. *J. Psychiat. Res.* **12**, 189–198.

Folstein, S. E. (1989). "Huntington's Disease: A Disorder of Families." Johns Hopkins University Press, Baltimore, MD.

Freedman, M. (1990). Object alternation and orbitofrontal system dysfunction in Alzheimer's and Parkinson's disease. *Brain Cogn.* **14**, 134–143.

Gordon, W. P., and Illes, J. (1987). Neurolinguistic characteristics of language production in Huntington's Disease: A preliminary report. *Brain Lang.* **31**, 1–10.

Graf, P., and Schachter, D. L. (1985). Implicit and explicit memory for new associations in normal and amnesic subjects. *J. Exp. Psychol.* **11**, 501–518.

Haaland, K. Y., Vranes, L. F., Goodwin, J. S. and Garry, P. J. (1987). Wisconsin card sorting test performance in healthy elderly population. *J. Gerontol.* **42**, 345–346.

Heaton, R. K., Chelune, G. J., Talley, J. L., Kay, G. C., and Curtis, G. (1993). "Wisconsin Card Sorting Test (WCST) Manual—Revised and Expanded." Psychological Assessment Resources, Odessa, FL.

Heindel, W. C., Salmon, D. P., and Butters, N. (1990). Pictorial priming and cued recall in Alzheimer's and Huntington's Disease. *Brain Cogn.* **13**, 282–295.

Huff, F. J., Growdon, J. H., Corkin, S., and Rosen, T. J. (1987). Age at onset and rate of progression of Alzheimer's disease. *J. Am. Geriatr. Soc.* **35**, 27–30.

Jastak, S. (1984). "The Wide-range Achievement Test—Revised." Jastak Associates, Wilmington, DE.

Kaszniak, A. W., Poon, L. W., and Riege, W. L. (1986). Assessing memory deficits: An information-processing approach. *In* "Clinical Memory Assessment of Older Adults." L. W. Poon ed., pp. 277–284. American Psychological Association, Washington, DC.

Katzman R. (1986). The prevalence and malignancy of Alzheimer's disease: A major killer. *Arch. Neurol. (Chicago)* **33**, 217–218.

Kausler, D. H. and Lair, C. V. (1966). Associative strength and paired-associate learning. *J. Gerontol.* **21**, 278–280.

Kemper, S. (1987). Life-span changes in syntactic complexity. *J. Gerontol.* **42**, 323–328.

Khachaturian, Z. S. (1985). Diagnosis of Alzhiemer's disease. *Arch. Neurol. (Chicago)* **42**, 1097–1105.

Lanska, D. J., Lanska, M. J., Lavine, L., and Schoenberg, B. S. (1988). Conditions associated with Huntington's Disease at death, A case-control study. *Arch. Neurol. (Chicago)* **45**, 878–880.

Lundervold, A. J., Reinvang, I., and Lundervold, A. (1994a). Characteristic patterns of verbal memory function in patients with Huntington's Disease. *Scand. J. Psychol.* **35**, 38–47.

Lundervold, A. J., Karlsen, N. R., and Reinvang, I. (1994b). Assessment of subcortical dementia in patients with Huntington's Disease, Parkinson's Disease, multiple sclerosis and AIDS by a neuropsychological screening battery. *Scand. J. Psychol.* **35**, 48–55.

Lupien, S. J., Gaudreau, S., Tchiteya, B. M., Maheu, F. Sharma, S., Nair, N. P., Hauger, R. L., McEwen, B. S., and Meaney, M. J. (1997). Stress-induced declarative memory impairment in healthy elderly subjects: Relationship to Cortisol reactivity. *J. Clin. Endocrinol. Metab.* **82**, 2070–2075.

Lyle, O. E., and Gottesman, I. I. (1977). Premorbid psychometric indicators of the gene for Huntington's Disease. *J. Consult. Clin. Psychol.* **45**, 1011–1022.

Martin, J. B. (1984). Huntington's Disease: New approaches to an old problem. *Neurology* **34**, 1059–1072.

Massman, P. J., Delis, D. C., Butters, N., Levin, B. E., and Salmon, D. P. (1990). Are all subcortical dementias alike? Verbal learning and memory in Parkinson's and Huntington's Disease patients. *J. Clin. Exp. Neuropsychol.* **12**, 729–744.

Mattis, S. (1976). Mental status exam for organic mental syndrome in the elderly patient. *In* ''Geriatric Psychiatry'' (L. Bellack and T. B. Karasu, eds.), pp. 77–121. Grune & Stratton, New York.

Mazauk, J. M. *et al*. (1995). Visuo-spatial attention and psychomotor performance in elderly community residents: Effects of age, gender, and education. *J. Clin. Exp. Neuropsychol.* **17**, 71–79.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., and Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology* **34**, 939–944.

Mendez, M. F. (1994). Huntington's Disease: Update and review of neuropsychiatric aspects. *Int. J. Psychiatry Med.* **24**, 189–208.

Mohr, E., Brouwers, P., Claus, J. J., Mann, U. M., Fedio, P., and Chase, T. N. (1991). Visuospatial cognition in Huntington's Disease. *Movement Disord.* **6**, 127–132.

Mohs, R. C. (1995). Neuropsychological assessment of patients with Alzheimer's disease. *In* ''Psychopharmacology: The Fourth Generation of Progress'' (F. E. Bloom and D. J. Kupfir, eds.), pp. 1377–1388. Raven Press, New York.

Morris, J. C., Edland, S., Clark, C., Galasko, D., Koss, E., Mohs, R., van Belle, G., Fillenbaum, G., and Heyman, A. (1993). The Consortium to establish a registry for Alzheimer's Disease (CERAD): Part IV. Rates of cognitive change in the longitudinal assessment of probable Alzheimer's disease. *Neurology* **43**, 2457–2465.

Morris, M. (1991). Psychiatric aspects of Huntington's Disease. *In* ''Huntington's Disease: Major Problems in Neurology'' (P. S. Harper, ed.), pp. 81–126. Saunders, London.

Nebes, R. D., and Brady, C. B. (1992). Generalized cognitive slowing and severity of dementia in Alzheimer's Disease: Implications for the interpretation of response-time data. *J. Clin. Exp. Neuropsychol.* **14**, 317–326.

Nebes, R. D., and Madden, D. J. (1988). Different patterns of cognitive slowing produced by Alzheimer's disease and normal aging. *Psychol. Aging* **3**, 102–104.

Nelson, H. E. (1976). A modified card sorting test sensitive to frontal lobe deficits. *Cortex* **12**, 313–324.

Obler, L. K. *et al*. (1985). On Comprehension across the adult lifespan. *Cortex* **21**, 273–280.

O'Carroll, R. E., Baikie, E. M., and Whittick, J. E. (1987). Does the NART hold in dementia? *Br. J. Clin. Psychol.* **26**, 315–316.

Owen, N. A. (1953). Age and mental abilities: A longitudinal study. *Genet. Psychol. Monogr.* **40**, 71–79.

Panek, P. E., Barrett, G. V., Sterns, H. L., and Alexander, R. A. (1977). A review of age changes in perceptual information processing ability with regard to driving. *Exp. Aging Res.* **3**, 387–449.

Paulsen, J. S., Butters, N., Sadek, J. R., Johnson, S. A., Salmon, D. P., Swerdlow, N. R., and Swenson, M. R. (1995a). Distinct cognitive profiles of cortical and subcortical dementia in advanced illness. *Neurology* **45**, 951–956.

Paulsen, J. S., Salmon, D. P, Monsch, A., Butters, N., Swenson, M. R., and Bondi, M. W. (1995b). Discrimination of cortical from subcortical dementias on the basis of memory and problem-solving tests. *J. Clin. Psychol.* **51**, 48–58.

Pillon, B., Dubois, B., Lhermitte, F., and Agid, Y. (1986). Heterogeneity of cognitive impairment in progressive supranuclear palsy, Parkinson's disease, and Alzheimer's disease. *Neurology* **36**, 1179–1185.

Pillon, B., Deweer, B., Agid, Y., and Dubois, B. (1993). Explicit memory in Alzheimer's, Huntington's, and Parkinson's Disease. *Arch. Neurol.* **50**, 374–379.

Plude, D. J. *et al.* (1986). Age differences in depicting and perceiving tridimensionality in sample line drawing. *Exp. Aging Res.* **12**, 221–225.

Podoll, K., Caspary, P., Lange, H. W., and Noth, J. (1988). Language functions in Huntington's Disease. *Brain* **111**, 1475–1503.

Poon, L. W., Fozard, J. L., Paulshock, D. R., and Thomas, J. C. (1979). A questionnaire assessment of age differences in retention of recent and remote events. *Exp. Aging Res.* **5**, 401–411.

Psychological Corporation. (1997). ''WAIS-3 and WMS-3 Technical Manual.'' Psychological Corporation, San Antonio, TX.

Rabbitt, P. M. A. (1985). An age decrement in the ability to ignore irrelevant information. *J. Gerontol.* **40**, 586–591.

Randolph, C., Braun, A. R., Goldberg, T. E., and Chase, T. N. (1993). Semantic fluency in Alzheimer's, Parkinson's, and Huntington's Disease: Dissociation of storage and retrieval failures. *Neuropsychology* **1**, 82–88.

Rebok, G. W., and Folstein, M. F. (1993). Dementia. *J. Neuropsychiatry Clin. Neurosci.* **5**, 265–276.

Reitan, R. M., and Wolfson, D. A. (1994). A selective and critical review of neuropsychological deficits and the frontal lobes. *Neuropsychol. Rev.* **4**, 161–198.

Rosen, W. G. (1980). Verbal fluency in aging and dementia. *J. Clin. Neuropsychol.* **2**, 135–146.

Rosen, W. G., Mohs, R. C., and Davis, K. L. (1984). A new rating scale for Alzheimer's Disease. *Am. J. Psychiatry* **141**, 1356–1364.

Rothlind, J., and Brandt, J. (1993). A brief assessment of frontal and subcortical functions in dementia. *J. Neuropsychiatry* **5**, 73–77.

Rothlind, J. C., Bylsma, F. W., Peyser, C., Folstein, S. E., and Brandt, J. (1993). Cognitive and motor correlates of everyday functioning in early Huntington's Disease. *J. Nerv. Ment. Dis.* **181**, 194–199.

Rowe, J. W., and Kahn, R. L. (1987). Human aging: Usual and successful. *Science* **237**, 143–149.

Salmon, D. P., Thal, L. J., Butters, N., and Heindel, W. C. (1990). Longitudinal evaluation of dementia of the Alzheimer type: A comparison of 3 standardized mental status examinations. *Neurology* **40**, 1225–1230.

Salthouse, T. A. (1984). Effects of age and skill in typing. *J. Exp. Psychol. Gen.* **113**, 345–371.

Saugstad, L., and Odegard, O. (1986). Huntingtons Chorea in Norway. *Psychol. Med.* **16**, 39–48.

Speedie, L. J., Brake, N., Folstein, S. E., Bowers, D., and Heilman, K. M. (1990). Comprehension of prosody in Huntington's Disease. *J. Neurol. Neurosurg. Psychiatry* **53**, 607–610.

Squire, L. R., and Cohen, N. J. (1982). Remote memory, retorgrade amnesia, and the neuropsychology of memory. *In* ''Human Memory and Amnesia'' L. J. Cermack, (ed.). Erlbaum, Hillsdale, NJ.

Teri, L., Rabins, P., Whitehouse, P., Berg, L., Riesberg, B., Sunderland, T., Eichelman, B., and Phelps, C. (1992). Management of behavior disturbance in Alzheimer's disease: Current knowledge and future directions. *Alzheimer's Dis. Assoc. Disord.* **6**, 77–88.

Terry, R., and Katzman, R. (1992). Alzheimer's disease and cognitive loss. *In* ''Principles of Geriatric Neurology'' (R. Katzman and J. W. Rowe, eds.), pp. 207–265. Davis, Philadelphia.

Warrington, E. K., and Sanders, H. (1971). The fate of old memories. *Q. J. Exp. Psychol.* **23**, 432–442.

Weingartner, H. R., Eckart, M., Grafman, J., Molchan, S., Putnam, K., Rawlings, R., and Sunderland, T. (1993). The effects of repetition on memory performance in cognitively impaired patients. *Neuropsychology* **7**, 385–395.

Welsh, K. A., Butters, N., Hughes, J., Mohs, R. C., and Heyman A. (1991). Detection of abnormal memory decline in mild cases of Alzheimer's disease using CERAD neuropsychological measures. *Arch. Neurol.* **48**, 278–281.

Welsh, K. A., Butters, N., Hughes, J., Mohs, R. C., and Heyman, A. (1992). Detection and staging of dementia in Alzheimers disease: use of the neuropsychological measures developed for the Consortium to Establish a Registry for Alzheimer's disease (CERAD). *Arch. Neurol. (Chicago)* **49**, 448–452.

World Health Organization. (1992). ''International Classification of Disease, 10th ed., WHO, Geneva.

Zec, R. F. (1993). Neuropsychological functioning in Alzheimer's Disease. *In* ''Neuropsychology of Alzheimer's Disease and Related Disorders'' (R. W. Parks, R. F. Zec, and R. S. Wilson, eds.). Oxford University Press, New York and Oxford.

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6

Types of Age-Related Brain Lesions and Relationship to Neuropathologic Diagnostic Systems of Alzheimer's Disease

Neurofibrillary tangles, senile plaques, and neuronal loss are the three major pathologic hallmarks of Alzheimer's disease (AD); however, they are also observed in the course of normal brain aging. In recent years, the classical neuropathological description of these changes has been tentatively replaced by an integrative view, including morphological, biochemical, and molecular characteristics. The first part of this chapter focuses on the description of AD-related lesions and attempts to highlight possible relationships between their structure and hypotheses regarding their etiopathogenesis. Moreover, the time evolution and codependence of these lesions in the aged brain is discussed. The second part of this chapter provides a critical review of diagnostic systems for the neuropathological diagnosis of AD. Comparative analysis of these systems is presented with particular reference to their theoretical framework, validity, and acceptance.

I. Introduction

The two classic lesions described by Alzheimer (1907) in his first report of an early-onset demented patient, neurofibrillary tangles (NFT) and senile plaques (SP), are those currently used for the routine neuropathological diagnosis of Alzheimer's disease (AD; Figs. 6.1–6.3). These alterations are also present in normal brain aging, but they are far less severe than in AD and occur in restricted regions of the cerebral cortex (Tomlinson *et al*., 1968; Ball, 1977; Mountjoy *et al*., 1983; Ulrich, 1985). Our understanding of these lesions has progressively changed to encompass complex molecular, biochemical, and structural issues. Moreover, other structural abnormalities have been described, and recent advances in neuron counting technology have provided information on the extent of synaptic and neuronal loss in normal aging and AD (Terry *et al*., 1991, 1994; Morrison and Hof, 1997). This chapter provides an overview of current knowledge of AD-related lesion structures as well as a critical examination of the concepts that underlie the neuropathological diagnosis of AD.

II. Histopathological Changes

A. Neurofibrillary Tangles

1. Microscopic and Biochemical Characteristics

Neurofibrillary tangles, described by Alzheimer (1907), represent the accumulation and abnormal biochemical modification of components of the neuronal cytoskeleton that form paired helical filaments. These lesions are composed of intracellular argentophilic fibers that are stained intensely by histochemical stains such as thioflavin-S (Figs. 6.1a and 6.1b). Ultrastructurally, NFT are formed by an apparent pair of strands, wound around one another, with crossover repeats around 75–80 nm and widths between 5 and 22 nm, called paired helical filaments (PHF; Fig. 6.2a; Terry *et al*., 1994). A great variety of cytoskeletal proteins, such as the microtubule-associated protein tau, ubiquitin, and neurofilament triplet protein, are associated with PHF (Vickers *et al*., 1992, 1994, 1996; Trojanowski *et al*., 1993). The major protein subunit of PHF is the microtubule-associated protein tau (Iqbal, 1998). Six different tau isoforms ranging from 352 to 441 amino acids can be generated from a single tau gene by alternative splicing. The expression of tau is developmentally regulated so that all six tau isoforms are found in the adult human brain, while only the smallest isoform, known as fetal tau, is present in the fetal human brain (Bramblett *et al*., 1992; Matsuo *et al*., 1994). PHF-tau has a higher molecular weight and a more acidic isoelectric charge than normal tau (Grundke-Iqbal *et al*., 1986). Although PHF-tau are hyperphosphorylated compared to normal adult brain tau (Bramblett *et al*., 1992; Matsuo *et al*., 1994), it has been demonstrated that biopsy-derived normal adult human tau is phosphorylated *in vivo* on sites that are similar to those found in PHF-tau and that a significant dephosphorylation of normal tau takes place during the postmortem

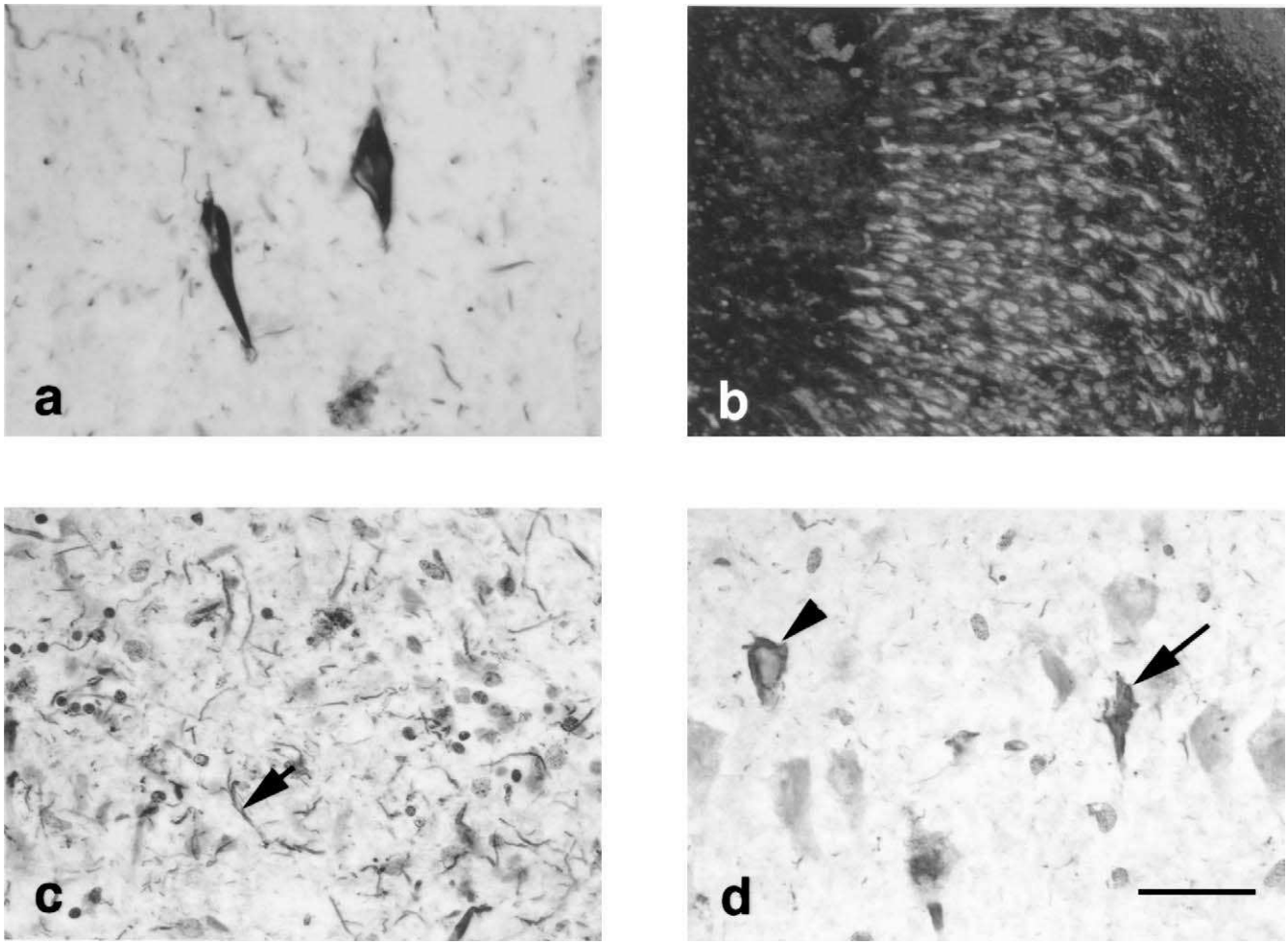

FIG. 6.1. Representative examples of cytoskeletal pathology in a 78-year-old AD case: NFT in high magnification (a), NFT in the CA1 field of hippocampus (b), neuropil threads (c; arrow), and intracellular (d; arrowhead) and extracellular (d; arrow) tangles. Materials were stained with an antibody against the microtubule-associated tau protein (a, c, d) and modified thioflavin-S stain (b) and were counterstained with Nissl stain (c, d). Scale bars: 50 (a, c, d) and 200 (b) μm.

period (Matsuo *et al*., 1994). PHF-tau inhibit the microtubule assembly-promoting activities of microtubule-associated proteins 1 and 2 and tubulin (for review, see Iqbal *et al*., 1998). It is thus possible that downregulation of phosphatases in the AD brain could induce the generation of maximally phosphorylated PHF-tau, causing disassembly of microtubules and consequently a retrograde neuronal degeneration (Matsuo *et al*., 1994). Alternatively, the formation of PHF-tau may be accelerated by key molecules, such as sulfated glycosaminoglycans (Goedert *et al*., 1996). Glycation of PHF-tau proteins, which itself could be the result of oxidative stress, induces an additional oxidative stress in neurons (Yen *et al*., 1995). In addition, NFT show immunoreactivity to a variety of proteins, such as casein kinase II, protease nexin I, fibroblast growth factor, microtubule-associated protein 5, amyloid Aβ protein, apolipoprotein E, and ubiquitin. Whether these proteins play a major role in the formation of NFT is still unclear (for review, see Terry *et al*., 1994).

2. Time Evolution of Cytoskeletal Pathology in AD: From Pretangles to Ghost Tangles

Several morphologically distinct types of cytoskeletal pathology have been described in AD brains corresponding to different evolutionary steps. At the beginning of the degenerative process, there is an accumulation of phosphorylated tau proteins in the somatodendritic compartment without PHF formation, refered to as pretangles (Bancher *et al*., 1989; Braak *et al*., 1994). Neurons displaying pretangles are ALZ-50 immunoreactive. At a following stage, neuropil threads may be identified by immunocytochemistry using both ALZ-50 and anti-PHF antibodies. Neuropil threads are abnormal neuronal processes that contain both PHF and straight filaments. They are mainly located in distal segments of axons and dendrites and share multiple tau epitopes with NFT (i.e., anti-tau and anti-ubiquitin reactivity), except those in the two high molecular weight neurofilament proteins (Fig. 6.1c; Perry *et al*.,

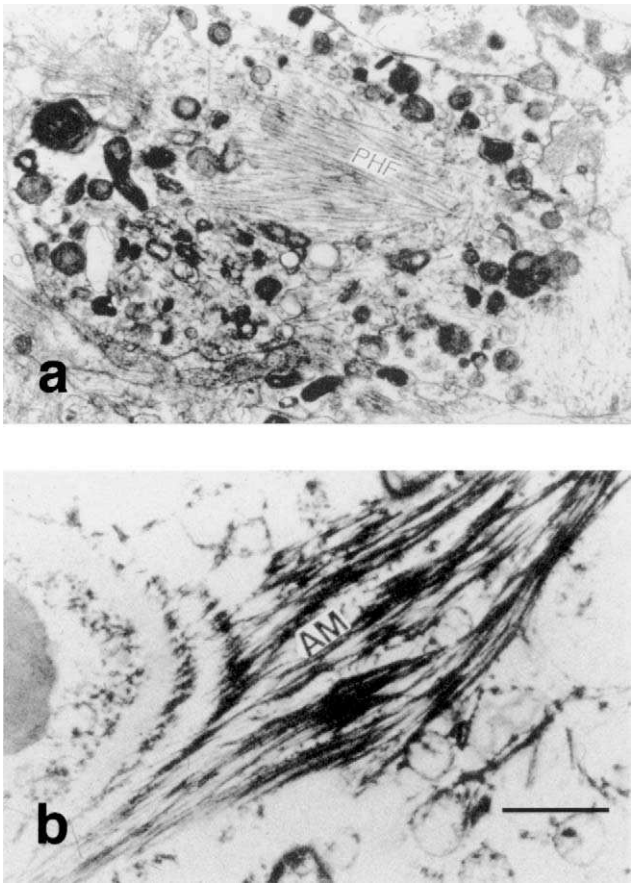

FIG. 6.2. Ultrastructural appearance of PHF in NFT (a) and amyloid fibrils (AM) in the core of a SP (b).

1991). The PHF immunoreactivity of neuropil threads progressively displaces the normal microtubule-associated protein 2 immunostaining in dendrites and is associated with a degeneration of the soma in vulnerable cortical areas (Ashford *et al*., 1998). In more advanced AD cases, fully developed NFT are seen as tightly packed bundles filling the cell body and extending into proximal dendrites (Fig. 6.1a). Following neuronal death, NFT in the cerebral cortex persist within the neuropil as extracellular or ghost tangles and are associated with degenerating neurites and neuropil threads (Fig. 6.1d; Yamagushi *et al*., 1991; Terry *et al*., 1994; Cras *et al*., 1995). Extracellular but not intracellular tangles are immunoreactive for the 40 carboxy-terminal sequence of amyloid Aβ protein (Schwab *et al*., 1998).

B. Senile Plaques

The histological description of SP has yet to be standardized, and there are substantial interlaboratory differences in the vocabulary used. Two main types of SP have been distinguished in the human brain (Fig. 6.3, see color insert). *Diffuse plaques* are formed by filamentous and nonstructured amyloid Aβ protein without a neuritic component, stain positively with silver stains but not with thioflavin-S, do not correlate with the severity of dementia, and are especially seen in oldest-old individuals (Fig. 6.3e; Hauw *et al*., 1986). In contrast, *neuritic plaques* contain dense bundles of amyloid fibrils stained by thioflavin-S and dystrophic neurites (Figs. 6.3a, 6.3b, and 6.3d). Neuritic plaques are further divided into *primitive plaques* without a dense Aβ protein core and *classic plaques*, which are composed of a central amyloid core surrounded by degenerating axon terminals, dendritic arborizations, and glial elements (Figs. 6.2a, and 6.2b; Brion, 1990; Dickson, 1997; Wisniewski and Silverman, 1997). Some of these neurites contain degenerating synaptic elements, laminated bodies, mitochondria, and lysosomes, whereas others contain PHF similar to those observed in NFT and neuropil threads. Neuritic plaques, which are PHF immunoreactive, are predominantly found in AD and are thought to be associated with clinical severity (Fig. 6.3b; Barcikowska *et al*., 1989; Dickson, 1997; Knowles *et al*., 1998). Neuritic elements are also immunolabeled for numerous proteins, such as amyloid precursor protein (APP), growth-associated protein 43, protein kinase C, tau, ubiquitin, brain spectrin, synaptophysin, and chromogranin, as well as a variety of neuropeptides (for review, see Terry *et al*., 1994). Although the exact significance of this immunoreactivity is unclear, the presence of growth-associated protein 43 indicates that regenerative sprouts occur in the vicinity of neuritic plaques. At the end of their evolution, SP are often present as a dense amyloid core with reactive astrocytes and microglial cells but without a neuritic component (Fig. 6.1c; i.e., burned-out plaques).

A prominent component of amyloid deposits is a polypeptide (Aβ) of 40–42 amino acids derived through proteolytic cleavage from a set of APP isoforms, which are encoded by a single gene on chromosome 21 (Fig. 6.2b; Brion, 1990). Three major isoforms of APP are generated from this gene by alternative splicing (APP 770, 751, 695). All of these isoforms are found in the human brain (Checler, 1995). Structurally, the APP is a protein of 110–135 kDa with a large extracellular N-terminal domain, a cell-surface domain, and a small intracellular carboxyl-terminal domain. The proteolytic cleavage of this molecule in the Aβ domain is catalyzed by a group of enzymes, known as α, β and γ secretases (Checler, 1995). It is thought that cleavage of the APP outside the Aβ domain could generate large C-terminal fragments containing the Aβ sequence, which are possibly amyloidogenic. Two distinct species of Aβ protein with different carboxyl terminals, Aβ40 and Aβ42(43), are deposited in the brains of patients with AD (Iwatsubo *et al*., 1994; 1996; Mann *et al*., 1996). In particular, diffuse plaques, which are commonly seen in normal brain aging, are immunoreactive for the Aβ42 fragment, whereas dense and reticular amyloid deposits in AD brains show an intense Aβ40 immunoreactivity (Arai *et al*., 1990; Gowing *et al*., 1994; Iwatsubo *et al*., 1994, 1996). Although this kind of distinction is not accepted unanimously, a better understanding of the molecular composition of amyloid deposits may make it possible to identify a subset of SP more closely related to AD than normal brain aging. Besides antibodies to Aβ protein, the amyloid core, as well as the amyloid fibrils in SP, is immunostained with antibodies to α_1-antichymotrypsin, protein kinase C, factors of complement, apolipoprotein E, and sulfated glycosaminoglycans. Most of these components are probably adsorbed secondarily to the amyloid in SP (Robakis, 1994).

The mechanism of SP formation is controversed. Classic SP may develop from preexistant diffuse Aβ deposits, although no consistent colocalization of these two forms of SP has been observed. Alternatively, SP may arise from amyloid in the vicinity of the blood vessel wall (see later). A third hypothesis involves a release of Aβ sequence from clusters of dystrophic neurites with the subsequent formation of diffuse Aβ deposits and classic SP (for review, see Terry *et al*., 1994).

C. NFT and SP: Concurrent or Causally Related Lesions?

Whether the consistent presence of NFT and SP in AD brains reflects a causal relationship between these lesions is still a very controversial issue. Several lines of evidence support an interdependence between these two types of lesions. In normal brain aging, AD, and Down syndrome, neuritic plaques coincide with NFT (Mann and Esiri, 1989; Bugiani *et al*., 1990); extracellular NFT contain several traits of SP such as Aβ40 protein, microglia, and astroglia (Cras *et al*., 1995; Schwab *et al*., 1998), and a subset of neuritic plaques show PHF immunoreactivity (see earlier discussion); the APP molecule contains a β-sheet conformation-dependent binding site for tau protein (Smith *et al*., 1995), and *in vitro* interaction between tau and APP proteins promotes fibrillogenesis (Giaconne *et al*., 1994); injection of PHF in rat brain induces amyloid deposits (Shin *et al*., 1993); and AD kindreds carrying APP mutations exhibit both amyloid deposition and NFT (for review, see Hardy, 1994). However, there are also strong arguments against such interdependence. For instance, amyloid deposition may occur in the absence of NFT, and the recent description of tauopathies and tau- and tangle pathology-related conditions clearly indicates that NFT may cause dementia in the absence of amyloid deposition (for review, see Jellinger and Bancher, 1998; Spillantini *et al*., 1998). Moreover, in AD brains there is no correlation between the regional distribution of NFT and SP within the cerebral cortex, at least at the early stages of the degenerative process (Giannakopoulos *et al*., 1993; van de Nes *et al*., 1998). One can conclude that although there is a high degree of pathological synergy between NFT and SP in AD, these lesions develop independently in the course of brain aging and their simultaneous presence is not a necessary condition for the clinical expression of dementia.

D. Synaptic and Neuronal Loss

Both types of loss are difficult to assess as they represent the inferred absence of something. Synaptic loss has been reported in the neocortex of elderly nondemented individuals, suggesting an age-dependent mechanism for the loss of synapses in the neocortex (Masliah *et al*., 1993). Terry and collaborators (1994) have proposed that the structural or functional loss of synapses may be the physical basis of dementia. In particular, synaptic loss has been revealed by electron microscopy studies, by confocal microscopy for synaptophysin immunostaining, and also by spectrophotometric analysis of enzyme-linked immunoassays (for review, see Terry *et al*., 1994). All these methods have demonstrated a close relationship between the extent of loss of synaptic integrity and AD severity. An average of 45% decrease in presynaptic terminal density, and a 27 to 42% synapse loss in the prefrontal cortex has been documented in AD cases (Brion *et al*., 1991; Masliah *et al*., 1991). This progressive synapse loss is accompanied by the development of an increased area of aposition between pre- and postsynaptic elements. While synaptic density is generally diminished in the cortical neuropil of AD compared to age-matched control cases, this reduction is not greater within the diffuse plaques than in the neuropil outside them. In contrast, synapse loss is more pronounced within neuritic plaques, and abnormal synapses are concentrated around dendritic neuropil threads. Numerous studies have analyzed in depth the relationships between synaptic changes and APP metabolism. APP has been localized in the presynaptic terminals in most SP dystrophic neurites, and it may be transported rapidly in the axon to the presynaptic site. These findings support a central role for synaptic alterations in AD pathogenesis. Whether synaptic changes precede the accumulation of abnormally processed APP and amyloid deposition or whether they are caused by amyloid neurotoxicity is still a matter of debate. More generally, the pathophysiology of synaptic alterations is poorly known. Possible explanations include the receptor-mediated neurotoxic effect of endogenous or exogenous substances, failure of neuronal plasticity due to altered intracellular signaling, and abnormal axonal transport (for review, see Terry *et al*., 1994).

Until recently, it was widely accepted that neuronal death is consistently associated with normal brain aging. This conviction was supported by several influential papers, some of which date back to the 1950s, demonstrating significant neuronal death in aged nondemented individuals as well as in nonhuman primates and rodents. All these studies estimated neuron densities but not the total number of neurons in a given structure. The field was reviewed extensively by Coleman and Flood (1987), who concluded that although there was an aging-related neuronal loss, the validity of data might be seriously compromised by species differences, tissue processing, and sampling design. This point of view has been further challenged by the development of stereological techniques to estimate the number of neurons in identifiable structures of interest, such as key hippocampal and neocortical areas (for review, see West and Gundersen, 1990). The main advantages of these techniques are that they obtain estimates of total neuron number within a given brain structure, which are not confounded by changes in the size of neurons, the size of the structure, and fixation parameters. Using stereological principles, it has been shown that there is only a 10% decrease in the total number of neurons in the neocortex across the age spectrum in normal individuals (Morrison and Hof, 1997; Pakkenberg and Gundersen, 1997). Most importantly, there was no evidence of age-related neuronal loss in the CA1 field and entorhinal cortex, two key areas in AD pathogenesis, in nondemented individuals (West, 1993; West *et al*., 1994; Gómez-Isla *et al*., 1996a). Although a certain decrement in neuron numbers has been reported in the hilus of the dentate gyrus and subiculum in the course of normal brain aging, this remains controversial (West, 1993; Simic *et al*., 1997). Conversely, even very mild AD cases (i.e., CDR of 0.5) display a 50% neuronal loss in layer II of the entorhinal cortex, and this percentage raises to 90% in severe AD cases (Gómez-Isla *et al*.,

1996a). Moreover, neuronal loss in the CA1 field of the hippocampus may quantitatively separate normal brain aging from AD (West *et al*., 1994) and correlate strongly with the duration and severity of AD (Bobinski *et al*., 1998). Decreased densities of larger neurons are observed not only in the AD cerebral cortex, but also in some subcortical nuclei, such as the basal nucleus of Meynert, locus coeruleus, and dorsal raphe (for review, see Terry *et al*., 1994).

The mechanisms surrounding cell death in AD are not clearly identified. Among the pyramidal neurons of the hippocampal formation, cell death appears closely related to the presence of NFT, many of which are visualized in the neuropil when the neuronal nucleus and cytoplasm disappear. In neocortical areas, both NFT-related and NFT-unrelated neuronal loss may take place, particularly in very old patients (Terry *et al*., 1987; Giannakopoulos *et al*., 1996). Several studies have indicated that neuronal apoptosis is an important mechanism of cell death in AD (for review, see Cotman and Anderson, 1995; Smale *et al*., 1995). An expanding family of genes encoding homologous proteins has been identified as the Bcl-2 family and appears to determine whether a cell will undergo apoptosis (Boise *et al*., 1993; Oltvai *et al*., 1993). Among them, the Bcl-2 and Bcl-X_L gene products can block apoptotic cell death and promote the survival of neurons (Boise *et al*., 1993; Zhong *et al*., 1993), whereas the Bax gene product (Bcl-2-associated X protein) may act as a promoter of cell death (Oltvai *et al*., 1993). In AD, Bcl-2 downregulation and Bax upregulation have been reported in neurons prone to degeneration (Su *et al*., 1996; MacGibbon *et al*., 1997), whereas a loss of Bax immunoreactivity may render neurons more resistant to programmed cell death (MacGibbon *et al*., 1997; Su *et al*., 1997). Subsequently, it has been proposed that an upregulation of Bax may act by promoting both NFT-related and NFT-unrelated neuronal loss (Su *et al*., 1997). However, Western blot analysis has shown an increase in the level of Bcl-X_L, but not Bax, protein in the temporal cortex in AD compared to nondemented cases (Kitamura *et al*., 1998). Moreover, an increase in the expression of Bcl-2 has been reported in AD brains, yet sparse and equivocal Bcl-2 staining is observed in neurons in this disorder (Satou *et al*., 1995; O'Barr *et al*., 1996). Apoptosis is not the only mechanism involved in cell death in AD (Cotman, 1998). The DNA fragmentation that occurs in the hippocampus of AD brains is not necessarily associated with an increased rate of cells displaying the morphological characteristics of apoptosis, and it has been postulated that necrosis may also play an equally important role in AD-related cell destruction (Stadelmann *et al*., 1998). Further studies, including both estimates of neurons showing DNA damage and expression of apoptosis-related proteins, are warranted to better understand the molecular mechanisms of neuronal loss in AD.

E. Other AD-Related Lesions

Two additional lesions frequently observed in AD brains are Hirano bodies and granulovacuolar degenerations. Hirano bodies are refractile eosinophilic rod-like structures that have been initially observed in specimens of Guamanian parkinsonism–dementia complex. They occur preferentially in neurons of the CA1 field of the hippocampus, and their number increases with age. In addition to their original description, they are also present in a variety of dementing conditions, including AD, Pick's disease, and dementia with Lewy bodies (Hirano, 1994). Immunocytochemical and electron microscopy studies have revealed that Hirano bodies are mainly formed by abnormal actin microfilaments, but also contain epitopes of tau protein, middle molecular weight neurofilament subunits, C-terminal fragment of β-APP, advanced glycation end products, stress-related proteins, and FAC1, a developmentally regulated protein (Hirano, 1994; Jordan-Sciutto *et al*., 1998; Munch *et al*., 1998). Although the exact role of Hirano bodies in aging pathophysiology is unknown, it is considered that they represent age-related alterations of the microfilamentous system, which are probably accelerated in various neurodegenerative diseases.

In classic histologic sections, granulovacuolar degenerations (GVD) appear as vacuoles in the cytoplasm of pyramidal neurons in the hippocampus, mainly in the CA1 field and prosubiculum. Each 3- or 4-μm-wide vacuole contains a dense hematoxylinophilic, argentophilic granule, 1 or 2 μm in diameter. Cells with GVD are scarce in normal brain aging, but their number increases markedly in several dementing conditions, such as AD, Pick's disease, multi-infarct dementia, and Fahr's syndrome (Xu *et al*., 1992). Electron microscopy shows a single membrane surrounding the vacuole and a dense granular mass. The molecular mechanism of GVD formation remains unknown. Evidence suggests a close relationship between these lesions and NFT. For instance, they are labeled by antibodies to tubulin, Alz-50, and neurofilament proteins. Moreover, they exhibit the same pattern of phosphorylated tau than NFT, yet are devoid of PHF-like structures. It has been proposed that GVD is formed through lysosomal autophagy of intraneuronal substances, in particular abnormally phosphorylated tau protein (Mena *et al*., 1992; Ikegami *et al*., 1996).

F. Vascular Pathology in AD

Pathological deposition of Aβ protein within the outer segments of the muscle layer in large vessels has long been recognized in AD and is referred to as congophilic amyloid angiopathy (Fig. 6.4a, see color insert; for review, see Kawai *et al*., 1993; Vinters *et al*., 1994). In addition, Aβ deposits occur in the abluminal aspects of the vascular basement membrane in capillaries and are often accompanied by the focal loss of endothelial cells (Fig 6.4b; for review, see Perlmutter, 1994). Although investigations of the chemical structure of Aβ vascular deposits have revealed a predominance of the 42 residue form, which represents almost 75% of the total Aβ protein in nondemented elderly individuals and patients with AD (Roher *et al*., 1993; Shinkai *et al*., 1995), widely varying amounts of the Aβ40 form are also deposited in AD vessel walls and may contribute decisively to the development of congophilic amyloid angiopathy. Furthermore, β-APP has been colocalized with white matter lesions in the human brain, and Aβ deposits have also been identified in AD white matter (Gravina *et al*., 1995). The putative source of vascular, and parenchymal Aβ deposits remains controversial in regard to whether they are of blood borne, local vascular, or central neuronal origin. The biochemical similarities between Aβ deposits

in SP and vessels and the well known permeability of the blood–brain barrier to soluble Aβ protein had initially suggested a possible blood origin of brain Aβ deposits (Joachim *et al*., 1988; Zlokovic *et al*., 1993). However, no regional correlation has been found between SP and Aβ vascular deposits within the cerebral cortex in AD (Lippa *et al*., 1993). Microglial-like perivascular cells within the vascular basement membrane express APP and may thus represent a possible source of vascular amyloid (Banati *et al*., 1994; Perlmutter, 1994; Wisniewski and Wiegel, 1994). In large vessels, Aβ protein may be produced in both APP-containing degenerating vascular muscle cells and microglial-like perivascular cells, and it is thought to be primarily deposited in periarterial interstitial fluid drainage pathways (Kawai *et al*., 1993; Wisniewski and Wiegel, 1994; Weller *et al*., 1998). Alternatively, an increased intraneuronal production and reduced clearance by microglia of the Aβ protein could contribute to a redirection of peptide processing to extracellular deposits (Frautschy *et al*., 1992).

Studies have also shown a substantial decrease in the density of capillaries in AD compared to nondemented individuals. This decrease is mainly observed in layers III and VI of the frontal and temporal cortex and correlates with the laminar densities of NFT and SP. In addition, twisted, coiled, and string vessels and glomerular loops have been reported in AD as well as in other dementing conditions, such as dementia pugilistica and Guam amyotrophic lateral sclerosis (Figs. 6.4c and 6.4d). Ultrastructurally, a conspicuous lack of endothelial cells in microvessels, as well as a significant increase in the number of normally occurring gaps in endothelial continuity, has been reported in AD compared to nondemented elderly people. Vascular endothelial cells show a decreased number of mitochondria and an increased number of pinocytotic vesicles, suggesting that a metabolic deficiency leading to an accumulation of transport carriers may be present in these cells in AD. The pathogenesis of these vascular alterations remains unclear, but it has been postulated that denervation angiopathy may be causally related to the microvascular pathology in AD. These pathologic changes may be associated with an increased blood–brain permeability and deposition of Aβ protein (for review, see Gold *et al*., 1998).

III. Neuropathological Diagnosis of Alzheimer's Disease

A. General Considerations

There is considerable subjectivity and disagreement among neuropathologists in determining the diagnosis of AD. Three main reasons may explain this phenomenon: the complex and uncertain relationship between normal brain aging and AD, the ambiguous role of the clinical history of dementia in the neuropathological diagnosis of AD, and differences in staining procedures. This section summarizes the conceptual problems related to these issues as well as their implication in the routine neuropathological diagnosis of AD.

Although there is broad agreement on the description of classical features of the AD brain, a substantial interindividual variation is seen across AD cases. Most importantly, studies have shown that the majority of elderly people displayed NFT formation in hippocampal formation even in the absence of cognitive impairment or with very mild memory impairment (Price *et al*., 1991; Arriagada *et al*., 1992a,b; Hof *et al*., 1992; West *et al*., 1994; Bierer *et al*., 1995). SP may appear early in the neocortex of intellectually preserved individuals, whereas the hippocampus is relatively spared by SP formation at the onset of the degenerative process (Pearson *et al*., 1985; Lewis *et al*., 1987; Hof *et al*., 1992). In addition, numerous neuropathological analyses of nondemented people have demonstrated the presence of NFT confined to the temporal neocortex, implying that the progression in NFT density within adjacent components of the medial and inferior aspects of the temporal cortex may take place in cognitively intact individuals (Hubbard *et al*., 1990; Arriagada *et al*., 1992b; Hof *et al*., 1992; Bouras *et al*., 1993, 1994; Bierer *et al*., 1995). Whether any observed NFT and SP represent a disease process or are age-related phenomena is still a matter of debate. It is well established that both NFT and SP frequencies in cognitively intact individuals are strongly age related (for review, see Giannakopoulos *et al*., 1996). This finding has been interpreted in very different ways. Similar to atherosclerosis in the elderly, NFT and SP in the aged brain have been considered as ubiquitous findings, which represent the same pathophysiological process as in AD (Hyman, 1997). This linear relationship between brain aging and AD is far from being generally accepted. Based on neuropathological studies of the oldest-old, which showed substantial differences in the pattern and densities of lesion distribution between very old and younger elderly people without dementia, it has been proposed that AD and brain aging are two separate conditions (for review, see Giannakopoulos *et al*., 1996). Despite their differences, both points of view imply that the diagnosis of AD should be based on the severity and topography of pathological changes rather than on the presence of a qualitative marker, at least as far as NFT and SP are concerned. In contrast, the presence of qualitative differences between brain aging and AD has been proposed (West *et al*., 1994; Gómez-Isla *et al*., 1996a; Dickson, 1997; Wisniewski and Silverman, 1997). These qualitative differences include the presence of Aβ40 immunoreactive reticular amyloid deposits and PHF-positive SP in AD, which, however, has been also reported in normal brain aging (Barcikowska *et al*., 1989; Dickson, 1997; Wisniewski and Silverman, 1997), and mainly the substantial neuronal loss in the CA1 field observed in AD but not in control cases (West *et al*., 1994; Gómez-Isla *et al*., 1996a). Both neuronal and synaptic loss in association cortices are good predictors of the severity of dementia. However, they are not specific for AD and are difficult to study because they are the inferred absence of a normal tissular structure. Moreover, tissue preparation and counting procedures influence their assessment (West and Gundersen, 1990; Terry *et al*., 1994; Gómez-Isla *et al*., 1996a).

The evolution of neuropathological criteria for AD is marked by a hesitation between two theoretical positions: one which considers that the neuropathological diagnosis of AD should be made only in the presence of clinically overt dementia and the other which proposes a clear separation between the neuropathological definition of the disease and its clinical expression. The rationale for linking neuropathological diagnostic criteria to the presence of dementia is a histor-

ical one: in Alzheimer's (1907) original case, the definition of AD is based on the correlation between the presence of a clinical history of dementia and abundant NFT and SP formation within the cerebral cortex. Although the inclusion of dementia as a sine qua non criterion for the neuropathological diagnosis of AD could eliminate the problem of false-positive neuropathological diagnoses, this practice is largely controversed in the current literature. In fact, if the neuropathological diagnosis of AD changes depending on a clinical impression, it would be of little value as a ''gold standard.'' Moreover, the clinical diagnosis of dementia may not be sensitive or reliable in the early stages of the disease. Because AD is a slowly progressive and global encephalopathy, it is probable that for a long period of time, perhaps years, the accumulation of lesions below a threshold is clinically silent. In this respect, several previous reports have indicated that a progression in NFT density within adjacent cortical components of the medial and inferior aspects of the temporal cortex may be a neuropathological hallmark of incipient dementia in elderly patients presenting with normal cognitive abilities or very mild cognitive impairment (Hubbard *et al*., 1990; Hof *et al*., 1992; Bouras *et al*., 1993). If these cases are ''negative'' because of the absence of dementia, crucial information about the very early stages of AD pathogenesis could go unrecognized (Hyman, 1997; Wisniewski and Silverman, 1997).

The third source of interlaboratory variability in the neuropathological diagnosis of AD is the use of different staining procedures, particularly in the assessment of SP densities (Vallet *et al*., 1992). The routine use of immunocytochemistry makes it possible to visualize increasing number, of lesions, including diffuse Aβ deposits, as well as the different types of SP. By striving for increased sensitivity, a more comprehensive and detailed description of positive cases has been achieved. However, although Aβ immunocytochemistry shows more alterations than classic histological methods, these changes are not as well correlated with the severity of dementia (Wisniewski and Silverman, 1997). A possible exception to this rule is the mean Aβ load in the hippocampus (i.e., the percentage of this cortical area occupied by Aβ immunoreactivity), which may be a good correlate of clinical status in AD (Cummings *et al*., 1996; Bartoo *et al*., 1997). In fact, a method that reveals the largest number of lesions is not necessarily the best one, and it should be kept in mind that less sensitive methods may allow a better distinction between classification categories. This heterogeneity in staining procedures is a major handicap when consistent quantitative criteria are used, as it may cause an unacceptable level of false-positive or -negative results.

B. Current Diagnostic Systems

The first cited diagnostic criteria for AD were elaborated by a National Institute on Aging (NIA) workshop (1997) and described by Khachaturian (1985). These criteria were intended to aid in the development of uniform procedures by proposing minimal SP densities as a function of age. Although this method may appear arbitrary, it was justified by the assumption that SP formation may be partly a benign age-related phenomenon. Implicitly, these criteria recognize that SP formation is not sufficient to cause dementia, as SP densities associated with AD at younger ages are unrelated to clinical symptoms at older ages. It is thus surprising that the presence of NFT is not considered for cases over 50 years of age. Several other criticisms of Khachaturian's criteria have been formulated: the type of SP is not specified; precise quantification of SP may be technically difficult and, most importantly, could change in function of apolipoprotein E genotype; only neocortical areas were taken into account; and the role of the clinical history remains vague (Table 6.1; Schmechel *et al*., 1993; Gómez-Isla *et al*., 1996b; Hyman, 1997; Markesbery, 1997). Khachaturian's recommendations were not broadly accepted and their validity has been questioned. In a survey

TABLE 6.1 Comparison of Diagnostic Systems for AD[a]

	Khachaturian	CERAD	Braak	NIA–Reagan
Areas of interest	Frontal cortex Parietal cortex Temporal cortex	Middle frontal gyrus Sup temporal gyrus Mid temporal gyrus Inf parietal lobule	Entorhinal cortex, hippocampus Amygdala Thalamus Isocortex	Sup temporal gyrus Inf parietal lobule Middle frontal gyrus, occipital cortex Hippocampus, substantia nigra, locus coezuleus
Staining	Silver stains Congo red Thioflavin-S	Silver or thioflavin stains	Silver stains	Silver or thioflavin stains Immunostaining (for research)
Quantification	Semiquantitative	Semiquantitative	Not specified	Semiquantitative
Effect of age	Age-related SP densities	Age-related NP densities	Not related	Not defined
Main criterion	SP formation in the neocortex	NP formation in the neocortex	NFT progression within the cerebral cortex	NFT and NP formation in the cerebral cortex
Relationship to clinical data	Unspecified	Clinical history included in the diagnostic algorithm	None	Applied to demented patients

[a]SP, senile plaques; NP, neuritic plaques; NFT, neurofibrillary tangles.

of neuropathological practices performed 2 years after the original publication, only 21% of the responders used these criteria on a routine basis (Wisniewski *et al*., 1989). Data presented by Katzman and collaborators (1988) showed that only 89% of clinically confirmed AD cases and 34% of cases with no history of dementia met Khachaturian's criteria for AD. In order to validate these criteria, Tierney and collaborators (1988) made the first attempt to integrate both NFT and neuritic plaques in the neuropathological diagnosis of AD. They proposed three sets of criteria with one or more lesions per 25 × microscopic field in the hippocampus alone, in the neocortex alone, or both. The accuracy of all three sets of criteria was relatively high (81 to 88%), yet they have not been widely used.

Subsequent to the 1985 workshop, the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) proposed another set of standardized neuropathological criteria (Mirra *et al*., 1991). These semiquantitative criteria were determined as a function of the development of neuritic plaques in three age groups (less than 50, 50 to 75, and over 75). The diagnosis was based on a combination of clinical information and an ''age-related plaque score'' that reflected the maximal neocortical involvement, and a level of diagnostic certainty was assessed (i.e., definite, probable, or possible AD). These criteria are uncomplicated, limit the sensitivity and specificity problems related to quantitative criteria, and do not require extensive training. The combined CERAD categories of possible, probable, and definite AD correspond closely to cases fulfilling Khachaturian's criteria for AD (Nagy *et al*., 1998). As in these latter criteria, the hippocampal formation is ignored, despite its involvement in the pathogenesis of AD. However, the major weakness of CERAD criteria resides in that they have been inspired somewhat unilaterally by the amyloid cascade hypothesis and do not consider NFT densities in the neocortex, even though these correlate very strongly with the severity of dementia (Table 6.1). Although neuritic plaques, which are used for the diagnosis, appear to be superior than Aβ deposits in predicting the degree of cognitive impairment, neither perform nearly as well in this respect as NFT densities in the neocortex or neuronal or synaptic loss (Gómez-Isla *et al*., 1996b; Haroutunian *et al*., 1998). CERAD criteria are widely accepted, yet their validity is still poorly documented (Wisniewski and Silverman, 1997).

In 1991, the year of the publication of the CERAD criteria, Braak and Braak proposed a staging scheme for AD that was entirely dependent on the progressive development of NFT within the cerebral cortex. This scheme presupposes a predictable temporal pattern in the evolution of NFT that can be ordered in a particular regional hierarchy. In stages I and II, the transentorhinal region is preferably affected with only mild involvement of the hippocampus, the neocortex is essentially spared. Stages III and IV, referred to as ''limbic stages,'' include more abundant NFT formation in the transentorhinal and entorhinal cortex, moderate involvement of the hippocampus, and only mild neocortical pathology. Cases with stages V and VI display high NFT densities in both the hippocampus and the neocortex. In their initial report, Braak and Braak suggested that the transentorhinal stages (stages I and II) correspond to a clinically silent initial phase of AD, limbic stages to early AD, and neocortical stages (stages V and VI) to fully developed AD (Table 6.1). Although this scheme appears plausible and correlates well with clinical data (Nagy *et al*., 1999), a considerable degree of overlap without a clear-cut threshold between normal and demented cases has raised the issue of its diagnostic value (Gertz *et al*., 1998; Gold *et al*., 2000). Furthermore, it has not been demonstrated whether the precise neuropathological hierarchy proposed by Braak and Braak corresponds to stepwise decrements in cognitive function. Despite these limitations, this NFT-based staging system is a useful instrument to ameliorate probabilistic estimates of degree of dementia from neuropathological data.

In 1997, the NIA–Reagan Consensus conference attempted to integrate the experience of neuropathologists from the United States and Europe in a comprehensive set of neuropathological criteria for AD (Table 6.1). In contrast to CERAD criteria, which incorporate clinical data to provide a neuropathological diagnosis, these new criteria aim to define the likelihood that a clinically overt dementia is due to AD lesions. In other words, the neuropathologist is asking to ascertain not the presence of AD but the most likely cause of dementia. This subtle theoretical change could guarantee the role of the neuropathological diagnosis as a ''gold standard.'' The procedure takes into account both the CERAD criteria and the Braak staging scheme (Table 6.1). The use of CERAD protocols for tissue processing was adopted, but sampling and semiquantitative assessment of AD lesions must be made in several neocortical areas, hippocampal formation, substantia nigra, and locus coeruleus. In addition, the assessment of Lewy bodies with immunocytochemical methods using antiubiquitin antibodies was recommended. Conceptually, the merit of these new criteria is their attempt to conciliate the amyloid cascade hypothesis with the key role of NFT in clinicopathological correlations. Furthermore, they are rapid and easy to apply in both AD and nondemented individuals (Jellinger, 1998). Whether they are more valid than the previous ones remains to be assessed.

C. Perspectives

It has been long considered that the neuropathological assessment of AD pathologic changes provides an easy and unequivocal way to confirm the clinical diagnosis of AD. Experience accumulated since the mid-1980s suggests that the morphological diagnosis of AD depends on theoretical beliefs with respect to ''normal'' and ''pathological'' brain aging, the role of different lesions in AD pathogenesis, and the pertinence of clinical informations. What is now crucial is to decrease interlaboratory variability and ensure that the neuropathological diagnosis of AD fulfills the criteria of a ''gold standard.'' We believe that the diagnostic procedure should be influenced less by theoretical positions and consider equally NFT and SP. NFT are a good indicator of clinical progression, and their formation follows a hierarchical pattern involving the entorhinal cortex, hippocampus, inferior temporal cortex, and, later, other neocortical areas. SP are distributed widely and nonspecifically within the neocortex and are correlated poorly with AD clinical severity, yet molecular genetic data support a central role for amyloid in AD pathogenesis. We also suggest that an exhaustive description of neuropathologist's findings independent of clinical data is the only way to establish unbiased clinicopathological correlations. In the light

of these statements, the criteria proposed by the NIA–Reagan Consensus conference merit strong support.

References

Alzheimer, A. (1907). Über eine eigenartige Erkrankung der Hirnrinde. *Allg. Z. Psychiat. u. i. Grenzgeb.* **64**, 146–148.

Arai, H., Lee, V. M., Otvos, L., Jr., Greenberg, B. D., Lowery, D. E., Sharma, S. K., Schmidt, M. L., and Trojanowski, J. Q. (1990). Defined neurofilament, tau, and beta-amyloid precursor protein epitopes distinguish Alzheimer from non-Alzheimer senile plaques. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 2249–2253.

Arriagada, P. V., Growdon, J. H., Hedley-White, E. T., and Hyman, B. T. (1992a). Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology* **42**, 631–639.

Arriagada, P. V., Marzloff, K., and Hyman, B. T. (1992b). Distribution of Alzheimer-type pathologic changes in nondemented elderly individuals matches the pattern in Alzheimer's disease. *Neurology* **42**, 1681–1688.

Ashford, J. W., Soultanian, N. S., Zhang, S. X., and Geddes, J. W. (1998). Neuropil threads are collinear with MAP2 immunostaining in neuronal dendrites of Alzheimer brain. *J. Neuropathol. Exp. Neurol.* **57**, 972–978.

Ball, M. J. (1977). Neuronal loss, neurofibrillary tangles and granulovacuolar degeneration in the hippocampus with ageing and dementia. *Acta Neuropathol.* **37**, 111–118.

Banati, R. B., Schubert, P., Rothe, G., Gehrmann, J., Rudolphi, K., Valet, G., and Kreutzberg, G. W. (1994). Modulation of intracellular formation of reactive oxygen intermediates in peritoneal macrophages and microglia/brain macrophages by propentofylline. *J. Cereb. Blood Flow Metab.* **14**, 145–149.

Bancher, C., Brunner, C., Lassmann, H., Budka, H., Jellinger, K., Wiche, G., Seitelberger, F., Grundke-Iqbal, I., Iqbal, K., and Wisniewski, H. M. (1989). Accumulation of abnormally phosphorylated tau precedes the formation of neurofibrillary tangles in Alzheimer's disease. *Brain Res.* **477**, 90–99.

Barcikowska, M., Wisniewski, H. M., Bancher, C., and Grundke-Iqbal, I. (1989). About the presence of paired helical filaments in dystrophic neurites participating in the plaque formation. *Acta Neuropathol.* **78**, 225–231.

Bartoo, G. T., Nochlin, D., Chang, D., Kim, Y., and Sumi, S. M. (1997). The mean A beta load in the hippocampus correlates with duration and severity of dementia in subgroups of Alzheimer disease. *J. Neuropathol. Exp. Neurol.* **56**, 531–540.

Bierer, L. M., Hof, P. R., Purohit, D. P., Carlin, L., Schmeidler, J., Davis, K.L., and Perl, D.P. (1995). Neocortical neurofibrillary tangles correlate with dementia severity in Alzheimer's disease. *Arch. Neurol. (Chicago)* **52**, 81–88.

Bobinski, M., de Leon, M. J., Tarnawski, M., Wegiel, J., Reisberg, B., Miller, D. C., and Wisniewski, H. M. (1998). Neuronal and volume loss in CA1 of the hippocampal formation uniquely predicts duration and severity of Alzheimer's disease. *Brain Res.* **805**, 267–269.

Boise, L. H., Gonzàlez-Garcìa, M., Postema, C. E., Ding, L., Lindsten, T., Turka, L. A., Mao, X., Nùnez, G., and Thompson, C. B. (1993). bcl-x, a bcl-2 related gene that functions as a dominant regulator of apoptotic cell death. *Cell (Cambridge, Mass.)* **74**, 597–608.

Bouras, C., Hof, P. R., and Morrison, J. H. (1993). Neurofibrillary tangle densities in the hippocampal formation in a non-demented population define subgroups of patients with differential early pathologic changes. *Neurosci. Lett.* **153**, 131–135.

Bouras, C., Hof, P. R., Giannakopoulos, P., Michel, J. P., and Morrison, J. H. (1994). Regional distribution of neurofibrillary tangles and senile plaques in the cerebral cortex of elderly patients: A quantitative evaluation of a one-year autopsy population from a geriatric hospital. *Cereb. Cortex* **4**, 138–150.

Braak, E., Braak, H., and Mandelkow, E.-M. (1994). A sequence of cytoskeleton changes related to the formation of neurofibrillary tangles and neuropil threads. *Acta Neuropathol.* **87**, 554–567.

Braak, H., and Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* **82**, 239–259.

Bramblett, G. T., Trojanowski, J. Q., and Lee, V. M.-Y. (1992). Regions with abundant neurofibrillary pathology in human brain exhibit a selective reduction in levels of binding-competent tau and accumulation of abnormal tau-isoforms (A68 proteins). *Lab. Invest.* **66**, 212–222.

Brion, J. P. (1990). Molecular pathology of Alzheimer amyloid and neurofibrillary tangles. *Semin. Neurosci.* **2**, 89–100.

Brion, J. P., Couck, A. M., Bruce, M., Anderton, B., and Flament-Durand, J. (1991). Synaptophysin and chromogranin. A immunoreactivities in senile plaques of Alzheimer's disease. *Brain Res.* **539**, 143–150.

Bugiani, O., Giaconne, G., Verga, L., Pollo, B., Ghetti, B., Frangione, B., and Tagliavini, F. (1990). Alzheimer patients and Down patients: Abnormal presynaptic terminals are related to cerebral preamyloid deposits. *Neurosci. Lett.* **119**, 56–59.

Checler, F. (1995). Processing of the β-amyloid precursor protein and its regulation in Alzheimer's disease. *J. Neurochem.* **65**, 1431–1444.

Coleman, P. D., and Flood, D. G. (1987). Neuron numbers and dendritic extent in normal aging and Alzheimer's disease. *Neurobiol. Aging* **8**, 521–545.

Cotman, C. W. (1998). Apoptosis decision cascades and neuronal degeneration in Alzheimer's disease. *Neurobiol. Aging* **19**, S29–S32.

Cotman, C. W., and Anderson, A. J. (1995). A potential role for apoptosis in neurodegeneration and Alzheimer's disease. *Mol. Neurobiol.* **10**, 19–45.

Cras, P., Smith, M. A., Richey, P. L., Siedlak, S. L., Mulvihill, P., and Perry, G. (1995). Extracellular neurofibrillary tangles reflect neuronal loss and provide further evidence of extensive protein cross-linking in Alzheimer disease. *Acta Neuropathol.* **89**, 291–295.

Cummings, B. J., Pike, C. J., Shankle, R., and Cotman, C. W. (1996). Beta amyloid deposition and other measures of neuropathology predict cognitive status in Alzheimer's disease. *Neurobiol Aging* **17**, 921–933.

Dickson, D.W. (1997). Neuropathological diagnosis of Alzheimer's disease: A perspective from longitudinal clinicopathological studies. *Neurobiol. Aging* **18**, S21–S26.

Frautschy, S. A., Cole, G. M., and Baird, A. (1992). Phagocytosis and deposition of vascular β-amyloid in rat brains injected with Alzheimer β-amyloid. *Am. J. Pathol.* **140**, 1389–1399.

Gertz, H. J., Xuereb, J., Huppert, F., Brayne, C., McGee, M. A., Paykel, E., Harrington, C., Mukaetova-Ladinska, E., Arendt, T., and Wischik, C. M. (1998). Examination of the validity of the hierarchical model of neuropathological staging in normal aging and Alzheimer's disease. *Acta Neuropathol.* **95**, 154–158.

Giaconne, G., Verga, L., Perez, J., Racagni, G., Pedrotti, B., Salmona, M., Ghiso, J., Frangione, B., Islam, K., Bugiani, O., and Tagliavini, F. (1994). Interaction between βAPP and tau: A possible link between β-amyloid and NFT formation. *Neurobiol. Aging* **15**, S77–S78.

Giannakopoulos, P., Hof, P. R., Surini, M., Michel, J.-P., and Bouras, C. (1993). Quantitative immunohistochemical analysis of the distribution of neurofibrillary tangles and senile plaques in the cerebral cortex of nonagenarians and centenarians. *Acta Neuropathol.* **85**, 601–610.

Giannakopoulos, P., Hof, P. R., Kövari, E., Vallet, P. G., and Bouras, C. (1996). Distinct patterns of neuronal loss and Alzheimer's disease lesion distribution in elderly individuals older than 90 years. *J. Neuropathol. Exp. Neurol.* **55**, 1210–1120.

Goedert, M., Jakes, R., Spillantini, M. G., Hasegawa, M., Smith, M. J., and Crowther, R. A. (1996). Assembly of microtubule-associated protein tau into Alzheimer-like filaments induced by sulfated glycosaminoglycans. *Nature (London)* **383**, 550–553.

Gold, G., Giannakopoulos, P., and Bouras, C. (1998). Re-evaluating the role of vascular changes in the differential diagnosis of Alzheimer's disease and vascular dementia. *Eur. Neurol.* **40**, 121–129.

Gold, G., Bouras, C., Kövari, E., Canuto, A., Gonzáles Glaría, B., Malky, A., Hof, P. R., Michel, J. P., and Giannakopoulos, P. (2000). Clinical validity of Braak neuropathological staging in the oldest-old. *Acta Neuropathol.* **99**, 579–582.

Gómez-Isla, T., Price, J. L., McKeel, D. W., Jr., Morris, J. C., Growdon, J. H., and Hyman, B. T. P. (1996a). Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease *J. Neurosci*. **16**, 4491–4500.

Gómez-Isla, T., West, H. L., Rebeck, G. W., Harr, S. D., Growdon, J. H., Locasio, J. T., Perls, T. T., Lipsitz, L. A., and Hyman, B. T. (1996b). Clinical and pathological correlates of apolipoprotein E ϵ4 in Alzheimer's disease. *Ann. Neurol*. **39**, 62–70.

Gowing, E., Roher, A. E., Woods, A. S., Cotter, R. J., Chaney, M., Little, S. P., and Ball, M. J. (1994). Chemical characterization of the Aβ17-42 peptide, a component of diffuse amyloid deposits of Alzheimer's disease. *J. Biol. Chem*. **269**, 10987–10990.

Gravina, S. A., Ho, L., Eckman, C. B., Long, K. E., Otvos, L., Jr., Younkin, L. H., Suzuki, N., and Younkin, S. G. (1995). Amyloid beta protein (A beta) in Alzheimer's disease brain. Biochemical and immunocytochemical analysis with antibodies specific for forms ending at A beta 40 or A beta 42(43). *J. Biol. Chem*. **270**, 7013–7016.

Grundke-Iqbal, I., Iqbal, K., Tung, Y. C., Quinlan, M., Wisniewski, H. M., and Binder, L.I. (1986). Abnormal phosphorylation of the microtubule-associated protein tau in Alzheimer cytoskeletal pathology. *Proc. Natl. Acad. Sci. U.S.A*. **83**, 4913–4917.

Hardy, J. (1994). Alzheimer's disease: The present situation and our tasks. *Neurobiol. Aging* **15**, S111–S112.

Haroutunian, V., Perl, D. P., Purohit, D. P., Marin, D., Khan, K., Lantz, M., Davis, K. L., and Mohs, R. C. (1998). Regional distribution of neuritic plaques in the nondemented elderly and subjects with very mild Alzheimer disease. *Arch. Neurol. (Chicago)* **55**, 1185–1191.

Hauw, J.-J., Vignolo, P., Duyckaerts, C., Beck, H., Forette, F., Henry, J.F., Laurent, M., Piette, F., Sachet, A., and Berthaux, P. (1986). Etude neuropathologique de 12 centenaires: La fréquence de la démence sénile de type Alzheimer n'est pas particulièrement élevée dans ce groupe de personnes très agées. *Rev. Neurol*. **142**, 107–115.

Hirano, A. (1994). Hirano bodies and related neuronal conclusions. *Neuropathol. Appl. Neurobiol*. **20**, 3–11.

Hof, P. R., Bierer, L. M., Perl, D. P., Delacourte, A., Buée, L., Bouras, C., and Morrison, J. H. (1992). Evidence for early vulnerability of the medial and inferior aspects of the temporal lobe in an 82-year-old patient with preclinical signs of dementia—Regional and laminar distribution of neurofibrillary tangles and senile plaques. *Arch. Neurol. (Chicago)*. **49**, 946–953.

Hubbard, B. M., Fenton, G. W., and Anderson, J. M. (1990). A quantitative histological study of early clinical and preclinical Alzheimer's disease. *Neuropathol. Appl. Neurobiol*. **16**, 111–121.

Hyman, B. T. (1997). The neuropathological diagnosis of Alzheimer's disease: Clinical-pathological studies. *Neurobiol. Aging* **18**, S27–S32.

Ikegami, K., Kimura, T., Katsuragi, S., Ono, T., Yamamoto, H., Miyamoto, E., and Miyawaka, T. (1996). Immunohistochemical examination of phosphorylated tau in granulovacuolar degeneration granules. *Psychiatry Clin. Neurosci*. **50**, 137–140.

Iqbal, K., Alonso, A., Gong, C. X., Khatoon, S., Pei., J. J., Wang, J. Z., and Grundke-Iqbal, I. (1998). Mechanisms of neurofibrillary degeneration and the formation of neurofibrillary tangles. *J. Neural Transm*. **53**, 169–180.

Iwatsubo, T., Odaka, A., Suzuki, N., Mizusawa, H., Nukina, N., and Ihara, Y. (1994). Visualization of Aβ42(43) and Aβ(40) in senile plaques with end-specific Aβ monoclonals: Evidence that an initially deposited species is Aβ42(43). *Neuron* **13**, 45–53.

Iwatsubo, T., Saido, T. C., Mann, D. M. A., Lee, V. M.-Y., and Trojanowski, J. Q. (1996). Full-length amyloid-β (1-42(43)) and amino-terminally modified and truncated amyloid β42(43) deposit in diffuse plaques. *Am. J. Pathol*. **149**, 1823–1830.

Jellinger, K. A. (1998). The neuropathological diagnosis of Alzheimer's disease. *J. Neural Transm., Suppl*. **53**, 97–118.

Jellinger, K. A., and Bancher, C. (1998). Senile dementia with tangles (tangle predominant form of senile dementia). *Brain Pathol*. **8**, 367–376.

Joachim, C. L., Morris, J. H., and Selkoe, D. J. (1988). Clinically diagnosed Alzheimer's disease: Autopsy results in 150 cases. *Ann. Neurol*. **24**, 50–56.

Jordan-Sciutto, K., Dragich, J., Walcott, D., and Bowser, R. (1998). The presence of FAC1 protein in Hirano bodies. *Neuropathol. Appl. Neurobiol*. **24**, 359–366.

Katzman, R., Terry, R., DeTeresa, R., Brown, T., Davies, P., Fuld, P., Renbing, X., and Peck, A. (1988). Clinical, pathological, and neurochemical changes in dementia: A subgroup with preserved mental status and numerous neocortical plaques. *Ann. Neurol*. **23**, 138–144.

Kawai, M., Kalaria, R. N., Cras, P., Siedlak, S. L., Velasco, M. E., Shelton, E. R., Chan, H. W., Greenberg, B. D., and Perry, G. (1993). Degeneration of vascular muscle cells in cerebral amyloid angiopathy of Alzheimer disease. *Brain Res*. **623**, 142–146.

Khachaturian, Z. S. (1985). Diagnosis of Alzheimer's disease. *Arch. Neurol. (Chicago)* **42**, 1097–1105.

Kitamura, Y., Shimohama, S., Kamoshima, W., Ota, T., Matsuoka, Y., Nomura, Y., Smith, M. A., Perry, G., Whitehouse, P. J., and Taniguchi, T. (1998). Alteration of proteins regulating apoptosis, Bcl-2, Bcl-x, Bax, Bak, Bad, ICH-1 and CPP32 in Alzheimer's disease. *Brain Res*. **780**, 260–269.

Knowles, R. B., Gómez-Isla, T., and Hyman, B. T. (1998). Aβ associated neuropil changes: Correlation with neuronal loss and dementia. *J. Neuropathol. Exp. Neurol*. **57**, 1122–1130.

Lewis, D. A., Campbell, M. J., Terry, R. D., and Morrison, J. H. (1987). Laminar and regional distribution of neurofibrillary tangles and neuritic plaques in Alzheimer's disease: A quantitative study of visual and auditory cortices. *J. Neurosci*. **7**, 1799–1808.

Lippa, C. F., Hamos, J. E., Smith, T. W., Pulaski-Salo, D., and Drachman, D. A. (1993). Vascular amyloid deposition in Alzheimer's disease. Neither necessary nor sufficient for the local formation of plaques and tangles. *Arch. Neurol. (Chicago)* **50**, 1088–1092.

MacGibbon, G. A., Lawlor, P. A., Sirimanne, E. S., Walton, M. R., Connor, B., Young, D., Williams, C., Gluckman, P., Faull, R. L., Hughes, P., and Dragunow, M. (1997). Bax expression in mammalian neurons undergoing apoptosis, and in Alzheimer's disease hippocampus. *Brain Res*. **750**, 223–234.

Mann, D. M., and Esiri, M. M. (1989). The pattern of acquisition of plaques and tangles in the brains of patients under 50 years of age with Down's syndrome. *J. Neurol. Sci*. **89**, 169–179.

Mann, D. M., Iwatsubo, T., Ihara, Y., Cairns, N. J., Lantos, P. L., Bogdanovic, N., Lannfelt, L., Winblad, B., Maat-Schieman, M. L. C., and Rossor, M. N. (1996). Predominant deposition of amyloid-beta (42(43)) in plaques in cases of Alzheimer's disease and hereditary cerebral hemorrhage associated with mutations in the amyloid precursor protein gene. *Am. J. Pathol*. **148**, 1257–1266.

Markesbery, W. R. (1997). Neuropathological criteria for the diagnosis of Alzheimer's disease. *Neurobiol. Aging* **18**, S13–S19.

Masliah, E., Terry, R. D., Aldorf, M., DeTeresa, R. M., and Hansen, L.A. (1991). Cortical and subcortical patterns of synaptophysin-like immunoreactivity in Alzheimer disease. *Am. J. Pathol*. **138**, 235–246.

Masliah, E., Mallory, M., Hansen, L., DeTeresa, R., and Terry, R.D. (1993). Quantitative synaptic alterations in the human neocortex during normal aging. *Neurology* **43**, 192–197.

Matsuo, S. E., Shi, R. W., Billingsley, M. L., Van de Voorde, A., O'Connor, M., Trojanowski, J. Q., and Lee, V. M.-Y. (1994). Biopsy-derived adult human brain tau is phosphorylated at many of the same sites as Alzheimer's disease paired helical filament tau. *Neuron* **13,** 989–1002.

Mena, R., Robitaille, Y., and Cuello, A. C. (1992). New patterns of intraneuronal accumulation of the microtubular binding domain of tau in granulovacuolar degeneration. *J. Geriatr. Psychiatry Neurol*. **5**, 132–141.

Mirra, S. S., Heyman, A., McKeel, D., Sumi, S. M., Crain, B. J., Brownlee, L. M., Vogel, F. S., Hughes, J. P., Van Belle, G., and Berg, L. (1991). The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* **41**, 479–486.

Morrison, J. H., and Hof, P. R. (1997). Life and death of neurons in the aging brain. *Science* **278**, 412–419.

Mountjoy, C. Q., Roth, M., Evans, N. J. R., and Evans, H. M. (1983). Cortical neuronal counts in normal elderly controls and demented patients. *Neurobiol. Aging* **4**, 1–11.

Munch, G., Cunnigham, A. M., Riederer, P., and Braak, E. (1998). Advanced glycation endproducts are associated with Hirano bodies in Alzheimer's disease. *Brain Res*. **796**, 307–310.

Nagy, Zs., Esiri, M. M., Joachim, C., Jobst, K. A., Morris, J. H., King, E. M., Hindley, N. J., McDonald, B., Litchfield, S., Barnetson, L., and Smith, A. D. (1998). Comparison of pathological diagnostic criteria for Alzheimer disease. *Alzheimer Dis. Assoc. Disord*. **12**, 182–189.

Nagy, Zs., Hindley, N. J., Braak, H., Braak, E., Yilmarez-Hanke, D. M., Schultz, C., Barnetson, L., King, E. M.-F., Jobst, K. A., and Smith, A. D. (1999). The progression of Alzheimer's disease from limbic regions to the neocortex: Clinical, radiological and pathological relationships. *Dementia* **10**, 115–120.

National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. (1997). Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. *Neurobiol. Aging* **18**, S1–S2.

O'Barr, S., Schultz, J., and Rogers, J. (1996). Expression of the protooncogene bcl-2 in Alzheimer's disease brain. *Neurobiol. Aging* **17**, 131–136.

Oltvai, Z. N., Milliman, C. L., and Korsmeyer, S. J. (1993). Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. *Cell (Cambridge, Mass.)* **74**, 609–619.

Pakkenberg, B., and Gundersen, H. J. (1997). Neocortical neuron number in humans: Effect of sex and age. *J. Comp. Neurol*. **384**, 312–320.

Pearson, R. C. A., Esiri, M. M., Hiorns, R. W., Wilcock, G. K., and Powell, T. P. S. (1985). Anatomical correlates of the distribution of the pathological changes in the neocortex in Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A*. **82**, 4531–4534.

Perlmutter, L. S. (1994). Microvascular pathology and vascular basement membrane components in Alzheimer's disease. *Mol. Neurobiol*. **9**, 33–40.

Perry, G., Kawai, M., and Tabaton, M. (1991). Neuropil threads of Alzheimer's disease show a marked alteration of the normal cytoskeleton. *J. Neurosci*. **11**, 1748–1755.

Price, J. L., Davis, P. B., Morris, J. C., and White, D. L. (1991). The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. *Neurobiol. Aging* **12**, 295–312.

Robakis, N. K. (1994). β-amyloid and amyloid precursor protein: Chemistry, molecular biology, and neuropathology. *In* "Alzheimer Disease" (R. D. Terry, R. Katzman, and K. L. Bick, eds.), pp. 317–326. Raven Press, New York.

Roher, A. E., Lowenson, G. D., Clarke, S., Woods, A. S., Cotter, R. J., Gowing, E., and Ball, M. J. (1993). β-amyloid-(1-42) is a major component of cerebrovascular amyloid deposits: Implications for the pathology of Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A*. **90**, 10836–10840.

Satou, T., Cummings, C. W., and Cotman, C. W. (1995). Immunoreactivity for Bcl-2 protein within neurons in the Alzheimer's disease brain increases with disease severity. *Brain Res*. **697**, 35–43.

Schmechel, D. E., Saunders, A. M., Strittmatter, W. J., Crain, B., Hulette, C., Joo, S. H., Pericak-Vance, M. A., Goldgaber, D., and Roses, A. D. (1993). Increased amyloid β-peptide deposition as a consequence of apolipoprotein E genotype in late-onset Alzheiemr's disease. *Proc. Natl. Acad. Sci. U.S.A*. **90**, 9649–9653.

Schwab, C., Akiyama, H., McGeer, E. G., and McGeer, P. L. (1998). Extracellular neurofibrillary tangles are immunopositive for the 40 carboxy-terminal sequence of beta-amyloid protein. *J. Neuropathol. Exp. Neurol*. **57**, 1131–1137.

Shin, R.-W., Bramblett, G. T., Lee, V. M.-Y., and Trojanowski, J. Q. (1993). Alzheimer disease A68 proteins injected into rat brain induce co-deposits of β-amyloid, ubiquitin, and a1-antichymotrypsin. *Proc. Natl. Acad. Sci. U.S.A*. **90**, 6825–6828.

Shinkai, Y., Yoshimura, M., Ito, Y., Odaka, A., Suzuki, N., Yanagisawa, K., and Ihara, Y. (1995). Amyloid β-protein 1-40 and 1-42(43) in the soluble fraction of extra- and intracranial blood vessels. *Ann. Neurol*. **38**, 421–428.

Simic, G., Kostovic, I., Winblad, B., and Bogdanovic, N. (1997). Volume and number of neurons of the human hippocampal formation in normal aging and Alzheimer's disease. *J. Comp. Neurol*. **379**, 482–494.

Smale, G., Nichols, N. R., Brady, D. R., Finch, C. E., and Horton, W. E., Jr. (1995). Evidence for apoptotic cell death in Alzheimer's disease. *Exp. Neurol*. **133**, 225–230.

Smith, M. A., Siedlak, S. L., Rickey, P. L., Mulvihill, P., Ghiso, J., Frangione, B., Tagliavini, F., Giaconne, G., Bugiani, O., Praprotnik, D., Kalaria, R. N., and Perry, G. (1995). Tau protein directly interacts with the amyloid β-protein precursor: Implications for Alzheimer disease. *Nature Med*. **1**, 365–369.

Spillantini M. G., Bird, T. D., and Ghetti, B. (1998). Frontotemporal dementia and Parkinsonism linked to chromosome 17: A new group of tauopathies. *Brain Pathol*. **8**, 387–402.

Stadelmann, C., Bruck, W., Bancher, C., Jellinger, K., and Lassmann, H. (1998). Alzheimer disease: DNA fragmentation indicates increased neuronal vulnerability, but not apoptosis. *J. Neuropathol. Exp. Neurol*. **57**, 456–464.

Su, J. H., Satou, T., Anderson, A. J., and Cotman, C. W. (1996.) Upregulation of Bcl-2 is associated with neuronal DNA damage in Alzheimer's disease. *NeuroReport* **7**, 437–440.

Su, J. H., Deng, G., and Cotman, C. W. (1997). Bax protein expression is increased in Alzheimer's brain: Correlations with DNA damage, Bcl-2 expression, and brain pathology. *J. Neuropathol. Exp. Neurol*. **56**, 86–93.

Terry, R. D., Hansen, L. A., DeTeresa, R., Davies, P., Tobias, H., and Katzman, R. (1987). Senile dementia of the Alzheimer type without neocortical neurofibrillary tangle. *J. Neuropathol. Exp. Neurol*. **46,** 262–268.

Terry, R. D., Masliah, E., Salmon, D. P., Butters, N., DeTeresa, R., Hill, R., Hansen, L. A., and Katzman, R. (1991). Physical basis of cognitive alterations in Alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. *Ann. Neurol*. **30**, 572–580.

Terry, R. D., Masliah, E., and Hansen, L. A. (1994). Structural basis of the cognitive alterations in Alzheimer disease. *In* "Alzheimer Disease" (R. D. Terry, R. Katzman, and K.L. Bick, eds.), pp. 179–196. Raven Press, New York.

Tierney, M. C., Fisher, R., Lewis, A., Zorzitto, M., Snow, G., Reid, D., and Nieuwstraten, P. (1988). The NINDCS-ADRDA Work Group criteria for the clinical diagnosis of probable Alzheimer's disease: A clinicopathologic study of 57 cases. *Neurology* **36**, 359–364.

Tomlinson, B. E., Blessed, G., and Roth, M. (1968). Observations on the brains of ND old people. *J. Neurol. Sci*. **7**, 331–356.

Trojanowski, J. Q., Schmidt, M. L., Shin, R. W., Bramblett, G. T., Rao, D., and Lee, V. M.-Y. (1993). Altered tau and neurofilament proteins in neurodegenerative diseases: Diagnostic implications for Alzheimer's disease and Lewy body dementias. *Brain Pathol*. **3**, 45–54.

Ulrich, J. (1985). Alzheimer changes in non-demented patients younger than sixty-five: Possible early stages of Alzheimer's disease and senile dementia of Alzheimer type. *Ann. Neurol*. **17**, 273–277.

Vallet, P. G., Guntern, R., Hof, P. R., Golaz, J., Delacourte, A., Robakis, N. K., and Bouras, C. (1992). A comparative study of histological and immunohistochemical methods for neurofibrillary tangles and senile plaques in Alzheimer's disease. *Acta Neuropathol*. **83**, 170–178.

van de Nes, J. A., Kamphorst, W., Ravid, R., and Swaab, D. F. (1998). Comparison of beta-protein/A4 deposits and Alz-50-stained cytoskele-

tal changes in the hypothalamus and adjoining areas of Alzheimer's disease patients: Amorphic plaques and cytoskeletal changes occur independently. *Acta Neuropathol*. **96**, 129–138.

Vickers, J. C., Delacourte, A., and Morrison, J. H. (1992). Progressive transformation of the cytoskeleton associated with normal aging and Alzheimer's disease. *Brain Res*. **594**, 273–278.

Vickers, J. C., Riederer, B. M., Marugg, R. A., Buée-Scherrer, V., Buée, L., Delacourte, A., and Morrison, J. H. (1994). Alterations in neurofilament protein immunoreactivity in human hippocampal neurons related to normal aging and Alzheimer's disease. *Neuroscience* **62**, 1–13.

Vickers, J. C., Chin, D., Edwards, A. M., Sampson, V., Harper, C. and Morrison, J. H. (1996). Dystrophic neurite formation associated with age-related β amyloid deposition in the neocortex: Clues to the genesis of neurofibrillary pathology. *Exp. Neurol*. **141**, 1–11.

Vinters, H. V., Secor, D. L., Read, S. L., Frazee, J. G., Tomiyasu, U., Stanley, T. M., Ferreiro, J. A., and Akers, M.-A. (1994). Microvasculature in brain biopsy specimens from patients with Alzheimer's disease: An immunohistochemical and ultrastructural study. *Ultrastruct. Pathol*. **18**, 333–348.

Weller, R. O., Massey, A., Newman, T. A., Hutchings, M., Kuo, Y. M., and Roher, A. E. (1998). Cerebral amyloid angiopathy: Amyloid beta accumulates in putative interstitial fluid drainage pathways in Alzheimer's disease. *Am. J. Pathol*. **153**, 725–733.

West, M. J. (1993). Regionally specific loss of neurons in the aging human hippocampus. *Neurobiol. Aging* **14**, 287–293.

West, M. J., and Gundersen, H. J. G. (1990). Unbiased stereological estimation of the number of neurons in the human hippocampus. *J. Comp. Neurol*. **296,** 1–22.

West, M. J., Coleman, P. D., Flood, D. G., and Troncoso, J.C. (1994). Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. *Lancet* **344**, 769–772.

Wisniewski, H. M., and Silverman, W. (1997). Diagnostic criteria for the neuropathological assessment of Alzheimer's disease: Current status and major issues. *Neurobiol. Aging* **18**, S43–S50.

Wisniewski, H. M., and Wiegel, J. (1994). β-amyloid formation by myocytes of leptomeningeal vessels. *Acta Neuropathol*. **87**, 233–241.

Wisniewki, H. M., Rabe, A., Zigman, W., and Silverman, W. (1989). Neuropathological diagnosis of Alzheimer disease. *J. Neuropathol. Exp. Neurol*. **48**, 606–609.

Xu, M., Shibayama, H., Kobayashi, H., Yamada, K., Ishihara, R., Zhao, P., Takeuchi, T., Yoshida, K., Inagaki, T., and Nokura, K. (1992). Granulovacuolar degeneration in the hippocampal cortex of aging and demented patients—a quantitative study. *Acta Neuropathol*. **85**, 1–9.

Yamagushi, H., Nakazato, Y., Kawarabayashi, T., Ishiguro, K., Ihara, Y., Morimatsu, M., and Hirai, S. (1991). Extracellular neurofibrillary tangles associated with degenerating neurites and neuropil threads in Alzheimer-type dementia. *Acta Neuropathol*. **81**, 603–609.

Yen, S.-H., Liu, W.-K., Hall, F. L., Yan, S.-D., Stern, D., and Dickson, D. W. (1995). Alzheimer neurofibrillary lesions: Molecular nature and potential role of different components. *Neurobiol. Aging* **16**, 381–387.

Zhong, L. T., Sarafian, T., Kane, D. J., Charles, A. C., Mah, S. P., Edwards, R. H., and Bredesen, D. E. (1993). bcl-2 inhibits death of central neural cells induced by multiple agents. *Proc. Natl. Acad. Sci. U.S.A*. **90**, 4533–4537.

Zlokovic, B. V., Ghiso, J., Mackic, J. B., McComb, J. G., Weiss, M. H., and Frangione, B. (1993). Blood-brain barrier transport of circulating Alzheimer's amyloid beta. *Biochem. Biophys. Res. Commun*. **197,** 1034–1040.

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7

Morphological Changes in Human Cerebral Cortex during Normal Aging

Alzheimer's disease (AD) is the most frequent cause of dementia in industrialized countries and has become one of the most studied age-related neuropsychiatric illnesses. However, the clinical assessment of AD in its early stages is still awaiting accurate and reliable tools that can be used to assess the progression of the disease and to evaluate emerging therapeutics interventions, although numerous studies have shown that results obtained from the neuropathological examination of AD brains are usually well correlated with clinical data. The major histopathologic hallmarks of AD are extracellular amyloid deposits referred to as senile plaques (SP), intraneuronal fibrillar inclusions referred to as neurofibrillary tangles (NFT), and neuronal and synaptic loss. The decline of cognitive functions observed in AD patients is reflected by the regional distribution and density of these lesions and by synapse loss and neuron death, which results in the disruption of major cortical circuits. However, SP and NFT are also present in brains from cognitively and intellectually preserved elderly individuals. A consistent neurofibrillary pathology restricted to the entorhinal cortex and the hippocampus occurs consistently in the brain of unaffected individuals and in patients with very mild cognitive impairment. Conversely, the distribution pattern of SP is variable among these individuals. Investigation of the regional and laminar localization of AD-related lesions simultaneously in nondemented subjects and patients with very mild cognitive impairment is important to understand the difference between normal aging and dementing process and to elucidate the pathogenetic mechanisms leading to the onset of the disease.

I. Histopathological Changes in Cerebral Cortex in Alzheimer's Disease (AD) and Aging

A. Neurofibrillary Tangles (NFT) and Senile Plaques (SP) Lesions in AD

Neuronal loss, NFT, and SP are the morphological signature of AD and of several neurodegenerative disorders (Mirra *et al*., 1993). NFT and SP have been extensively studied biochemically, and it is now well established that their major components are the modified microtubule-associated protein tau and the amyloid peptide, respectively (Delacourte and Buée, 1997; Hof *et al*., 1999). Conversely, it is still unclear how these compounds are involved in the pathogenic cascade leading to the onset of the disease and how they interact with each other. Neuropathological studies of brains from patients presenting with AD have shown that NFT and SP are not distributed uniformly in the cerebral cortex. Thus, NFT and SP display a preferential laminar and regional distribution and do not affect equally the different types of neurons. In fact, in severe AD cases, NFT are present in a high density in the medial temporal cortex, especially in layer III of the transentorhinal cortex, in layer II of the entorhinal cortex, and in the hippocampal formation where the CA1 field and the subiculum display the highest densities of NFT. The neocortical association areas are also severely affected in these cases, with high densities of NFT in prefrontal and temporal cortices. The NFT formation occurs selectively in the pyramidal cells of supragranular layers II–III and infragranular layers V–VI. The primary sensory and motor cortices are relatively spared by the neurofibrillary degeneration. Conversely, amyloid deposition may be present in these areas and are commonly observed in the neocortical association areas, where SP tend to be more numerous in supragranular layers than in infragranular layers (Rogers and Morrison, 1985; Arnold *et al*., 1991; Braak and Braak, 1991; Bouras *et al*., 1994).

The severity of dementia has been correlated to the distribution and density of both NFT and SP in the cerebral cortex. A significant correlation has been established between the SP frequency in several cortical regions and the dementia severity assessed within 6 weeks of death using the Blessed Dementia Scale (Tomlinson *et al*., 1970). Nevertheless, the most significant correlation that has been found is related to the density of NFT, especially in the temporal neocortex, entorhinal cortex, and hippocampus (Arriagada *et al*., 1992a; Bouras *et al*., 1993; 1994), as well as to synapse loss in neocortical areas

(Terry *et al*., 1991). Finally, the specific distribution of NFT and SP within the cerebral cortex clearly argues in favor of a vulnerability to degeneration restricted to a subset of identifiable cortical neurons. Thus, the distribution of lesions indicates that the neurons of origin of long corticocortical and hippocampal projections are highly vulnerable in AD. The dysfunction of such pathways between hippocampus and neocortex and between the neocortical association areas may represent the morphological substrate for the impairment of cognitive functions in AD (see Chapter 9).

B. NFT and SP Lesions in Normal Aging

The presence of the characteristic cerebral lesions of NFT and SP are required to ascertain the neuropathological diagnosis of AD. Nevertheless, these pathological hallmarks are also observed in the cerebral cortex of nondemented cases as well as in elderly subjects with mild cognitive impairment. Studies of cognitively intact elderly people have demonstrated that NFT formation and amyloid deposition are common features of normal brain aging and that the number of NFT, but not of SP, is increased significantly with age (Fig. 7.2). NFT regional distribution is relatively consistent across individuals, whereas the SP pattern could be heterogeneous (Crystal *et al*., 1988; Braak and Braak, 1990; Price *et al*., 1991; Arriagada et al., 1992a,b; Bouras *et al*., 1993, 1994). Thus, NFT are present in the entorhinal cortex and the CA1 in nondemented older cases, and only very rare lesions are observed in the temporal and superior frontal neocortex (Braak and Braak, 1990; Price *et al*., 1991; Arriagada *et al*., 1992b; Bouras *et al*., 1993). In cases presenting with mild symptoms of cognitive decline, NFT are always present in the medial and inferior temporal cortex, including hippocampus, entorhinal, and perirhinal cortices (Figs. 7.1 and 7.2). Lesions occur preferentially in layer II of the entorhinal cortex and, to a lesser extent, in layers III and V. Other neocortical regions display only sparse NFT (Hof *et al*., 1992; Bouras *et al*., 1993, 1994; Bierer *et al*., 1995). NFT spread out progressively in the neocortex as the dementia worsens, leading in these cases to the typical neuropathologic profile of AD with a severe involvement of association neocortical areas (Arnold *et al*., 1991). The topogaphic distribution of SP in nondemented elderly individuals shows a different pattern than NFT, as SP occur primarily in the inferior temporal neocortex (Brodmann's area 20) and in the occipital cortex. In this latter case, SP are distributed preferentially throughout layer IV (Braak *et al*., 1989). The hippocampal formation, the entorhinal and perirhinal cortices, and the frontal cortex are less often affected. SP are exclusively primitive plaques without any amyloid-dense core or crown of dystrophic neurites. In very mild or mildly demented cases, higher densities of plaques may be observed in neocortical areas and also in subcortical structures such as basal ganglia. These amyloid deposits are mainly primitive plaques that are localized preferentially in the supragranular cortical layers. While neurodegeneration is evolving, amyloid deposits affect all cortical areas and many subcortical structures and are mainly mature plaques with a dense amyloid core and dystrophic neurites. The visual areas of the occipital cortex have relatively high SP densities (Price *et al*., 1991; Arriagada *et al*., 1992a; Bouras *et al*., 1994). Finally, it should be noted that the SP distribution is not as much consistent as the NFT regional and laminar pattern described earlier.

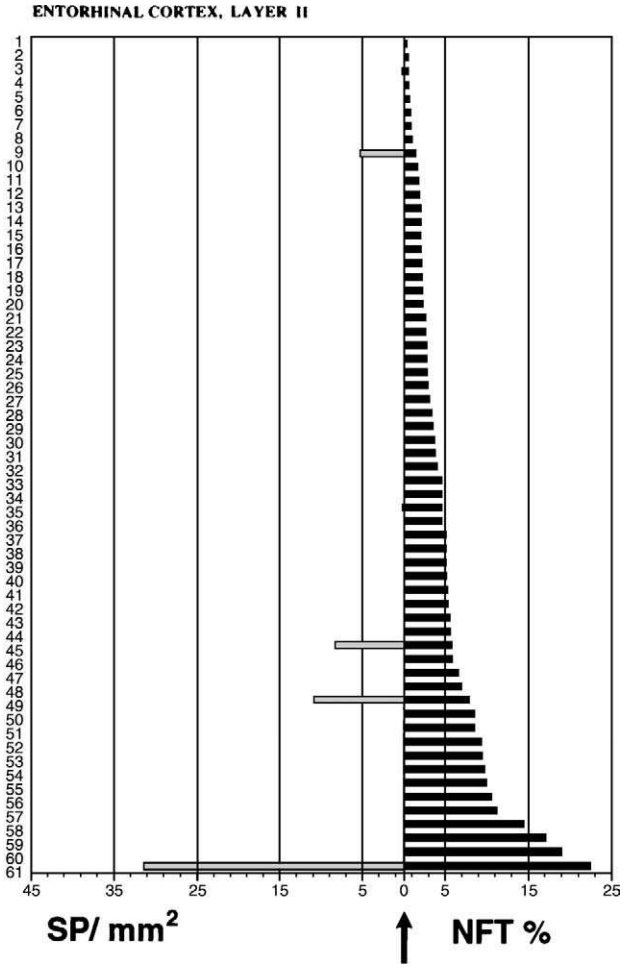

FIG. 7.1. Severity of pathologic changes in an unselected series of 61 elderly cases with no cognitive impairment (Bouras *et al*., 1993). NFT% is the proportion of neurons in layer II of the entorhinal cortex that contain a NFT (black bars at right). SP densities are shown at left and are far less prevalent. Note that all cases had at least a few NFT (cases are ordered by increasing NFT numbers).

II. Neuronal Loss in Normal Aging and AD

In addition to the amyloid deposition and NFT formation, neuronal loss also reflects the degenerative process that occurs in the brain of demented patients. Neuronal loss also takes place in the brain of patients with very mild cognitive impairment, and this pattern of minimal neuronal loss is well correlated with that of NFT distribution in the entorhinal cortex. Furthermore, the presence of more severe dementing symptoms is correlated with neuronal loss in hippocampal formation and neocortical regions (West *et al*., 1994; Gómez-Isla *et al*., 1996, 1997; Simic *et al*., 1997). Using a stereologic quantitative assessment of neuron numbers, Gómez-Isla and colleagues have compared the occurrence of neuronal loss in the entorh-

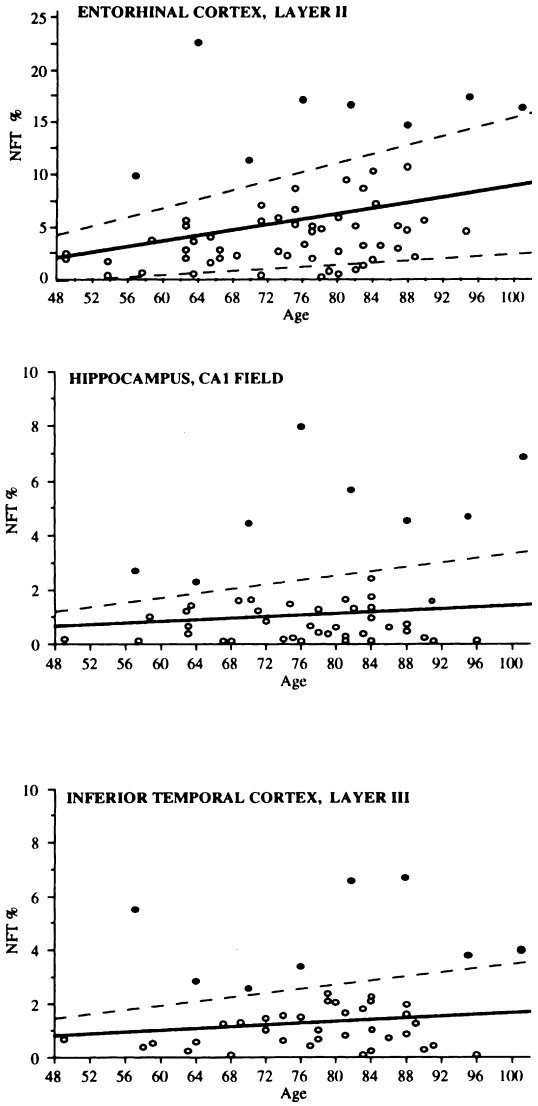

FIG. 7.2. Proportion of neurons containing an NFT in layer II of the entorhinal cortex, the CA1 field of the hippocampus, and layer III of area 20 (inferior temporal cortex) in the same population as in Fig. 7.1. There is a weak positive correlation with the age of the patients in the entorhinal cortex. Interestingly, in all three regions the same eight cases exhibit higher NFT numbers than the rest of the group (the dashed lines correspond to 1 SD of the data). These eight cases may represent individuals at higher risk to develop overt AD (Bouras *et al*., 1993).

inal cortex in subjects with different cognitive status. In the first group of cognitively normal subjects, with a clinical dementia rating (CDR) score of 0, there is no variation of the total number of neurons or neuronal densities in any layer of the entorhinal cortex between the sixth and ninth decades. Brains from patients diagnosed with very mild AD (CDR of 0.5) may show a quite extensive loss of neurons in the entorhinal cortex, especially in layers II and IV where the number of neurons is decreased by 57% in layer II and by 41% in layer IV compared with controls. Other layers of entorhinal cortex are also affected, but to a lesser extent. Finally, in severe AD cases (CDR of 3 or higher), 87% of the neurons in layer II and 69% in layer IV of the entorhinal cortex are missing, with many presumably having degenerated to end-stage NFT (Gómez-Isla *et al*., 1996). A similar stereologic study performed in our laboratory indicates that a significant neuronal loss is seen for CDR 2 cases, which seems to be a pivotal stage in the neurodegenerative process (Fig. 7.3) (Bussière *et al*., 1999b; Hof *et al*., 1999b). These two studies take into account small populations and cannot overcome the neuropathological interindividual variation. This fact might explain the discrepancy between the two studies. Nevertheless, these data suggest that a marked neuronal loss in layer II of entorhinal cortex distinguishes very mild AD from nondemented aging. Similarly, stereologic estimates of neuronal loss in the different parts of the hippocampal formation have been reported in different studies, leading to controversial data (West *et al*., 1994; Simic *et al*., 1997). In fact, the work performed by West and colleagues indicates a quantitative and qualitative difference in the regional pattern of neuronal loss between normal aging and AD. Losses in the hilus and subiculum are, respectively, 37 and 43% over the ages studied (13 to 101 years), and these hippocampal subdivisions also exhibit an AD-related neuronal loss. The major point raised by this study is the absence of evidence of neuronal loss in the CA1 field in normal aging cases, while this latter area displays the most important neuronal loss in AD. An average of 68% of neurons is lost compared to an age-matched control group (Fig. 7.4) (West *et al*., 1994). Even though data obtained by Simic and colleagues show a similar magnitude of neuron numbers in the different regions of hippocampal formation, they are conflicting with those reported by West (Simic *et al*., 1997). These authors described a significant negative regression between age and neuron numbers in CA1 and subiculum (loss of 67 and 32%, respectively), but not in other subdivisions, especially in the hilus, as described previously. In addition, neuronal loss restricted to certain regions of the CA1 field is reported as an almost constant feature of normal older brains. Furthermore, neuronal loss assessment in the CA1 of AD cases does not appear as significantly differ-

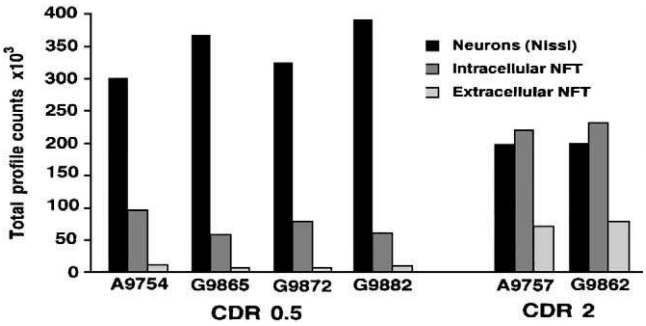

FIG. 7.3. Total number of neurons in layer II of the entorhinal cortex in CDR 0.5 and CDR 2 cases. Neuron counts were obtained stereologically and have been subdivided into normal neurons revealed by Nissl stain, neurons containing an intracellular NFT (i.e., a Nissl-stained cell labeled with an antibody to hyperphosphorylated tau protein), and extracellular ghost NFT. Note that a significant proportion of the neurons demonstrate transitional pathology in CDR 0.5 cases, but that the majority remains unaffected by NFT formation. However, in CDR 2 cases, the majority of neurons has progressed to NFT formation, as revealed by the higher proportion of intra- and extracellular NFT (Bussière *et al*., 1999b).

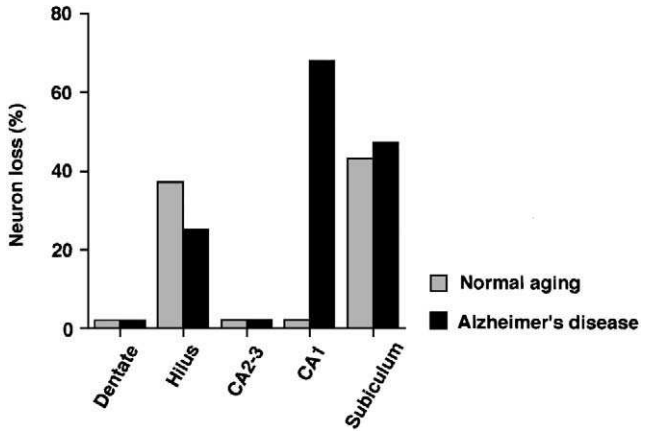

FIG. 7.4. Percentage of neuronal loss in the various subfields of hippocampal formation in normal aging and AD based on stereologic data of West *et al*. (1994). This study revealed that if a large enough population is analyzed to circumvent the problem of interindividual variability in neuronal numbers, the neurons in the hilus of the dentate gyrus and the pyramidal neurons of the subiculum are affected as severely in cognitively normal patients as in AD cases.

ent when compared to an age-matched control group, and a pronounced neuronal loss is found only in the subiculum and the dentate gyrus. Once again, the substantial anatomical variability that exists among individual human brains may explain the discrepancy. New studies including larger populations are needed to overcome the problem of interindividual heterogeneity. In this context, the stereological approach represents a powerful way to analyze the transitional state between normal aging and pathological degeneration concomitant to early AD, with the ultimate goal of such studies being to determine if neurodegeneration in AD and aging involves different processes.

III. Dynamic Neuronal Changes during Aging and AD

Existing stereologic data on the neuronal alterations in AD consist almost exclusively of total neuron counts based on the Nissl stain or of NFT counts. Both of these measurements may be misleading because they do not take into account a large and dynamic population of transitional alterations that can be identified by several neurochemical markers, such as neurofilament proteins or modified tau proteins (Morrison and Hof, 1997; Vickers, 1997; Vickers *et al*., 2000). In addition, most available studies do not take into consideration the very high variability in neuron numbers that exists among human brains and generally do not provide accurate estimates of numbers in cytoarchitecturally defined regions in the neocortex or rely on density measurements. In order to provide a more accurate reflection of the dynamic changes occurring in certain subpopulations of vulnerable neurons during aging and in the course of AD, detailed stereological analyses of several cell morphologic parameters are needed that will underline the progressive cellular pathology leading to the selective neuronal loss. These indices of cellular degeneration are investigated in correlation with specific neuropsychological measures assessing the cognitive status of the cases. Such immunohistochemical studies will permit subdividing the neuronal profiles in a manner directly relevant to the degenerative process at a much finer level of resolution than a Nissl-based quantification of total cell loss alone (Bussière *et al*., 1999b; Hof *et al*., 1999b).

Stereological measurements of pyramidal neurons containing neurofilament proteins in the superior frontal cortex may represent reliable indices to follow the progressive cellular pathology. Neurofilament as well as other cytoskeletal proteins have been implicated in NFT formation (Ksiezak-Reding *et al*., 1987; Morrison *et al*., 1987; Zhang *et al*., 1989; Vickers *et al*., 1992, 1994; Trojanowski *et al*., 1993; Morrison and Hof, 1997; Hof and Morrison, 1999), and pyramidal cells with a high content of nonphosphorylated neurofilament protein emerge as a neuron type highly susceptible to NFT formation (see Chapter 9). The laminar distribution of this subset of neurons in visual association, prefrontal, and anterior cingulate human cerebral cortices is very similar to the distribution of NFT, and the layers that have high NFT density in an AD brain also display a high density of neurofilament protein-immunoreactive neurons (Hof and Morrison, 1990, 1999; Hof *et al*., 1990, 1999b). Pyramidal neurons of the subiculum and hippocampal formation, especially layers II, III, and V of entorhinal cortex, exhibit the same profile, having a very high density of neurofilament protein-immunoreactive neurons in the normally aging human brain and presenting with a dramatic loss of these neurons in AD (Fig. 7.5, see color insert) (Vickers *et al*., 1992; 1994; Morrison and Hof, 1997). The most recent data obtained indicate that in area 9, neurofilament protein-containing pyramidal cells already undergo notable neuronal loss in CDR 2 cases, whereas a more dramatic loss of neurons overall occurs in CDR 3 cases. Accordingly, this result suggests that CDR 2 may be considered as the clinical transition stage at which considerable development of AD degenerative changes takes place in the prefrontal cortex.

IV. Neuronal Loss and Early Markers of Neuronal Degeneration

The hypothesis that considers CDR 2 stage as pivotal in the development of the neurodegenerative process is supported by quantitative analyses of early markers of the neurodegeneration, such as calpain immunoreactivity or modified tau proteins (Morrison and Hof, 1997; Gimmel *et al*., 1998; Perl *et al*., 1998; Bussière *et al*., 1999b; Hof *et al*., 1999b). Calpains constitute a family of ubiquitous calcium-dependent cysteine proteases and occur in the brain as two isoenzymes, μcalpain and mcalpain, which require micromolar or millimolar calcium concentrations, respectively, for activation *in vitro*. Calpains are involved in selective proteolytic cleavages and in the regulation of structure and dynamics of major cytoskeletal proteins that are also constituents of NFT, such as neurofilament proteins or tau proteins (Nixon *et al*., 1994; Suzuki *et al*., 1995). Pathological modifications of tau proteins, such as abnormal phosphorylation, may reduce their susceptibility to calpain-mediated proteolysis and subsequently lead to their accumulation into PHF. Finally, a direct and extensive associa-

tion of μ- or mcalpain with neurofibrillary pathology and its early involvement in the cytoskeletal disorganization and degeneration of neurons in AD has been reported (Iwamoto *et al.*, 1991; Grynspan *et al.*, 1997). Some early biochemical changes of tau proteins also constitute reliable indices of the degenerative process that occurs in neurons. Thus, the intramolecular association between the amino-terminal end and the third microtubule-binding domain of tau proteins results in a specific conformation, and the appearance of this conformation seems to precede the pathological aggregation of tau proteins into PHF and the resultant neurofibrillary degeneration. The MC1 and Alz-50 monoclonal antibodies are useful to pursue such early pathological modifications (Jicha *et al.*, 1997, 1999). TG3 is another antibody of interest to analyze early modifications of neuronal cytoskeleton, as it recognizes simultaneously the previously described altered conformation and a phosphorylated epitope of tau proteins (Vincent *et al.*, 1996; Jicha *et al.*, 1997). Previous studies realized at light and electron microscopic levels with MC1 and TG3 antibodies reveal that MC1 staining appears earlier than the TG3 staining in hippocampal CA1 neurons, suggesting that the conformational changes in tau proteins may precede the accumulation of hyperphosphorylated proteins (Anderton *et al.*, 1998).

A stereologic approach taking into account the total number of neuronal cells, indicated by the Nissl stain, as well as by the number of NFT at different stages of development, is an efficient procedure to describe the dynamic and transitional modifications that occur during aging and neurodegenerative diseases. Preliminary studies using this stereologic design have been reported (Bussière *et al.*, 1999b; Hof *et al.*, 1999b). A small number of brains from patients with different cognitive status (CDR scores ranging between 0–0.5 and 3) have been investigated for early markers of pathology revealing intracellular NFT (mcalpain and tau proteins with the MC1 epitope; Grynspan *et al.*, 1997; Jicha *et al.*, 1997, 1999) and for hyperphosphorylated tau proteins revealing extracellular NFT (AD2 or 988; Buée-Scherrer *et al.*, 1996; Bussière *et al.*, 1999a). Preliminary evidence from layer II of the entorhinal cortex and from layers III and V of area 9 suggests that the early stages of degeneration (CDR scores of 0–0.5) involve primarily the formation of neuritic changes (Fig. 7.6). Numerous dystrophic neurites show immunoreactivity for μcalpain and the MC1 epitope, but are not double labeled by antibodies AD2 or 988. Furthermore, this is observed first in the entorhinal cortex and occurs only at later stages (CDR 2) in the prefrontal cortex. Degeneration takes place secondarily in neuronal perikarya, which may represent a transitional stage prior to the final evolution of the lesion to an extracellular NFT. Moreover, the stereologic determination of the number of NFT-free cells and NFT-bearing cells in the entorhinal cortex and its relationship to the cognitive status of the cases confirm that CDR 2 represents a pivotal stage in the neurodegenerative process and subsequent neuronal loss. With respect to CDR 0 and 0.5 cases, moderate numbers of intracellular NFT are observed, whereas extracellular NFT are rare. The total number of neurons is not decreased significantly in these cases (Figs. 7.3 and 7.5). Neurofibrillary pathology becomes predominant in CDR 2 cases, with a severe increase of intracellular and extracellular NFT, whereas the total number of neurons is decreased dramatically (Bussière *et al.*, 1999b; Hof *et al.*, 1999b).

V. Synapse Loss

In addition to the classical neuropathological lesions and to the neuronal loss described previously, synapse loss is also involved in normal aging and in the pathogenesis of AD.

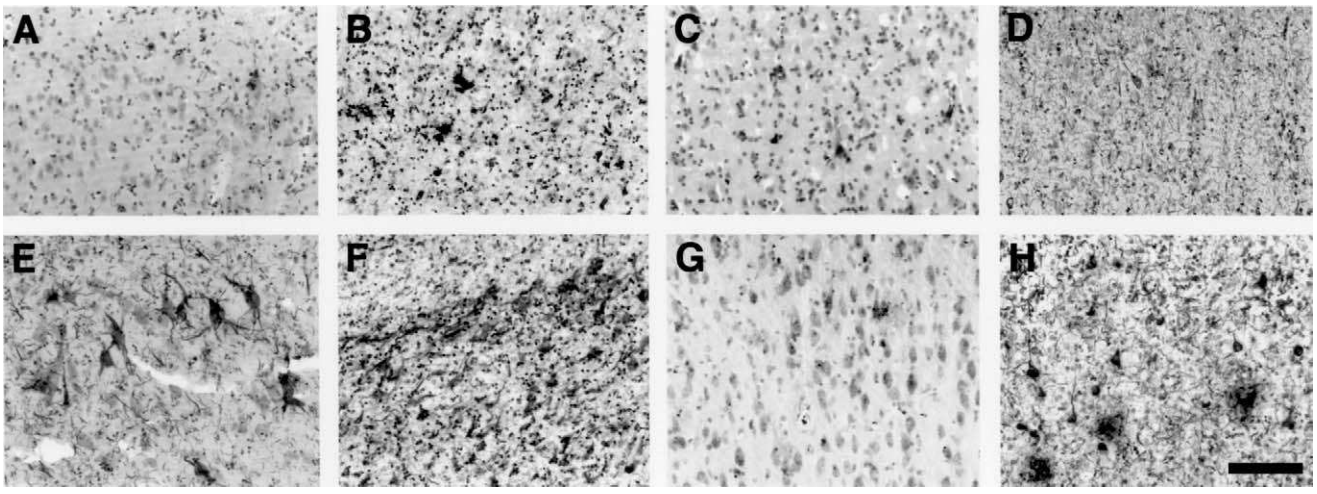

FIG. 7.6. Development of neurofibrillary changes labeled by antibodies MC1 (A–D) and AD2 (E–H) in layer II of the entorhinal cortex (A, B, E, and F) and layer III of area 9 (C, D, G, and H). For both regions each pair of photomicrographs shows a CDR 0.5 case on the left and a CDR 3 on the right. Note the parallel increase in immunoreactive profiles for these markers with disease severity. In CDR 3 cases, there is overall more profiles labeled with antibody AD2 to hyperphosphorylated tau. In CDR 0.5 cases, MC1 labels preferentially thin neurites, which may represent the earliest stage of neurofibrillary degeneration. As demonstrated previously for hyperphosphorylated tau, the pathologic changes identified with MC1 appear at later stages of the disease in the prefrontal cortex, with densities at CDR 3 that are comparable to those observed in CDR 0.5–1 cases in the entorhinal cortex. Scale bar (on H): 100 μm.

Moreover, the correlation between synapse loss and the severity of dementia assessed by the Mini Mental State or Blessed score has been shown to be stronger than the correlation with the distribution and density of NFT (DeKosky and Scheff, 1990; Terry *et al*., 1991; Sze *et al*., 1997). The quantification of synaptophysin immunoreactivity, reflecting presynaptic terminal densities, has been used by Masliah and colleagues to evaluate synaptic loss in the frontal cortex of individuals without dementia. These authors reported a significant decrease in synaptophysin immunoreactivity, with an average of 20% after 60 years of age compared to younger individuals (Masliah *et al*., 1993). Furthermore, immunochemical and immunohistochemical analyses have shown that synaptic loss is even more pronounced in AD brains, with the frontal and parietal cortices presenting the most severe and widespread loss (45%) (Fig. 7.7, see color insert; Masliah *et al*., 1991, 1993). Similarly, ultrastructural studies revealed that the number of synapses in layers III and V of Brodmann's area 9 is decreased significantly in brains from patients with mild to moderate AD compared to the number in age-matched control brains. A negative correlation has also been shown between the decrease in synapse numbers and the enlargement of postsynaptic densities, suggesting a compensatory mechanism that allows for the total synaptic contact area to remain stable (DeKosky and Scheff, 1990; Scheff *et al*., 1990). These data indicate that synaptic loss occurs in physiological conditions during aging, but to a lesser extent than in AD, and might be compensated by an increase in synaptic contact area. In AD, this compensatory process is inefficient and the decline in both synaptic number and synaptic contact area leads to the disconnection of corticocortical pathways.

VI. Conclusion

Even in elderly subjects with clinically intact intellectual functions, AD-related neuropathologic changes are a common finding. In particular, layer II of the entorhinal cortex is always affected by the neurofibrillary degeneration. Severe involvement of neocortical association areas results in the degeneration of corticocortical circuits and is required for the clinical expression of the dementia. Thus, the key to dementia may reside in the mechanisms by which pathological changes that are relatively limited to the hippocampal formation at early stages of the disease begin to involve neocortical circuits (Morrison and Hof, 1997), thereby differentiating the memory defects associated with normal aging (also known as ''benign senescent forgetfulness'') from the more generalized memory and cognitive deficits that characterize dementing disorders. Importantly, these observations imply that elderly individuals can maintain a high level of cognitive performance while sustaining a significant compromise of hippocampal circuits and that they may rely more on neocortical than on hippocampal circuits for memories essential for daily activities. It is worth mentioning that in terms of cognitive performance, healthy elders may describe difficulties in learning and retrieving new information, although in reality, the problem lies in the amount of information that one such individual can learn within a given period of time in comparison to younger subjects. The major difference with early AD patients is that after a delay, healthy elders will have retained the new information, whereas patients with very mild impairment retain little of the new information (Albert, 1996). Thus, the relatively minor cellular changes associated with normal brain aging are likely to be sufficient to cause the learning deficits experienced by healthy elders. It will be important to gain knowledge on the morphologic and molecular phenotype of the neurons affected by these minimal changes to understand the mechanisms of normal aging and develop therapeutic interventions to protect the vulnerable neuronal circuits.

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References

Albert, M. S. (1996). Cognitive and neurobiologic markers of early Alzheimer's disease, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 13547–13551.

Anderton, B. H., Callahan, L., Coleman, P., Davies, P., Flood, D., Jicha, G. A., Ohm, T., and Weaver, C. (1998). Dendritic changes in Alzheimer's disease and factors that may underlie these changes. *Prog. Neurobiol.* **55**, 595–609.

Arnold, S. E., Hyman, B. T., Flory, J., Damasio, A. R., and Van Hoesen, G. W. (1991). The topographical and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer's disease. *Cereb. Cortex* **1**, 103–116.

Arriagada, P. V., Growdon, J. H., Hedley-Whyte, E. T., and Hyman, B. T. (1992a). Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology* **42**, 631–639.

Arriagada, P. V., Marzloff, K., and Hyman, B. T. (1992b). Distribution of Alzheimer-type pathologic changes in nondemented elderly individuals matches the pattern in Alzheimer's disease. *Neurology* **42**, 1681–1688.

Bierer, L. M., Hof, P. R., Purohit, D. P., Carlin, L., Schmeidler, J., Davis, K. L., and Perl, D. P. (1995). Neocortical neurofibrillary tangles correlate with dementia severity in Alzheimer's disease. *Arch. Neurol. (Chicago)* **52**, 81–88.

Bouras, C., Hof, P. R., and Morrison, J. H. (1993). Neurofibrillary tangle densities in the hippocampal formation in a non-demented population define subgroups of patients with differential early pathologic changes. *Neurosci. Lett.* **153**, 131–135.

Bouras, C., Hof, P. R., Giannakopoulos, P., Michel, J. P., and Morrison, J. H. (1994). Regional distribution of neurofibrillary tangles and senile plaques in the cerebral cortex of elderly patients: A quantitative evaluation of a one-year autopsy population from a geriatric hospital. *Cereb. Cortex* **4**, 138–150.

Braak, H., and Braak, E. (1990). Neurofibrillary changes confined to the entorhinal region and an abundance of cortical amyloid in cases of presenile and senile dementia. *Acta Neuropathol.* **80**, 479–486.

Braak, H., and Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* **82**, 239–259.

Braak, H., Braak, E., and Kalus, P. (1989). Alzheimer's disease: Areal and laminar pathology in the occipital isocortex. *Acta Neuropathol.* **77**, 494–506.

Buée-Scherrer, V., Condamines, O., Mourton-Gilles, C., Jakes, R., Goedert, M., Pau, B., and Delacourte, A. (1996). AD2, a phosphorylation-dependent monoclonal antibody directed against tau proteins found in Alzheimer's disease. *Mol. Brain Res.* **39**, 79–88.

Bussière, T., Hof, P. R., Mailliot, C., Brown, C. D., Caillet-Boudin, M. L., Perl, D. P., Buée, L., and Delacourte, A. (1999a). Phosphorylated serine422 on tau proteins is a pathological epitope found in several

diseases with neurofibrillary degeneration. *Acta Neuropathol.* **97**, 221–230.

Bussière, T., Wicinski, B., Lin, G. I., Perl, D. P., Davies, P., Nixon, R. A., Morrison, J. H., and Hof, P. R. (1999b). Early neurodegenerative alterations in the cerebral cortex during normal aging and Alzheimer's disease. *Soc. Neurosci. Abstr.* **25,** 593.

Crystal, H., Dickson, D. W., Fuld, P., Masur, D., Scott, R., Mehler, M., Masdeu, J., Kawas, C., Aronson, M., and Wolfson, L. (1988). Clinico-pathologic studies in dementia: Nondemented subjects with pathologically confirmed Alzheimer's disease. *Neurology* **38**, 1682–1687.

DeKosky, S. T., and Scheff, S. W. (1990). Synapse loss in frontal cortex biopsies in Alzheimer's disease: Correlation with cognitive severity. *Ann. Neurol.* **27**, 457–464.

Delacourte, A., and Buée, L. (1997). Normal and pathological Tau proteins as factors for microtubule assembly. *Int. Rev. Cytol.* **171**, 167–224.

Gimmel, D., Morrison, J. H., Perl, D. P., Bouras, C., and Hof, P. R. (1998). Development of stereologic indices of neurodegeneration in the cerebral cortex during normal aging and Alzheimer's disease. *Soc. Neurosci. Abstr.* **24,** 960.

Gómez-Isla, T., Price, J. L., McKeel, D. W., Jr., Morris, J. C., Growdon, J. H., and Hyman, B. T. (1996). Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *J. Neurosci.* **16**, 4491–4500.

Gómez-Isla, T., Hollister, R., West, H., Mui, S., Growdon, J. H., Petersen, R. C., Parisi, J. E., and Hyman, B. T. (1997). Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. *Ann. Neurol.* **41**, 17–24.

Grynspan, F., Griffin, W. R., Cataldo, A., Katayama, S., and Nixon, R. A. (1997). Active site-directed antibodies identify calpain II as an early-appearing and pervasive component of neurofibrillary pathology in Alzheimer's disease. *Brain Res.* **763**, 145–158.

Hof, P. R., and Morrison, J. H. (1990). Quantitative analysis of a vulnerable subset of pyramidal neurons in Alzheimer's disease: II. Primary and secondary visual cortex. *J. Comp. Neurol.* **301**, 55–64.

Hof, P. R., and Morrison, J. H., (1999). The cellular basis of cortical disconnection in Alzheimer disease and related dementing conditions. *In "Alzheimer Disease"* (R. D. Terry, R. Katzman, K. L. Bick, and S. S. Sisodia, eds.), pp. 207–232. Lippincott/Williams & Wilkins, Philadelphia.

Hof, P. R., Cox, K., and Morrison, J. H. (1990). Quantitative analysis of a vulnerable subset of pyramidal neurons in Alzheimer's disease: I. Superior frontal and inferior temporal cortex. *J. Comp. Neurol.* **301**, 44–54.

Hof, P. R., Bierer, L. M., Perl, D. P., Delacourte, A., Buée, L., Bouras, C., and Morrison, J. H. (1992). Evidence for early vulnerability of the medial and inferior aspects of the temporal lobe in an 82-year-old patient with preclinical signs of dementia. Regional and laminar distribution of neurofibrillary tangles and senile plaques. *Arch. Neurol. (Chicago)* **49**, 946–953.

Hof, P. R., Bouras, C., and Morrison, J. H., (1999a). Cortical neuropathology in aging and dementing disorders: Neuronal typology, connectivity, and selective vulnerability. *In* "Neurodegenerative and Age-related Changes in Structure and Function of Cerebral Cortex" (A. Peters and J. H. Morrison, eds.), Cerebral Cortex, Vol. 14, pp. 175–312. Kluwer Academic Plenum, New York.

Hof, P. R., Wicinski, B., Lin, G. I., Bussière, T., Giannakopoulos, P., Bouras, C., Perl, D. P., and Morrison, J. H. (1999b). Neurofilament proteins identify vulnerable neocortical neurons: Stereologic analysis in normal aging and Alzheimer's disease. *Soc. Neurosci. Abstr.* **25,** 593.

Iwamoto, N., Thangnipon, W., Crawford, C., and Emson, P. C. (1991). Localization of calpain immunoreactivity in senile plaques and in neurones undergoing neurofibrillary degeneration in Alzheimer's disease. *Brain Res.* **561**, 177–180.

Jicha, G. A., Lane, E., Vincent, I., Otvos, L., Jr., Hoffmann, R., and Davies, P. (1997). A conformation- and phosphorylation-dependent antibody recognizing the paired helical filaments of Alzheimer's disease. *J. Neurochem.* **69**, 2087–2095.

Jicha, G. A., Berenfeld, B., and Davies, P. (1999). Sequence requirements for formation of conformational variants of tau similar to those found in Alzheimer's disease. *J. Neurosci. Res.* **55**, 713–723.

Ksiezak-Reding, H., Dickson, D. W., Davies, P., and Yen, S. H. (1987). Recognition of tau epitopes by anti-neurofilament antibodies that bind to Alzheimer neurofibrillary tangles. *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3410–3414.

Masliah, E., Terry, R. D., Alford, M., DeTeresa, R., and Hansen, L. A. (1991). Cortical and subcortical patterns of synaptophysinlike immunoreactivity in Alzheimer's disease. *Am. J. Pathol.* **138**, 235–246.

Masliah, E., Mallory, M., Hansen, L., DeTeresa, R., and Terry, R. D. (1993). Quantitative synaptic alterations in the human neocortex during normal aging. *Neurology* **43**, 192–197.

Mirra, S. S., Hart, M. N., and Terry, R. D. (1993). Making the diagnosis of Alzheimer's disease. A primer for practicing pathologists. *Arch. Pathol. Lab. Med.* **117**, 132–144.

Morrison, J. H., and Hof, P. R. (1997). Life and death of neurons in the aging brain. *Science* **278**, 412–419.

Morrison, J. H., Lewis, D. A., Campbell, M. J., Huntley, G. W., Benson, D. L., and Bouras, C. (1987). A monoclonal antibody to non-phosphorylated neurofilament protein marks the vulnerable cortical neurons in Alzheimer's disease. *Brain Res.* **416**, 331–336.

Nixon, R. A., Saito, K. I., Grynspan, F., Griffin, W. R., Katayama, S., Honda, T., Mohan, P. S., Shea, T. B., and Beermann, M. (1994). Calcium-activated neutral proteinase (calpain) system in aging and Alzheimer's disease. *Ann. N.Y. Acad. Sci.* **747**, 77–91.

Perl, D. P., Good, P. F., and Hof, P. R. (1998). Practical approaches to stereology in the setting of a human disease-related brain bank. In "Quantitative Neuroanatomy: A Picture is Worth a Thousand Words, but a Number is Worth a Thousand Pictures" (J. H. Morrison and P. R. Hof, eds.), Soc. Neurosci. Short Course Syllabus, pp. 47–55. Society for Neuroscience, Washington, DC.

Price, J. L., Davis, P. B., Morris, J. C., and White, D. L. (1991). The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. *Neurobiol. Aging* **12**, 295–312.

Rogers, J., and Morrison, J. H. (1985). Quantitative morphology and regional and laminar distributions of senile plaques in Alzheimer's disease. *J. Neurosci.* **5**, 2801–2808.

Scheff, S. W., DeKosky, S. T., and Price, D. A. (1990). Quantitative assessment of cortical synaptic density in Alzheimer's disease. *Neurobiol. Aging* **11**, 29–37.

Simic, G., Kostovic, I., Winblad, B., and Bogdanovic, N. (1997). Volume and number of neurons of the human hippocampal formation in normal aging and Alzheimer's disease. *J. Comp. Neurol.* **379**, 482–494.

Suzuki, K., Sorimachi, H., Yoshizawa, T., Kinbara, K., and Ishiura, S. (1995). Calpain: Novel family members, activation, and physiologic function. *Biol. Chem. Hoppe-Seyler* **376**, 523–529.

Sze, C. I., Troncoso, J. C., Kawas, C., Mouton, P., Price, D. L., and Martin, L. J. (1997). Loss of the presynaptic vesicle protein synaptophysin in hippocampus correlates with cognitive decline in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* **56**, 933–944.

Terry, R. D., Masliah, E., Salmon, D. P., Butters, N., DeTeresa, R., Hill, R., Hansen, L. A., and Katzman, R. (1991). Physical basis of cognitive alterations in Alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. *Ann. Neurol.* **30**, 572–580.

Tomlinson, B. E., Blessed, G., and Roth, M. (1970). Observations on the brains of demented old people. *J. Neurol. Sci.* **11**, 205–242.

Trojanowski, J. Q., Schmidt, M. L., Shin, R. W., Bramblett, G. T., Rao, D., and Lee, V. M. Y. (1993). Altered tau and neurofilament proteins in neurodegenerative diseases: Diagnostic implications for Alzheimer's disease and Lewy body dementias. *Brain Pathol.* **3**, 45–54.

Vickers, J. C. (1997). A cellular mechanism for the neuronal changes underlying Alzheimer's disease. *Neuroscience* **78**, 629–639.

Vickers, J. C., Delacourte, A., and Morrison, J. H. (1992). Progressive transformation of the cytoskeleton associated with normal aging and Alzheimer's disease. *Brain Res.* **594**, 273–278.

Vickers, J. C., Riederer, B. M., Marugg, R. A., Buée-Scherrer, V., Buée, L., Delacourte, A., and Morrison, J. H. (1994). Alterations in neurofilament protein immunoreactivity in human hippocampal neurons related to normal aging and Alzheimer's disease. *Neuroscience* **62**, 1–13.

Vickers, J. C., Dickson, T. C., Adlard, P. A., Saunders, H. L., King, C. E., and McCormack, G. (2000). The cause of neuronal degeneration in Alzheimer's disease. *Prog. Neurobiol.* **60**, 139–165.

Vincent, I., Rosado, M., and Davies, P. (1996). Mitotic mechanisms in Alzheimer's disease? *J. Cell Biol.* **132**, 413–425.

West, M. J., Coleman, P. D., Flood, D. G., and Troncoso, J. C. (1994). Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. *Lancet* **344**, 769–772.

Zhang, H., Sternberger, N. H., Rubinstein, L. J., Herman, M. M., Binder, L. I., and Sternberger, L. A. (1989). Abnormal processing of multiple proteins in Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8045–8049.

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8

Longevity and Brain Aging: The Paradigm of Centenarians

The exponential increase in the prevalence of Alzheimer's disease with age, as well as the consistent presence of Alzheimer's disease pathologic changes in elderly people, has induced much debate regarding the relationships between brain aging and Alzheimer's disease. In this context, the study of nonagenarians and centenarians is of particular interest because it allows one to define the spectrum and extent of changes in brain morphology that occur near the upper age limit of life and to assess whether Alzheimer's disease is on a continuum with normal brain aging. The epidemiological and neuropathological evidence summarized in this chapter supports that Alzheimer's disease is an age-related pathologic condition, which does not represent an inevitable consequence of ageing. Moreover, it suggests the presence of a particular subgroup of oldest-old individuals with high resistance to the neurodegenerative process. These observations are discussed within the theoretical framework of human longevity.

I. Introduction

The continued expansion of the elderly population and a growing awareness of age-related diseases such as dementia have prompted considerable interest in the study of the aging human brian. The aging population is rapidly growing as a result of increased accessibility of advanced medical technology and declining birth rates. By 2020 it is estimated that at least 1 billion people will be older than 60 years, representing more than 20% of the total population. The few individuals who reach very old ages are called ''longevity outliers.'' This particular group survives to challenge today's maximum of 121 years (Smith, 1997).

From a neurobiological point of view, the study of oldest-old individuals may permit to define the spectrum and extent of changes in brain morphology that occur with normal brain aging and to assess correlations between the neuropathological definition of normal brain aging and the clinical development of dementing process (Mizutani and Shimada, 1992; Giannakopoulos *et al*., 1993; Green *et al*., 2000). The increase in the number of elderly people in the general population is accompanied by high prevalence and incidence rates of Alzheimer's disease (AD), and several community-based studies of AD epidemiology have suggested that the number of affected nonagenarians and centenarians may rise steadily in the next decade (Moss and Albert, 1988; Brayne, 1991; Hofman *et al*., 1991; Evans *et al*., 1992). Whether or not AD is on a continuum with normal brain aging is thus a major public health issue. After some epidemiological and clinical considerations, this chapter summarizes the neuropathological features of nonagenarians and centenarians with particular reference to the patterns of cerebral cortex pathology in this age group and discusses their relevance with respect to the dichotomy between AD and brain aging.

II. Epidemiological Data

The increase in the number of centenarians can be, at least partly, the consequence of the demographic transition and high quality of medical care provided for the elderly (Larkin, 1999). Methodologically, census data are preferred over sample data in very old cohorts because of their completeness and the absence of sampling errors. Several census studies have revealed an exponential increase in the number of centenarians (Beregi, 1987; IPSEN Foundation, 1990; Manton and Vaupel, 1995; Perls, 1995; Asada *et al*., 1996; Geneva, 1998; Wilkinson and Sainsbury, 1998; Larkin, 1999) (Fig. 8.1, see color insert). For instance, in France there were 2104 centenarians in 1985, 3853 in 1990 (6.9 per 100,000), 3232 in 1995, and 4968 are expected in 2005 and 8201 in 2025 (13.7 per 100,000) (IPSEN Foundation, 1990; Larkin, 1999). At the time of the last census in 1990, the state of Geneva in Switzerland had 38 centenarians among its 383,000 inhabitants, which corresponds to a prevalence of 9.9 per 100,000; in 1997 there were 60 for 400,860 inhabitants (15.0 per 100,000), which

represents a 50% increase in 7 years (Geneva, 1998), Wilkinson provided data on 297 centenarians in New Zealand in 1990, giving a prevalence of 8.8 per 100,000 inhabitants, and compared these data to the 15.0 centenarians per 100,000 inhabitants in the United States population in 1990 (Wilkinson and Sainsbury, 1998). In 1987, there were 218 living centenarians in Hungary for a population of a little over 10 millions (Beregi, 1987). In the United States, there is to date almost 70,000 centenarians and between 500,000 and 4 millions are expected to be living in 2050 (Perls, 1995). A well-known paradox is the higher proportion of centenarians in the African American community (19.6 per 100,000) with a higher life expectancy after the age of 85, despite a lower life expectancy at birth (Manton and Vaupel, 1995; Perls, 1997; Wilkinson and Sainsbury, 1998). The validity of data pertaining to U.S. African Americans obtained from the census has been criticized, but has been confirmed through other sources, such as Medicare data (Manton and Vaupel, 1995). Importantly, the survival rate of siblings of centenarians in the United States is higher than in a control population of the same birth cohort who died around their 75 years, thus underlying the role of heredity to reach an advanced age (Perls *et al*., 1998). Finally, in Japan, 5593 centenarians were living in 1994 (4.5 per 100,000), with a prefectural prevalence rate ranging from 1.9 to 18.5 (Asada *et al*., 1996).

The growing evidence for a steady increase in the number of centenarians worldwide is paralleled by several studies aiming to determine possible psychobiological particularities of these individuals. For instance, centenarians were less prone to oxidative stress and are thought to have better antioxidant defenses, nutritional status, immunological profile, and endocrinological and metabolic characteristics than younger elderly cohorts (Paolisso *et al*., 1998). Psychologically, they report greater satisfaction with life and social and family relations and display lower scores for anxiety and depression and better coping abilities compared to younger elderly individuals (Dello Buono *et al*., 1998). In this age group, good health and not moving home are associated with greater intellectual activity, whereas extraversion and negative life events are associated with greater social activity (Hilleras *et al*., 1999). Moreover, an autopsy study of 490 very old individuals revealed that the frequency of cardiac causes of death decreases after 80 years with a parallel increase of noncardiac, nonvascular causes (Roberts and Shirani, 1998). Also the proportion of death in patients with cancer decreases from 83% in those diagnosed in their six decade to 49% in centenarians. A very large cancer registry study, including more than 14,000 cases, showed that colorectal cancer becomes the most common localization after the age of 90 (21%), whereas this rank is occupied by lung cancer before this age (Saltzstein *et al*., 1998). Altogether, these data support the possibility that centenarians form a select cohort with relatively slow rates of aging and increased resistance to biological and psychological stress and age-related diseases such as cancer, stroke, and heart disease (Perls, 1995, 1997; Perls *et al*., 1998).

III. Dementia in the Oldest-Old

Although prevalence and incidence data are still scarce in this age group, it has long been considered that very old age is associated with the highest prevalence of dementia (Schneider, 1999). Several earlier studies reported an increased prevalence of dementia with aging, to 100% in the 100-year age group (Adolfsson, 1986; Gottfries, 1986; Bohl *et al*., 1987). Methodological biases were present in most of these studies and limit the validity of their conclusions. First, the samples included only a limited number of nonagenarians and centenarians. Second, the clinical diagnosis of dementia made by the physician was based on the global decline of cognitive performances rather than on a detailed analysis of each cognitive function, leading to an overestimation of the prevalence of dementia in the oldest-old. In fact, epidemiological studies in larger cohorts of very old individuals showed prevalence rates that varied from 27 to 70% (Asada *et al*., 1996; Samuelsson *et al*., 1997), and Ritchie and Kildea (1995), in their meta-analysis of numerous previous studies, demonstrated that the prevalence of dementia levels off at age 95, reaching only 40%. In an epidemiological survey of 1694 patients who met criteria for probable or definite AD, Lautenschlager and colleagues (1996) also reported that the risk of AD decreases significantly after age 90. Similar results were drawn by a community-based study of 402 individuals older than 85 years who had been assessed using structured interviews in Munich (Fichter *et al*., 1995), A relative resistance of centenarians to the degenerative process is also suggested by the observations of Howieson and collaborators (1997), who performed a longitudinal study of 31 nondemented individuals older than 80 years and reported a preservation of cognitive abilities during a follow-up period of 5 years. Furthermore, a lack of association between AD and apolipoprotein E allele $\epsilon 4$, a major risk factor for late-onset AD in younger cohorts, has been demonstrated in centenarians (Rebeck *et al*., 1994; Schachter *et al*., 1994; Sobel *et al*., 1995; Asada *et al*., 1996). However, one may argue that these epidemiological findings reflect only the fact that younger cohorts are at higher risk to develop AD and do not necessarily support differential neuronal aging in centenarians.

IV. Neuropathological Changes in the Oldest-Old: Relationship to AD

A major but yet unanswered question is whether AD is on a continuum with normal brain aging. In this respect, it is of particular interest to define the final stages of normal brain aging and neuropathological characteristics of AD in the ''oldest-old'' population (Hauw *et al*., 1986; Mizutani and Shimada, 1990, 1992; Delaère *et al*., 1993; Giannakopoulos *et al*., 1993, 1994b,c, 1995, 1996, 1997). Brain aging is characterized by the formation of neurofibrillary tangles (NFT) and senile plaques (SP), as well as neuronal and synaptic loss in both cognitively intact individuals and patients with AD (Mountjoy *et al*., 1983; Braak and Braak, 1991; Arriagada *et al*., 1992b; Masliah *et al*., 1992; Bouras *et al*., 1994; Bierer *et al*., 1995). In nondemented cases, NFT are usually restricted to the hippocampal formation, whereas the progressive involvement of the association areas in the temporal neocortex parallels the development of overt clinical signs of dementia (Arnold *et al*., 1991; Arriagada *et al*., 1992b; Hof *et al*., 1992; Bouras *et al*., 1993, 1994; Giannakopoulos *et al*., 1994b; Bierer *et al*., 1995). In contrast, severe SP formation may take place in several neocortical

areas in the presence of very mild cognitive impairment (Morris *et al*., 1991, 1996), and there is no correlation between the quantitative distribution of SP and the severity of AD (Crystal *et al*., 1988; Arnold *et al*., 1991; Dickson *et al*., 1991; Price *et al*., 1991; Arriagada *et al*., 1992b; Hof *et al*., 1992; Bouras *et al*., 1994; Giannakopoulos *et al*., 1994b). With regard to neuronal loss, stereological analyses have revealed age-related decreases in total neuron number of 30 and 50% in the dentate hilus of the hippocampus and subiculum, respectively, between ages 13 and 85. Conversely, no neuronal loss was found in CA1–3 fields and entorhinal cortex where AD lesions are also observed (West, 1993; West *et al*., 1994; Gómez-Isla *et al*., 1996). These studies also showed that in AD, there is an additional depletion of neuronal cell bodies in the dentate hilus and subiculum, as well as a massive reduction in the numbers of pyramidal neurons in the CA1 field and layers II and V of the entorhinal cortex (West, 1993; O'Banion *et al*., 1994; West *et al*., 1994; Gómez-Isla *et al*., 1996). Moreover, earlier and recent studies have shown a neuronal reduction in temporal, inferior, and superior parietal and frontal cortices of AD cases (Lippa *et al*., 1992; O'Banion *et al*., 1994). Does the pattern of lesion distribution and neuronal loss change in extreme aging? To address this question, we performed several neuropathological analyses of a large cohort of prospectively assessed nonagenarians and centenarians who died and were autopsied at the geriatric or psychiatric hospitals of the University of Geneva during the years 1976–1993 (Giannakopoulos *et al*., 1993, 1994a, 1995, 1996, 1997). The results of these studies, as well as previous and recent contributions in this field, are discussed in the following paragraphs.

A first observation is that among centenarians there is a limited number of cognitively intact individuals who display low NFT densities confined to the hippocampal formation and rare neocortical diffuse SP (Karasawa and Goto, 1985; Mizutani and Shimada, 1992; Giannakopoulos *et al*., 1993; Green *et al*., 2000). These individuals, also called "supernormal centenarians," represent a rare phenotype relatively protected from AD pathology and may be thus an exemple of successful aging near the upper age limit of life. Two additional findings should be mentioned. First, centenarians display only mild synaptic loss and cerebral amyloid angiopathy compared to younger individuals (Itoh *et al*., 1998). Second, very old people with AD show a marked decrease in NFT densities in the subiculum, entorhinal cortex, and neocortical areas compared to younger cases with the same clinical diagnosis (Giannakopoulos *et al*., 1993; Green *et al*., 2000). These observations indicate that the occurrence and progression of AD-related pathologic changes are not a necessary concomitant of brain aging.

Generally, the regional patterns of NFT and SP distribution in nondemented centenarians did not differ from that reported by Braak and Braak as well as other investigators in younger subjects (Bouras *et al*., 1993, 1994; Giannakopoulos *et al*., 1994b). However, in contrast to previous observations in younger cohorts (Hof *et al*., 1992), there was a frequent involvement of areas 22, 23, and 24 in intellectually preserved centenarians, suggesting the presence of a differential cortical vulnerability to NFT after 90 years. This hypothesis has been confirmed by our quantitative analysis, which revealed several particularities in the distribution of AD lesions in this age group. For instance, the anterior, but not the posterior, CA1 field of the hippocampus showed significantly higher NFT densities in AD compared to nondemented centenarians (Table 8.1). This implies that the massive NFT formation in the anterior part of the Ammon's horn may be a crucial step in the development of AD symptomatology in this age group, whereas the posterior part of this area can be affected severely without compromise of the higher cortical functions. In contrast to previous findings in younger AD cases (Bouras *et al*., 1994), NFT densities in the entorhinal cortex were comparable between demented and nondemented centenarians. In areas 7, 22, 23, and 24, AD cases displayed significantly higher NFT densities compared to nondemented cases, whereas no signifi-

TABLE 8.1 Comparison of NFT Counts in Nondemented and AD Centenarians[a]

Area/layers	Nondemented $n = 24$ (98.4 ± 1.4)	AD $n = 19$ (97.2 ± 1.2)	P
Ant CA1	34.1 ± 4.8	67.6 ± 12.3	<0.01
Post CA1	59.9 ± 9.5	59.5 ± 7.8	ns
CA2–3	20.3 ± 3.7	21.2 ± 3.9	ns
Hilus	2.1 ± 0.5	2.3 ± 0.8	ns
Subiculum	17.6 ± 2.7	19.9 ± 2.8	ns
Entorhinal II	35.5 ± 4.8	35.3 ± 3.7	ns
Entorhinal V	16.5 ± 2.7	16.1 ± 1.9	ns
20 II–III	16.7 ± 2.4	21.5 ± 4.7	ns
V–VI	11.4 ± 1.9	16.1 ± 3.0	ns
9 II–III	4.4 ± 1.5	6.9 ± 2.6	ns
V–VI	1.6 ± 0.6	5.3 ± 2.2	ns
7 II–III	1.1 ± 0.3	16.8 ± 5.2	<0.01
V–VI	0 ± 0	14.4 ± 4.1	<0.005
22 II–III	8.7 ± 3.7	22.8 ± 8.4	<0.05
V–VI	4.8 ± 2.6	16.7 ± 6.3	<0.01
17 II–III	0.1 ± 0.1	6.7 ± 2.4	ns
V–VI	0 ± 0	2.0 ± 0.9	ns
18 II–III	0.2 ± 0.1	6.9 ± 2.7	ns
V–VI	0 ± 0	4.8 ± 2.6	ns
24 II–III	4.5 ± 1.0	23.0 ± 4.0	<0.005
V–VI	1.5 ± 0.5	11.7 ± 2.6	<0.005
23 II–III	6.2 ± 2.5	17.5 ± 6.4	<0.05
V–VI	0.7 ± 0.2	8.6 ± 2.4	<0.005
Amygdala	21.8 ± 4.2	23.9 ± 3.5	ns
NBM	5.34 ± 1.20	11.24 ± 1.58	<0.01
LC	4.26 ± 0.61	4.72 ± 0.52	ns

[a]Results represent NFT counts/mm^2 (± SEM) in each area. The mean age of each group is indicated in parentheses. Statistical analysis was performed by analysis of covariance controlling for age at death. Ant CA1, anterior part of the CA1 field; Post CA1, posterior part of the CA1 field; NBM, nucleus basalis of Meynert; LC, locus coeruleus. Layers are indicated by Roman numerals. ns, not statistically significant (adapted from Giannakopoulos *et al*., 1995).

cant difference was observed in NFT densities in areas 9 and 20, as well as in areas 17 and 18 between AD and nondemented cases. Interestingly, when a distinction between cognitively intact cases and cases with age-associated memory impairment included in the nondemented group was made, an association between substantial NFT formation in layers V–VI of area 20 and age-associated memory impairment was found (Giannakopoulos *et al*., 1996, 1997). In the subcortical nuclei, a statistically significant difference in NFT counts between nondemented and AD cases was observed in the nucleus basalis of Meynert, but not in the locus coeruleus and amygdala (Table 8.1). These data indicate that the regional patterns of cytoskeletal pathology differ in several aspects among centenarians and younger subjects. First, the apparent progression of NFT formation from the hippocampus to the temporal neocortex may play a less important role in the clinical expression of AD in centenarians compared to younger patients in whom it has been associated with overt dementia symptomatology (Hof *et al*., 1992; Bouras *et al*., 1994; Giannakopoulos *et al*., 1994b). Although the involvement of area 20 may account for age-associated memory impairment in this age group, overt clinical signs of AD in oldest-old individuals require both high NFT densities in the CA1 field and progressive damage of areas 7, 22, 23, and 24, which are spared in most of the cases at the early stages of the dementing process (Hof *et al*., 1992; Giannakopoulos *et al*., 1996). This distribution of NFT can be regarded as a displacement of these lesions, such that parietal and cingulate areas are more affected than is usually the case in AD, whereas superior frontal and inferior temporal association areas are relatively preserved.

The severe damage of the anterior and posterior cingulate cortex in AD centenarians could be associated with a particular clinical course of the disease in this age group. It is well documented in experimental animals that this region participates in the acquisition and performance of active avoidance and spatial memory tasks, as well as in attentional processing (Watson *et al*., 1973; Gabriel *et al*., 1980; Sutherland *et al*., 1988; Matsunami *et al*., 1989). Pardo and collaborators (1990) found that the performance of the Stroop color/word test results on a selective activation of the anterior cingulate cortex and proposed that the selection and recruitment of processing centers appropriate for task execution may depend on the integrity of this area. Moreover, the anterior cingulate cortex possesses motor areas connected in a somatotopic fashion to the primary motor cortex and is involved in the integration of complex somatic and visceral activities (Morecraft and Van Hoesen, 1992; Nimchinsky *et al*., 1996). The posterior cingulate cortex is involved in triggering appropriate motor responses to specific visuomotor stimuli (Vogt *et al*., 1990; Olson and Musil, 1992; Olson *et al*., 1993). Detailed neuropsychological testing is needed to define if these cortical functions are preferentially compromised in AD centenarians.

Another particularity of the centenarian brain resides in the laminar distribution of NFT and SP within the cerebral cortex (Fig. 8.2). Several lines of evidence suggest that the long corticocortical projection system is likely to degenerate in the course of AD, evolving to a syndrome of cortical disconnection (Pearson *et al*., 1985; Lewis *et al*., 1987; Hof *et al*., 1990; Hof and Morrison, 1990). Higher NFT densities generally observed in layers V and VI than in superficial layers of association cortices indicate that feedback and lateral corticocortical projections are affected predominantly by the degenerative process in younger AD cases (Pearson *et al*., 1985; Lewis *et al*., 1987; Hof *et al*., 1990; Hof and Morrison, 1990). In contrast, the preferential localization of NFT in layers II and III in the neocortex of demented and nondemented centenarians suggests that the feedforward corticocortical projections that are furnished principally by layer III neurons are damaged early in very old patients (Pearson *et al*., 1985; Lewis *et al*., 1987; Hof *et al*., 1990; Giannakopoulos *et al*., 1994a, 1995, 1996).

With regard to SP, several studies of centenarians indicate that there is no relationship between their formation in the neocortex and the early stages of the dementing process, as is the case in younger populations (Delaère *et al*., 1993; Giannakopoulos *et al*., 1993). However, our evaluations of centenarians with severe AD, as well as longitudinal data of Green and collaborators (2000), demonstrated a clear correlation between SP densities in areas 7, 9, 20, 22, 23, and 24 and the severity of dementia (Table 8.2) (Giannakopoulos *et al*., 1994a, 1995, 1996). These findings differ from previous studies in younger demented and nondemented cases that reported a lack of correlation between the clinical severity of dementia and SP densities (Arriagada *et al*., 1992a,b; Berg *et al*., 1993; Bierer *et al*., 1995) and imply that SP formation may not be etiologically linked to the early stages of cognitive impairment but may represent a reliable pathological hallmark of severe AD in this age group.

V. Patterns of Neuronal Loss in the Centenarian Brain

On addition to these particularities in NFT and SP distribution, very old people show a differential pattern of neuronal loss within the cerebral cortex compared to younger cohorts (Giannakopoulos *et al*., 1996). Estimates of neuron densities are summarized in Table 8.3. After correction for differential cortical shrinkage, no statistically significant difference was found in neuron densities in the hippocampus, hilus of the dentate gyrus, and subiculum between nondemented and AD cases. In contrast, nondemented centenarians displayed significantly higher neuron densities in layers II and V of the entorhinal cortex and in layers II–III and V–VI of areas 9 and 20 compared to AD cases. In comparison to nondemented cases, AD cases showed a 30.8% neuronal loss in layer II and 17.8% in layer V of the entorhinal cortex, 26.7% in layers II–III and 27.5% in layers V–VI of area 20, and 5.1% in layers II-III and 8.3% in layers V–VI of area 9. No correlation was found between NFT and neuron densities in each area and among different cortical areas, whereas SP counts in layers II–III of area 20 were correlated negatively with neuron densities in layers II–III and layers V–VI of area 20. There was no correlation between SP densities and neuron numbers in other cortical areas. The negative correlation between SP and neuron densities in area 20 was further confirmed using a multiple regression model with neuron densities as the dependent variable and NFT and SP densities as independent variables.

The absence of AD-related neuronal loss in hippocampus fields and subiculum of centenarians differs from data showing

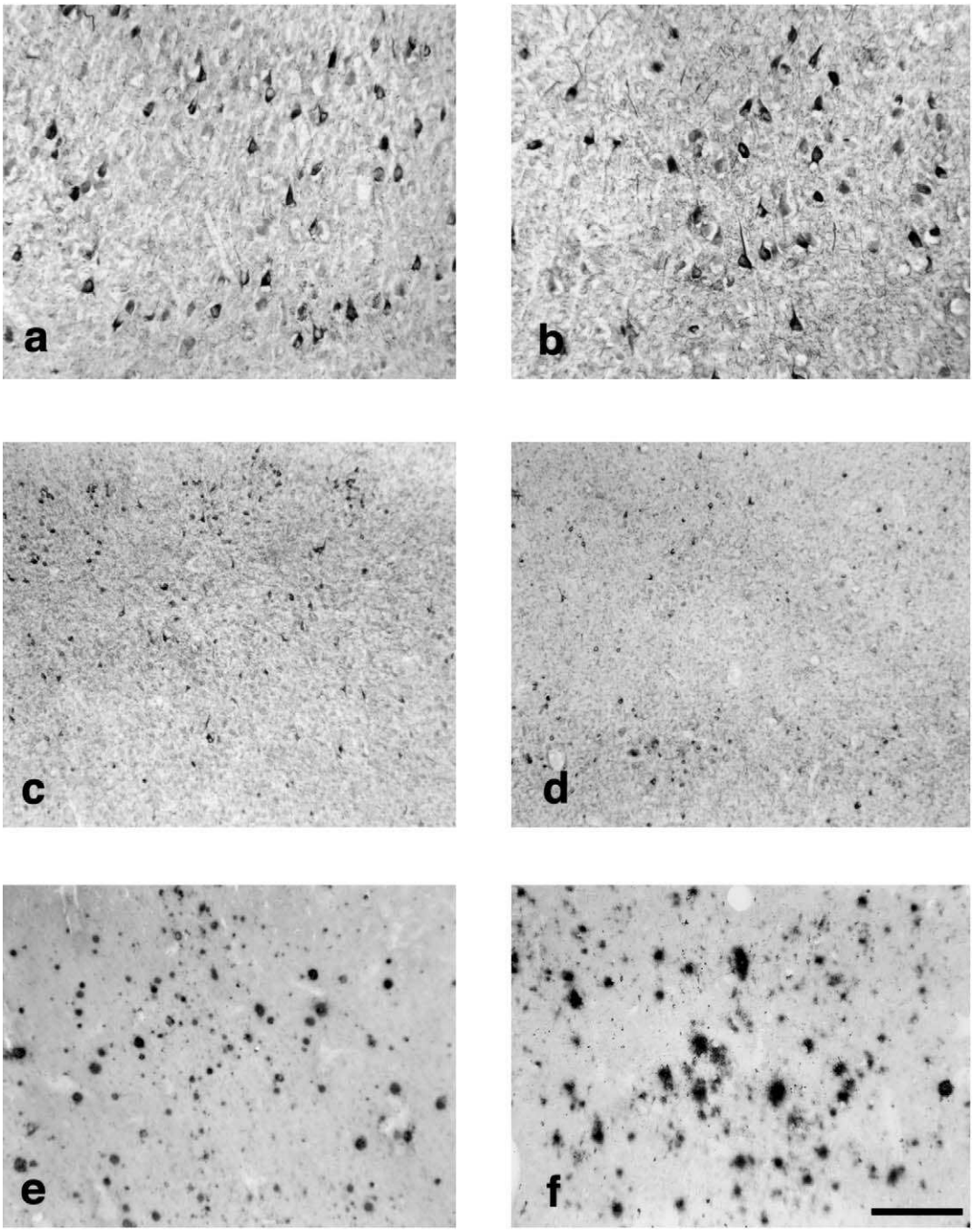

FIG. 8.2. Neurofibrillary tangles in the CA1 (posterior part) in a nondemented centenarian (a) and a centenarian with AD (b). There is no significant difference in NFT densities. Neurofibrillary tangles in the frontal cortex (area 9) of a 97-year-old AD (c) and a 65-year old AD (d). The old AD displays the highest density of NFT in layers II and III, whereas the ''younger'' AD case presents a high density of NFT in layers V and VI. The number of senile plaques in area 9 is lower in the nondemented centenarian (e) than in the centenarian with AD (f). Scale bar on a,b: 125 μm; on c,d: 500 μm; and on e,f: 250 μm.

a neuronal loss of 68% in the CA1 field and 47% in the subiculum in younger AD cohorts (West *et al*., 1994). In particular, these authors postulated that substantial neuronal depletion in the CA1 field represents a qualitative difference between normal aging and AD processes. These results suggest that despite the presence of high NFT densities, very old AD patients display no significant neuronal loss in the CA1 field. In contrast, the significant neuronal loss observed in layers, II and V of the entorhinal cortex and areas 9 and 20 AD centenarians is an agreement with earlier observations in younger demented individuals (Lippa *et al*., 1992; O'Banion *et al*., 1994; Gómez-Isla *et al*., 1996) and implies that decreasing

TABLE 8.2 Comparison of SP Counts in Nondemented and AD Centenarians[a]

Area/layers	Nondemented $n = 24$ (98.4 ± 1.4)	AD $n = 19$ (97.2 ± 1.2)	P
Ant CA1	2.4 ± 0.5	4.2 ± 0.8	ns
Post CA1	3.4 ± 0.8	3.3 ± 0.3	ns
CA2–3	4.8 ± 1.2	6.7 ± 0.5	ns
Hilus	1.2 ± 0.3	2.3 ± 0.4	ns
Subiculum	1.8 ± 0.6	3.7 ± 0.7	ns
Entorhinal II	4.4 ± 0.8	9.0 ± 1.8	ns
Entorhinal V	1.5 ± 0.4	2.8 ± 0.5	ns
20 II–III	10.5 ± 2.0	22.1 ± 2.9	< 0.05
V–VI	4.4 ± 1.1	7.4 ± 1.3	ns
9 II–III	5.7 ± 1.5	12.8 ± 2.3	< 0.05
V–VI	3.9 ± 1.1	7.7 ± 1.2	ns
7 II–III	13.2 ± 2.3	26.0 ± 3.0	< 0.05
V–VI	4.7 ± 1.0	9.9 ± 1.5	< 0.05
22 II–III	21.4 ± 8.6	27.4 ± 5.6	ns
V–VI	6.4 ± 1.9	12.5 ± 3.7	< 0.05
17 II–III	10.0 ± 1.5	11.1 ± 1.5	ns
V–VI	11.3 ± 2.2	7.1 ± 1.0	ns
18 II–III	9.1 ± 1.5	12.2 ± 1.5	ns
V–VI	6.6 ± 1.2	8.8 ± 1.8	ns
24 II–III	22.8 ± 3.2	33.8 ± 3.7	< 0.05
V–VI	12.1 ± 3.1	12.1 ± 1.6	ns
23 II–III	13.9 ± 2.1	26.4 ± 2.9	< 0.05
V–VI	4.4 ± 0.8	8.2 ± 0.9	< 0.05
Amygdala	9.2 ± 1.7	10.6 ± 1.0	ns
NBM	12.36 ± 4.89	10.73 ± 0.32	ns
LC	2.73 ± 1.23	1.03 ± 0.21	ns

[a] Results represent SP counts/mm^2 (± SEM) in each area. The mean age of each group is indicated in parentheses. Statistical analysis was performed by analysis of covariance controlling for age at death. Ant CA1, anterior part of the CA1 field; Post CA1, posterior part of the CA1 field; NBM, nucleus basalis of Meynert; LC, locus coeruleus. Layers are indicated by Roman numerals. ns, not statistically significant (adapted from Giannakopoulos *et al*., 1995).

TABLE 8.3 Comparison of Neuron Densities between Nondemented and AD Centenarians[a]

Area/layers	Nondemented $n = 24$ (98.4 ± 1.4)	AD $n = 19$ (97.2 ± 1.2)	P
CA1	34810 ± 1100	36012 ± 1339	ns
CA2–3	54200 ± 1285	53112 ± 1760	ns
Hilus	26663 ± 811	27211 ± 1144	ns
Subiculum	38344 ± 778	36788 ± 1123	ns
Entorhinal II	39204 ± 1270	28802 ± 1490	< 0.01
Entorhinal V	61106 ± 1228	51036 ± 2443	< 0.05
20 II–III	68897 ± 1338	55519 ± 2220	< 0.005
V–VI	66776 ± 1166	50966 ± 2112	< 0.005
9 II–III	57186 ± 855	52298 ± 1557	< 0.05
V–VI	63460 ± 1349	58600 ± 1562	< 0.05

[a] Results represent neuron number/mm^3 (± SEM) in each area. These densities were estimated using the optical disector method. A statistically significant neuronal loss was observed in layers II and V of the entorhinal cortex and in areas 9 and 20 of AD cases. The mean age of each group is indicated in parentheses. Layers are indicated by Roman numerals. Statistical analysis was performed by analysis of covariance controlling for age at death. ns, not statistically significant (adapted from Giannakopoulos *et al*., 1996).

neuron densities in these areas are associated with AD symptomatology in any age group. More precisely, our findings for the entorhinal cortex are consistent with a report by Gómez-Isla and co-workers (1996), who estimated total neuron numbers in the entorhinal cortex of patients younger than 95 years, and reported a decrease of 60% in the number of neurons in layer II of the entorhinal cortex in patients with nondemented and of 90% in severe AD cases. However, the magnitude of neuronal loss in AD centenarians is significantly lower than that reported in younger AD cases (Lippa *et al*., 1992; Gómez-Isla *et al*., 1996), suggesting that a mild decrease in neuron numbers in cortical regions, such as the entorhinal cortex and areas 9 and 20, may be sufficient to cause dementia after 95 years of age (Hansen *et al*., 1988). The neuropathological distinction between very old and younger people is further supported by the results of our correlation analysis. First, the dissociation between NFT and neuron densities in all of the areas studied indicates that neuronal loss unrelated to the presence of NFT is the rule in the oldest-old, in contrast to elderly subjects younger than 85 where a correlation was documented (Gómez-Isla *et al*., 1996). Moreover, the negative relationship between SP densities and neuron counts in area 20 suggests that SP formation may be related to neuronal depletion within certain cortical regions in centenarians, although the biological mechanism surrounding this phenomenon is still poorly understood.

VI. Conclusions

The debate on whether there is continuity or discontinuity between normal aging and dementia has a long history and

TABLE 8.4 List of Longitudinal Studies of Centenarians

Country	Name of project	>100	90–100	Genetics
Denmark	The Danish Centenarian Study	Yes	Yes	Yes
France	Chronos	Yes	Yes	Yes
Sweden	The Swedish Centenarian Study	Yes	No	?
United States	New England Centenarian Study	Yes	No	Yes

is not merely of academic interest. The hypothesis that AD is an aging-related condition has been supported by the quasi ubiquitous presence of AD pathologic changes in the course of brain aging and the exponential increase of AD prevalence after 65 years of age. Contrasting with this concept of aging, the study of oldest-old individuals indicates that the occurrence of AD pathology is not a mandatory phenomenon of increasing chronologic age. In particular, the neuropathology of advanced aging strongly suggests that very old individuals display differential neuronal aging and susceptibility to the degenerative process of AD (Hauw *et al*., 1986; Mizutani and Shimada, 1990, 1992; Delaère *et al*., 1993; Giannakopoulos *et al*., 1993, 1994b,c, 1995, 1996, 1997). In this respect, the absence of significant neuronal loss in the CA1 field, as well as the low rate of NFT formation in areas 9 and 20 in centenarians with AD, may be related to a genetically determined resistance of these neuronal subpopulations in very old people. Importantly, evidence from genetic studies of aging and AD implies that there are a number of susceptibility genes that may modify or delay the onset of late-life brain failure. These gene families form a natural target for devising strategies for significantly delaying the onset of late-life dementia (Kaye, 1997). More generally, several prospective studies of oldest-old people have been initiated, searching to define the possible genetic background of longevity (Table 8.4) (Schachter *et al*., 1994; Manton and Vaupel, 1995; Jeune *et al*., 1996; Perls, 1997; Samuelsson *et al*., 1997; Perls *et al*., 1998). The first one started in France in 1991 and is known as the ''Chronos Project.'' Its main goal is to identify genetic factors associated with human longevity. It consists of a follow-up of more than 1200 nonagenarians and centenarians with living siblings 90 years or older (Schachter *et al*., 1994) One of the most recent, the ''New England Centenarian Study,'' includes four main axes: a population-based medical and demographic study of all centenarians living within the Boston area, neuropsychological–neuropathological correlations to define the limits of normal brain aging, population genetics to define familial patterns relating to the phenotype of extreme longevity, and molecular genetics to identify both nuclear and mitochondrial longevity enabling genes among centenarian sibling pairs, centenarian members of families highly clustered for longevity, and sporadic centenarian cases compared to younger controls. In the near future, these complex longitudinal studies may provide us with clues for a better understanding of both genetic and neurobiological dimensions of extreme aging.

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References

Adolfsson, R. (1986). Neurochemical, genetic and clinical aspects of Alzheimer disease. *In* ''Dimensions in Aging'' (M. Bergener, M. Ermini, and H. B. Stahelin, eds.), pp. 183–207, Academic Press, San Diego.

Arnold, S. E., Hyman, B. T., Flory, J., Damasio, A. R., and Van Hoesen, G. W. (1991). The topographical and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer's disease. *Cereb. Cortex* **1**, 103–106.

Arriagada, P. V., Growdon, J. H., Hedley-White, E. T., and Hyman, B. T. (1992a). Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology* **42**, 631–639.

Arriagada, P. V., Marzloff, K., and Hyman, B. T. (1992b). Distribution of Alzheimer-type pathologic changes in nondemented elderly individuals matches the pattern in Alzheimer's disease. *Neurology* **42**, 1681–1688.

Asada, T., Yamagata, Z., Kinoshita, T., Kinoshita, A., Kariya, T., Asaka, A., and Kakuma, T. (1996). Prevalence of dementia and distribution of ApoE alleles in Japanese centenarians: An almost-complete survey in Yamanashi Prefecture, Japan. *J. Am. Geriatr. Soc*. **44**, 151–155.

Beregi, E. (1990). Centenarians in Hungary—A sociomedical and demographic study. *Interdiscip. Top. Gerontol*. **27**, 1–164.

Berg, L., McKeel, D. W., Miller, P., Baty, J., and Morris, J. C. (1993). Neuropathological indexes of Alzheimer's disease in demented and ND persons aged 80 years and older. *Arch. Neurol. (Chicago)* **50**, 349–358.

Bierer, L. M., Hof, P. R., Purohit, D. P., Carlin, L., Schmeidler, J., Davis, K. L., and Perl, D. P. (1995). Neocortical neurofibrillary tangles correlate with dementia severity in Alzheimer's disease. *Arch. Neurol. (Chicago)* **52**, 81–88.

Bohl, J., Störkel, S., and Steinmetz, H. (1987). Senile Demenz vom Alzheimer Type. *Z. Alternsforsch*. **63**, 65–74.

Bouras, C., Hof, P. R., and Morrison, J. H. (1993). Neurofibrillary tangle densities in the hippocampal formation in a non-demented population define subgroups of patients with differential early pathologic changes. *Neurosci. Lett*. **153**, 131–135.

Bouras, C., Hof, P. R., Giannakopoulos, P., Michel, J. P., and Morrison, J. H. (1994). Regional distribution of neurofibrillary tangles and senile plaques in the cerebral cortex of elderly patients: A quantitative evaluation of a one-year autopsy population from a geriatric hospital. *Cereb. Cortex* **4**, 138–150.

Braak, H., and Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol*. **82**, 239–259.

Brayne, C. (1991). The EURODEM collaborative re-analysis of case-control studies of Alzheimer's disease: Implications for public health. *Int. J. Epidemiol*. **20**, S68–S71.

Crystal, H., Dickson, D., Fuld, P., Masur, D., Scott, R., Mehler, M., Masdeu, J., Kawas, C., Aronson, M., and Wolfson, L. (1988). Clinico-pathologic studies in dementia: Nondemented subjects with pathologically confirmed Alzheimer's disease. *Neurology* **38**, 682–687.

Delaère, P., He, Y., Fayet, G., Duyckaerts, C., and Hauw, J. J. (1993). Beta A4 deposits are constant in the brain of the oldest-old: An immunocytochemical study of 20 French centenarians. *Neurobiol. Aging* **14**, 191–194.

Dello Buono, M., Urciuoli, O., and De Leo, D. (1998). Quality of life and longevity: A study of centenarians. *Age Ageing* **27**, 207–261.

Dickson, D. W., Crystal, H. A., Mattiace, L. A., Masur, D. M., Blau, A. D., Davies, P., Yen, S. H., and Aronson, M. K. (1991). Identification of normal and pathological aging in prospectively studied nondemented elderly humans. *Neurobiol. Aging* **13**, 179–189.

Evans, D. A., Scherr, P. A., Cook, N. A., Albert, M. S., Funkenstein, H. H., Beckett, L. A., Hebert, L. E., Wetle, T. T. Branch, L. G., Chown, M. J., Hennekens, C. H., and Taylor, J. O. (1992). The impact of Alzheimer's disease in the United States population. *In* ''The Oldest-Old'' (R. M. Suzman, D. P. Willis, and K. G. Manton, eds.), pp. 283–299. Oxford University Press, New York.

Fichter, M. M., Meller, I., Schroppel, H., and Steinkirchner, R. (1995). Dementia and cognitive impairment in the oldest-old in the community. Prevalence and comorbidity. *Br. J. Psychiatry* **166**, 621–629.

Gabriel, M., Foster, K., Orona, E., Saltwick, S. E., and Stanton, M. (1980). Neuronal activity of cingulate cortex, anteroventral thalamus, and hippocampal formation in discriminative conditioning: Encoding and extraction of the significance of conditional stimuli. *Prog. Psychobiol. Physiol. Psychol*. **9**, 125–131.

Geneva (1998). *In* ''Annuaire Statistique du Canton de Genève.'' Office Cantonal de la Statistique (OCSTAT), 36th ed., p. 56.

Giannakopoulos, P., Hof, P. R., Surini, M., Michel, J. P., and Bouras, C. (1993). Quantitative immunohistochemical analysis of the distribution of neurofibrillary tangles and senile plaques in the cerebral cortex of nonagenarians and centenarians. *Acta Neuropathol*. **85**, 601–610.

Giannakopoulos, P., Hof, P. R., Giannakopoulos, A. S., Buée-Scherrer, V., Surini, M., Delacourte, A., and Bouras, C. (1994a). Dementia in the oldest-old: Quantitative analysis of 12 cases from a psychiatric hospital. *Dementia* **5**, 348–356.

Giannakopoulos, P., Hof, P. R., Mottier, S., Michel, J. P., and Bouras, C. (1994b). Neuropathologic changes in the neocortex of 1258 cases from a geriatric hospital: Retrospective clinicopathologic evaluation of a ten year autopsy population. *Acta Neuropathol*. **87**, 456–468.

Giannakopoulos, P., Hof, P. R., Vallet, P. G., Giannakopoulos, A. S., Charnay, Y., and Bouras, C. (1994c). The quantitative analysis of neuropathological changes in the cerebral cortex of centenarians. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **19**, 577–592.

Giannakopoulos, P., Hof, P. R., Giannakopoulos, A. S., Hermann, F., Michel, J. P., and Bouras, C. (1995). Regional distribution of neurofibrillary tangles and senile plaques in the cerebral cortex of very old patients. *Arch. Neurol. (Chicago)* **52**, 1150–1159.

Giannakopoulos, P., Hof, P. R., Kövari, E., Vallet, P. G., and Bouras, C. (1996). Distinct patterns of neuronal loss and Alzheimer's disease lesion distribution in elderly individuals older than 90 years. *J. Neuropathol. Exp. Neurol*. **55**, 1210–1220.

Giannakopoulos, P., Hof, P. R., Michel, J. P., Guimon, J., and Bouras, C. (1997). Cerebral cortex pathology in aging and Alzheimer's disease: A quantitative survey of large hospital-based geriatric and psychiatric cohorts. *Brain Res. Rev.* **25**, 217–245.

Gómez-Isla, T., Price, J. L., McKeel, D. W., Jr., Morris, J. C., Growdon, J. H., and Hyman, B. T. (1996). Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *J. Neurosci*. **16**, 4491–4500.

Gottfries, C. G., (1986). Nosological aspects of differential typology of dementia of Alzheimer type. *In* ''Dimensions in Aging'' (M. Bergener, M. Ermini, and H. B. Stahelin, eds.), pp. 207–221. Academic Press, New York.

Green, M. S., Kaye, J. A., and Ball, M. J. (2000). The Oregon Brain Aging study: Neuropathology accompanying healthy aging in the oldest-old. *Neurology* **54**, 105–113.

Hansen, L. A., DeTeresa, R., Davies, P., and Terry R. D. (1988). Neocortical morphometry, lesion counts, and choline acetyltransferance levels in the age spectrum of Alzheimer's disease. *Neurology* **38**, 48–54.

Hauw, J. J., Vignolo, P., Duyckaerts, C., Beck, H., Forette, F., Henry, J. F., Laurent, M., Piette, F., Sachet, A., and Berthaux P. (1986). Etude neuropathologique de 12 centenaires: La fréquence de la démence sénile de type Alzheimer n'est pas particulièrement élevée dans ce groupe de personnes très agées. *Rev. Neurol*. **142**, 107–115.

Hilleras, P. K., Jorm, A. F., Herlitz, A., and Winblad, B. (1999). Activity patterns in very old people: A Survey of cognitively intact subjects aged 90 years or older. *Age Ageing* **28**, 147–152.

Hof, P. R., and Morrison, J. H. (1990). Quantitative analysis of a vulnerable subset of pyramidal neurons in Alzheimer's disease: II. Primary and secondary visual cortex. *J. Comp. Neurol*. **301**, 55–64.

Hof, P. R., Cox, K., and Morrison, J. H. (1990). Quantitative analysis of a vulnerable subset of pyramidal neurons in Alzheimer's disease: I. Superior frontal and inferior temporal cortex. *J. Comp. Neurol*. **301**, 44–54.

Hof, P. R., Bierer, L. M., Perl, D. P., Delacourte, A., Buée, L., Bouras, C., and Morrison, J. H. (1992). Evidence for early vulnerability of the medial and inferior aspects of the temporal lobe in an 82-year-old patient with preclinical signs of dementia—Regional and laminar distribution of neurofibrillary tangles and senile plaques. *Arch. Neurol. (Chicago)* **49**, 946–953.

Hofman, A., Rocca, W. A., Brayne, C, Breteler, M. M., Clarke, M., Cooper, B., Copeland, J. R., Dartigues, J. F., da Silva Droux, A., and Hagnell O. (1991). The prevalence of dementia in Europe: A collaborative study of 1980–1990 findings. Eurodem Prevalence Research Group. *Int. J. Epidemiol*. **20**, 736–748.

Howieson, D. B., Dame, A., Camicioli, R., Sexton, G., Payami, H., and Kaye, J. A. (1997). Cognitive markers preceding Alzheimer's dementia in the healthy oldest-old. *J. Am. Geriatr. Soc*. **45**, 584–589.

IPSEN Foundation. (1990). ''Rapport: Les personnes âgées dans la communauté européenne: Présent et avenir. '' IPSEN Foundation, Paris.

Itoh, Y., Yamada, M., Suematsu, N., Matsushita, M., and Otomo, E. (1998). An immunohistochemical study of centenarian brains: A comparison. *J. Neurol. Sci*. **157**, 73–81.

Jeune, B., Skytthe, A., and Vaupel, J. W. (1996). The demography of centenarians in Denmark. *Ugeskr. Laeg*. **158**, 7392–7396.

Karasawa, A., and Goto, S. (1985). [Bedside care of the aged with senile dementia. Direction in the care of the demented patients—care to preserve human dignity]. *Kankyo Gijutsu* **31**, 1557–1566.

Kaye, J. A. (1997). Oldest-old healthy brain function. The genomic potential. *Arch. Neurol. (Chicago)* **54**, 1217–1221.

Larkin, M. (1999). Centenarians point the way to healthy ageing. *Lancet* **353**, 1074.

Lautenschlager, N. T., Cupples, L. A., Rao, V. S., Auerbach, R., Becker, J., Burke, H., Chui, H. C., Duara, R., Glatt, S. L., Green, R. C., Jones, R., Karlisky, H., Kukull, W. A., Kurz, A., Larsen, E. B., Martelli, K., Sadovnick, A. D., Volicer, L., Waring, S. C., Growdon, J. H., and Farrer, L. A. (1996) Risk of dementia among relatives of Alzheimer's disease patients in the MIRAGE study: What is in store for the oldest-old? *Neurology* **46**, 641–650.

Lewis, D. A., Campbell, M. J., Terry, R. D., and Morrison, J. H. (1987). Laminar and regional distribution of neurofibrillary tangles and neuritic plaques in Alzheimer's disease: A quantitative study of visual and auditory cortices. *J. Neurosci*. **7**, 1799–1808.

Lippa, C. F., Hamos, J. E., Pulaski-Salo, D., Degennaro, L. G., and Drachman, D. A. (1992). Alzheimer's disease and aging: Effects on perforant pathway perikarya and synapses. *Neurobiol. Aging* **13**, 405–411.

Manton, K. G., and Vaupel, J. W. (1995). Survival after the age of 80 in the United States, Sweden, France, England, and Japan. *N. Engl. J. Med.* **333**, 1232–1235.

Masliah, E., Ellisman, M., and Carragher, B. (1992). Three-dimensional analysis of the relationship between synaptic pathology and neuropil threads in Alzheimer's disease. *J. Neuropathol. Exp. Neurol*. **51**, 404–414.

Matsunami, K., Kawashima, T., and Satake, H. (1989). Mode of [^{14}C]2-deoxy-D-glucose uptake into retrospenial cortex and other memory-related structures of the monkey during a delayed response. *Brain Res. Bull*. **22**, 829–838.

Mizutani, T., and Shimada, H. (1990). Neuropathological aspects of centenarian brains: Study of 24 autopsy cases. *In* ''Psychogeriatrics: Biomedical and Social Advances'' (K. Hasegawa and A. Homma, eds.), pp. 128–133. Excerpta Medica, Tokyo.

Mizutani, T., and Shimada, H. (1992). Neuropathological background of twenty-seven centenarian brains. *J. Neurol. Sci*. **108**, 168–177.

Morecraft, R. J., and Van Hoesen, G. W. (1992). Cingulate input to the primary and supplementary motor cortices in the rhesus monkey: Evidence for somatotopy in areas 24 and 23c. *J. Comp. Neurol*. **322**, 471–489.

Morris, J. C., McKeel, D. W., Jr., Storandt, M., Rubin, E. H., Price, J. L., Grant, E. A., Ball, M. J., and Berg, L. (1991). Very mild Alzheimer's disease: Informant-based clinical, psychometric, and pathologic distinction from normal aging. *Neurology* **41**, 469–478.

Morris, J. C., Storandt, M., and McKeel, D. W. (1996). Cerebral amyloid deposition and diffuse plaques in ''normal'' aging: Evidence for presymptomatic and very mild Alzheimer's disease. *Neurology* **46**, 707–719.

Moss, M., and Albert, M. (1988). Alzheimer's disease and other dementing disorders. *In* ''Geriatric Neuropsychology'' (M. Albert and M. Moss, eds.), pp. 145–177. Guilford Press, New York.

Mountjoy, C. Q., Roth, M., Evans, N. J. R., and Evans, H. M. (1983). Cortical neuronal counts in normal elderly controls and demented patients. *Neurobiol. Aging* **4**, 1–11.

Nimchinsky, E. A., Hof, P. R., Young, W. G., and Morrison, J. H. (1996). Neurochemical, morphologic and laminar characterization of cortical projection neurons in the cingulate motor areas of the macaque monkey. *J. Comp. Neurol*. **374**, 136–160.

O'Banion, M. K., Coleman, P. D., and Callahan, L. M. (1994). Regional neuronal loss in aging and Alzheimer's disease: A brief review. *Semin. Neurosci*. **6**, 307–314.

Olson, C. R., and Musil, S. Y. (1992). Topographic organization of cortical and subcortical projections to posterior cingulate cortex in the cat: Evidence for somatic, ocular, and complex subregions. *J. Comp. Neurol*. **324**, 237–260.

Olson, C. R., Musil, S. Y., and Goldberg, M. (1993). Posterior cingulate cortex and visuospatial cognition: Properties of single neurons in the behaving monkey. *In* "The Neurobiology of Cingulate Cortex and Limbic Thalamus" (B. A. Vogt, and M. Gabriel, eds.), pp. 336–380. Birkhäuser, Boston.

Paolisso, G., Tagliamonte, M. R., Rizzo, M. R., Manzella, D., Gambardella, A., and Varricchio, M. (1998). Oxidative stress and advancing age: Results in healthy centenarians. *J. Am. Geriatr. Soc*. **46**, 833–838.

Pardo, J. V., Pardo, P. J., Janer, K. W., and Raichle, M. E. (1990). The anterior cingulate cortex mediates processing selection in the Stroop attentional conflict paradigm. *Proc. Natl. Acad. Sci. U.S.A*. **87**, 256–259.

Pearson, R. C. A., Esiri, M. M., Hiorns, R. W., Wilcock, G. K., and Powell, T. P. S. (1985). Anatomical correlates of the distribution of the pathological changes in the neocortex in Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A*. **82**, 4531–4534.

Perls, T. T. (1995). The oldest-old. *Sci. Am*. **272**, 70–75.

Perls, T. T. (1997). Centenarians prove the compression of morbidity hypothesis, but what about the rest of us who are genetically less fortunate? *Med. Hypotheses* **49**, 405–407.

Perls, T. T., Bubrick, E., Wager, C. G., Vijg, J., and Kruglyak, L. (1998). Siblings of centenarians live longer. *Lancet* **351**, 1560.

Price, J. L., Davis, P. B., Morris, J. C., and White, D. L. (1991). The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. *Neurobiol. Aging* **12**, 295–312.

Rebeck, G. W., Perls, T. T., West, H. L., Sodhi, P., Lipsitz, L. A., and Hyman, B. T. (1994). Reduced apolipoprotein ϵ4 allele frequency in the oldest-old Alzheimer's patients and cognitively normal individuals. *Neurology* **44**, 1513–1516.

Ritchie, K., and Kildea, D. (1995). Is senile dementia "age-related" or "ageing-related"?—Evidence from meta-analysis of dementia prevalence in the oldest-old. *Lancet* **346**, 931–933.

Roberts, W. C., and Shirani, J. (1998). Comparison of cardiac findings at necropsy in octogenarians, nonagenarians, and centenarians. *Am. J. Cardiol*. **82**, 627–631.

Saltzstein, S. L., Behling, C. A., and Baergen, R. N. (1998). Features of cancer in nonagenarians and centenarians. *J. Am. Geriatr. Soc*. **46**, 994–998.

Samuelsson, S. M., Alfredson, B. B., Hagberg, B., Samuelsson, G., Nordbeck, B., Brun A., Gustafson, L., and Risberg, J. (1997). The Swedish Centenarian Study: A multidisciplinary study of five consecutive cohorts at the age of 100. *Int. J. Aging Hum. Dev*. **45**, 223–253.

Schachter, F., Faure-Delanef, L., Guenot, F., Rouger, H., Froguel, P., Lesueur-Ginot, L., and Cohen, D. (1994). Genetic associations with human longevity at the APOE and ACE loci. *Nat. Genet*. **6**, 29–32.

Schneider, E. (1999). Aging in the third millenium. *Science* **283**, 796–797.

Smith, D. W. (1997). Centenarians: Human longevity outliers. *Gerontologist* **37**, 200–206.

Sobel, E., Louhija, J., and Sulkava, R. (1995). Lack of association of apolipoprotein E allele epsilon 4 with late-onset Azheimer's disease among Finnish centenarians. *Neurology* **45**, 903–907.

Sutherland, R. J., Whishaw, I. Q., and Kolb, B. (1988). Contributions of cingulate cortex to two forms of spatial learning and memory. *J. Neurosci*. **8**, 1863–1872.

Vogt, B. A., Van Hoesen, G. W., and Vogt, L. J. (1990). Laminar distribution of neuron degeneration in posterior cingulate cortex in Alzheimer's disease. *Acta Neuropathol*. **80**, 581–589.

Watson, R. T., Heilman, K. M., Cauthen, J. C., and King, F. A. (1973). Neglect after cingulectomy. *Neurology* **23**, 1003–1007.

West, M. J. (1993). Regionally specific loss of neurons in the aging human hippocampus. *Neurobiol. Aging* **14**, 287–293.

West, M. J., Coleman, P. D., Flood, D. G., and Troncoso, J. D. (1994). Differences in the pattern of hippocampal neuronal loss in normal aging and Alzheimer's disease. *Lancet* **344**, 769–772.

Wilkinson, T. J., and Sainsbury, R. (1998). A census-based comparison of centenarians in New Zealand with those in the United States. *J. Am. Geriatr. Soc*. **46**, 488–491.

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Patrick R. Hof

9

Regional and Laminar Patterns of Selective Neuronal Vulnerability in Alzheimer's Disease

The pathogenetic events that lead to Alzheimer's disease (AD) are not fully understood, although the current knowledge of the pathological changes that occur in AD suggests that structurally and functionally AD is predominantly a disease of the cerebral cortex that involves only certain populations of neurons displaying specific regional and laminar distribution and connectivity patterns, whereas other neuron types are spared. Thus, differential neuronal vulnerability exists in AD that can be related to the morphologic and biochemical characteristics of identifiable neuronal populations and cortical connections. This chapter provides an overview of the relationships between the distribution of pathologic changes in AD and the localization of specific elements of the cortical circuitry that are affected by these alterations. This chapter also discusses observations that relate the neurochemical characteristics of particular neuronal types to their relative vulnerability or resistance to the degenerative process. It is possible that through such morphologic and molecular analyses, correlations will emerge among distribution of cellular pathologic changes, neurochemical characteristics related to vulnerability, and cortical circuits at risk. Such correlations may be useful for the development of preventive or protective interventions against the specific neuronal degenerative events that occur during the progression of the dementing illness.

I. Lesion Types and Distribution in Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative disorder classically characterized by the presence of two major types of histopathologic alterations in the cerebral cortex: neurofibrillary tangles (NFT) and senile plaques (SP) (Fig. 9.1). The distribution and density of NFT and SP have been analyzed in great detail and constitute the basis of the neuropathologic diagnosis of AD (Mirra *et al*., 1993). NFT are characterized by the accumulation of abnormal components of the neuronal cytoskeleton that form paired helical filaments, whereas SP are composed of dystrophic neurites and glial elements with or without a central amyloid core (Brion, 1990; Vickers *et al*., 1992, 2000; Hof *et al*., 1999a). These lesions are consistently observed throughout the brain and are predominant in the cerebral cortex, where NFT are located in the perikaryon of large pyramidal neurons and SP are distributed throughout the cortical regions, but are particularly numerous in association areas (Arnold *et al*., 1991; Hof *et al*., 1999a). In subcortical regions, NFT are found in large numbers in many structures connected with the cerebral cortex, including the amygdala, nucleus basalis of Meynert, ventral tegmental area, dorsal raphe, locus coeruleus, certain midline thalamic nuclei, some hypothalamic nuclei, and the olfactory bulb (Braak and Braak, 1991; Tomlinson, 1992; Kovács *et al*., 1999). Variable densities of SP are also observed in other subcortical structures, such as cerebellum and basal ganglia (Wisniewski *et al*., 1989). Other pathologic alterations commonly seen in the brain of AD patients include neuropil threads, which also contain paired helical filaments and appear early in the course of the disease, granulovacuolar degenerations, diffuse amyloid deposits, and amyloid angiopathy.

A considerable degree of neuronal loss in hippocampal formation and association regions of the neocortex, leaving primary sensory and motor areas relatively spared, is usually observed in the brain of demented patients (Ball, 1977; Terry *et al*., 1981; Mountjoy *et al*., 1983; Morrison and Hof, 1997). This neuronal loss involves large cortical neurons and is correlated with the presence of NFT in neocortical association areas (Morrison and Hof, 1997). Also, synapse loss may represent an early marker of the dementing process. In fact, a strong association between loss of neocortical synapses estimated on the basis of synaptophysin immunoreactivity and cognitive impairment has been documented (Terry *et al*., 1991; Masliah *et al*., 1991), which was stronger than that between NFT and

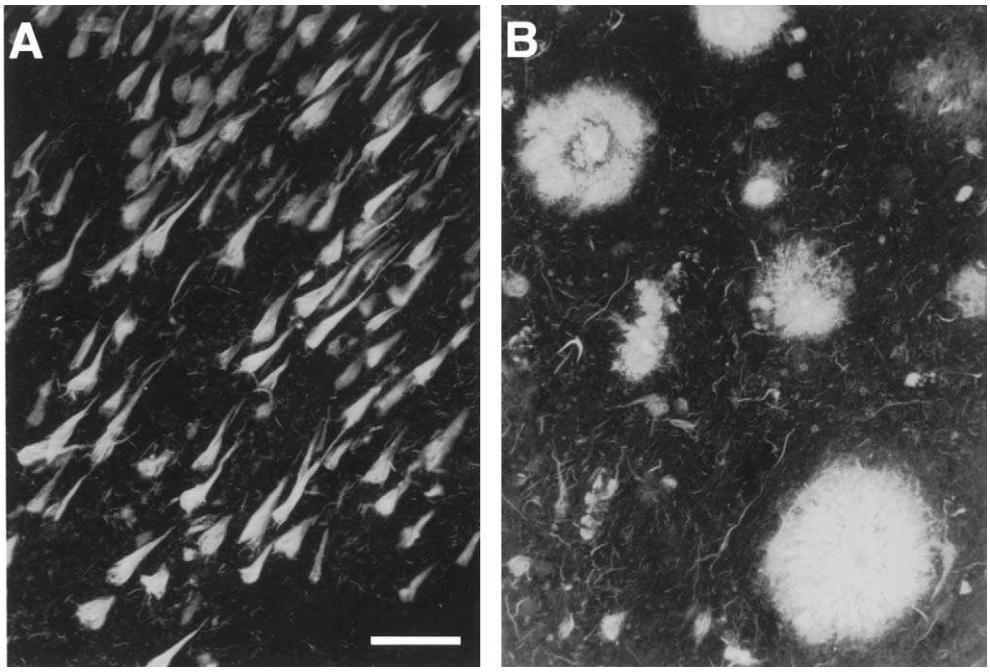

FIG. 9.1. Examples of NFT (A) and SP (B) from the hippocampus of a severe AD case. Note the flame-shaped morphology of NFT and the more variable features of SP. (B) A classical plaque with a central amyloid core and a rim of degenerating neurites, an isolated dense amyloid core, a predominantly neuritic plaque, and a few small diffuse deposits are visible. Scale bar: 50 μm.

cognitive deficit, indicating that synapse loss might be a more accurate neuropathologic indicator of dementia. Interestingly, an increase in synaptic size has been documented in the neocortex of AD cases, suggesting that despite a decrease in synaptic density, the total area of synaptic contact may remain unchanged in AD (Scheff and Price, 1993), although it is not clear how this seemingly compensatory mechanism affects the distribution of postsynaptic receptor molecules. Synaptic damage and synapse loss have also been reported in the neocortex of elderly nondemented individuals, suggesting an age-dependent mechanism for the loss of synapses in the neocortex (Masliah *et al*., 1993).

Strong correlations exist between the distribution of SP, NFT, and neuron loss in identifiable regions and layers of the cerebral cortex and the neurons of origin of certain long corticocortical and hippocampal projections (Rogers and Morrison, 1985; Pearson *et al*., 1985; Duyckaerts *et al*., 1986; Hyman *et al*., 1986; Lewis *et al*., 1987; Braak *et al*., 1989; Hof *et al*., 1990; Hof and Morrison, 1990; De Lacoste and White, 1993; Morrison and Hof, 1997). Overall, the distribution and severity of neuron loss follow closely that of NFT in hippocampal formation and neocortex (Ball, 1977; Gómez-Isla *et al*., 1996, 1997; Morrison and Hof, 1997). It has been shown that in certain regions, NFT densities are not consistently correlated to neuron loss and that differential laminar alterations have to be considered to estimate the severity of the pathologic changes. For example, it has been known for a long time that layer II of the entorhinal cortex, the subiculum, and the CA1 field of the hippocampus represent particularly vulnerable cortical domains that consistently display very high NFT densities in AD and that the most consistent observation in AD cases is the presence of large numbers of NFT in layers II and V of the entorhinal cortex (Hirano and Zimmerman, 1962; Hyman *et al*., 1984) (Figs. 9.2A and 9.2B). In the hippocampus, the most severely affected zones are the CA1 and subiculum, whereas the presubiculum, CA2 and CA3 fields, and dentate hilus are much less affected. In addition, the transentorhinal cortex located between the allocortex and the inferior temporal cortex shows a distinctive pattern of NFT formation very early in the course of AD (Braak and Braak, 1985). The distribution of SP in the hippocampal formation is variable, with certain zones displaying high SP densities, such as layer III of the entorhinal cortex, the molecular layer of the dentate gyrus, and the superficial layer of the subiculum (Hyman *et al*., 1990) (Figs. 9.2D and 9.2E).

Regionally, NFT are more numerous in the temporal cortex than in other cortical regions, the frontal cortex displays lower NFT densities, followed by the parietal cortex and the occipital cortex, and certain cortical regions, such as the posterior cingulate cortex, exhibit substantial case-to-case variability (Vogt *et al*., 1990, 1998; Arnold *et al*., 1991). NFT are found primarily within layers III and V in the neocortex (Fig. 9.2C), although their density varies considerably among cortical regions, with primary sensory and motor regions having much fewer NFT than association areas (Pearson *et al*., 1985; Lewis *et al*., 1987; Hof and Morrison, 1990; Arnold *et al*., 1991). Thus, when comparing the primary visual cortex (area 17) to the secondary visual cortex (area 18), there is an approximately 20-fold increase in the density of NFT area 18, and regions in the inferior temporal cortex that comprise high-order visual association areas are characterized by a further doubling of the NFT densities (Lewis *et al*., 1987). Similar differences

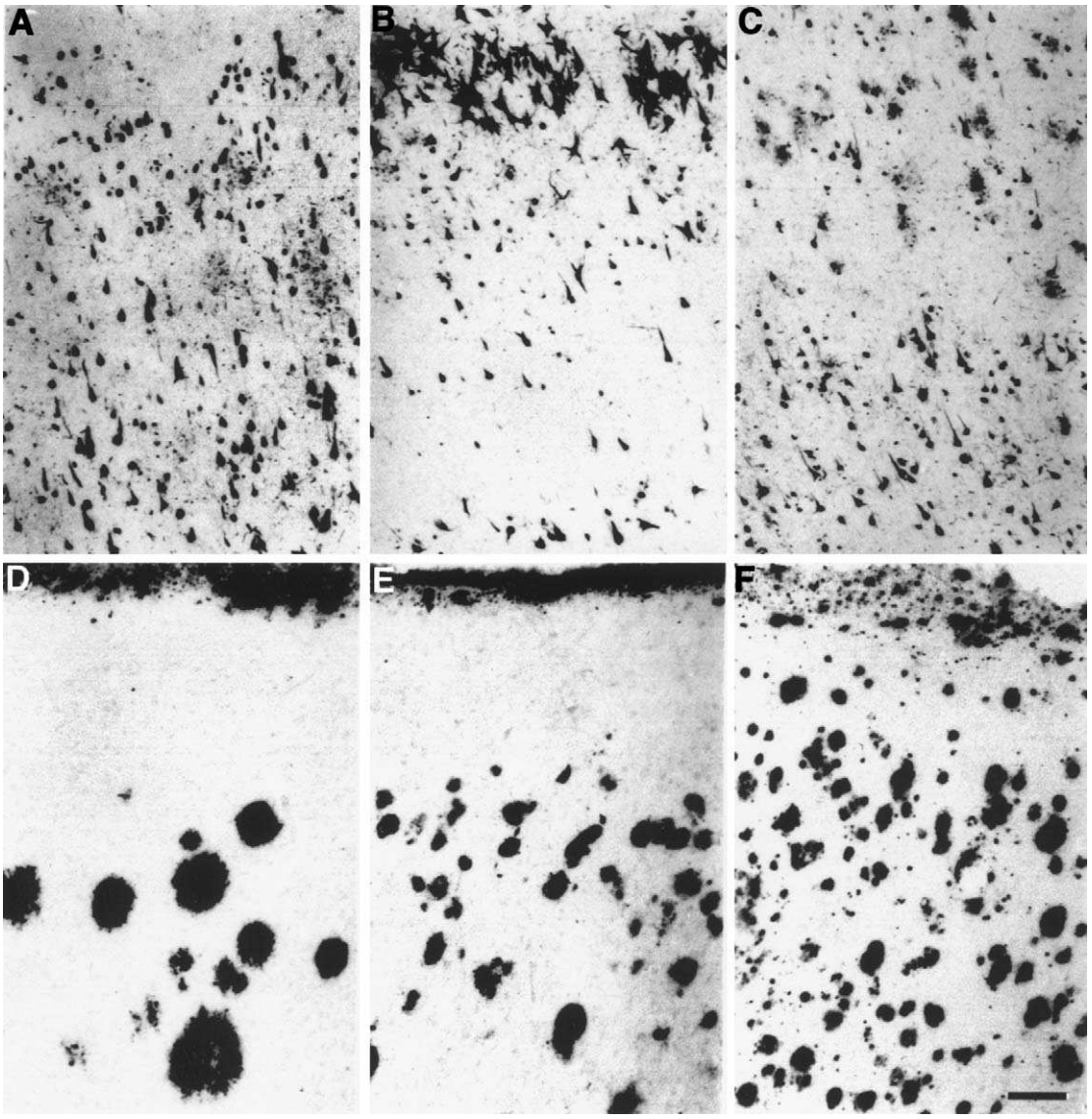

FIG. 9.2. Regional patterns of NFT (A–C) and SP (D–F) distribution in the CA1 field of the hippocampus (A and D), layers I–III of the entorhinal cortex (B and E), and layers III–V (C) and I–III (F) of area 20 in an AD case. There are high NFT densities in these regions. Layer II of the entorhinal cortex contains particularly high numbers of large NFT (B) and there are more NFT in layer V of area 20 (C). There are fewer SP in the CA1 field compared to the other regions (D–F). Scale bar (on F): 50 μm.

are found between the primary auditory cortex and auditory association areas, which display a 10-fold increase in NFT density (Esiri *et al*., 1986; Lewis *et al*., 1987). Considerable differences in laminar NFT distribution exist among neocortical regions. In areas 17 and 18, the majority of NFT are located in layer III, whereas in the inferior temporal cortex, only 40% of the NFT are found in layer III. A more striking difference exists in the auditory association cortex where only 27% of the NFT are observed in the supragranular layers (Lewis *et al*., 1987).

In contrast to NFT, SP show a relatively homogeneous distribution among cortical areas (Lewis *et al*., 1987; Arnold *et al*., 1991), although the frontal and anterior cingulate cortex contain more SP than temporal cortices (Rogers and Morrison, 1985). In the neocortex, SP are generally more numerous in layers II to IV than in layers V and VI (Rogers and Morrison, 1985) (Fig. 9.2F). Certain cortical areas are characterized by typical SP distribution patterns, such as the retrosplenial cortex, where SP occur in layer III, and area 17, where they are concentrated at the border of layer IVC and V (Braak *et al*., 1989, 1992; Beach and McGeer, 1992).

II. Alzheimer's Disease Affects Specific Elements of Cortical Circuits

The neurons of origin of corticocortical projections can be classified into three distinct categories based on their distribu-

tion in the cortical layers and by the distribution of their axonal terminals in their projection areas. Using these criteria, corticocortical projections can be categorized as feedforward, feedback, and lateral connections (Felleman and Van Essen, 1991). Feedforward connections ascend within the hierarchy of a given modality (i.e., from a primary sensory area to an association area), feedback projections descend the same hierarchy, and lateral connections link cortical regions at the same hierarchical level. Feedforward connections originate mostly from neurons located in the superficial layers and terminate in the deep portion of layer III and in layer IV of the target cortical region, feedback projection neurons are found principally in layers V and VI and project in layers I and VI, and lateral connections arise from layers V–VI and project to layers III to VI.

The preferential distribution of NFT in layers III and V indicates that elements of feedforward, lateral, and feedback projections area likely to be affected by the degenerating process of AD (Fig. 9.3). Considering that layer V contains generally higher NFT densities than layer III in association areas suggests that feedback as well as lateral projections may be at higher risk in AD than feedforward systems. Interestingly, most of the projection neurons from the occipital and temporal association cortex to the frontal and from the occipital cortex to the temporal cortex are located in layer III. However, the ratio of densities of projection neurons in layer III to layer V progressively decreases from the occipital to inferior temporal and further to superior temporal regions (Barbas, 1986): this change in laminar distribution is paralleled by a progressive shift of NFT densities from supragranular layers to infragranular layers (Lewis *et al*., 1987). In severe AD cases, only feedforward projections are affected in areas 17 and 18, as NFT predominate in layer III, which contains most of the efferent corticocortical neurons in these areas (Figs. 9.3 and 9.4). The regional and laminar distribution of SP suggests that they may be related to NFT formation. It has been proposed that, in fact, SP reflects degeneration of the terminations of projections from neurons that contain NFT (Pearson *et al*., 1985) and that the slightly higher incidence of SP in the supragranular layers and in layer IV in the association cortex reflects the involvement of feedforward projections (Lewis *et al*., 1987), although there are clear indications that multiple systems are involved in SP formation (Morrison *et al*., 1985; Walker *et al*., 1988; Vickers, 1997; Mochizuki *et al*., 2000).

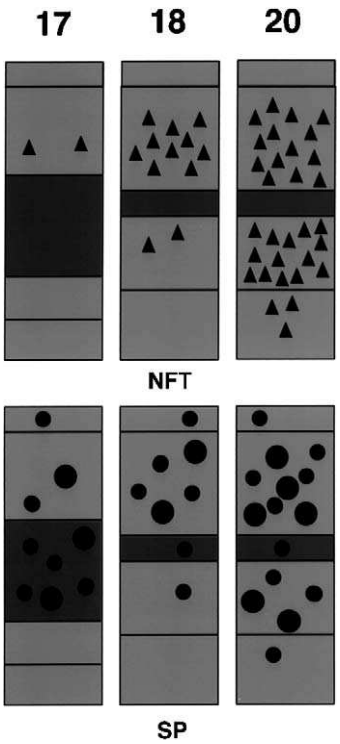

FIG. 9.3. The laminar distribution of NFT (▲) and SP (•) matches the distribution of corticocortically projecting neurons along neocortical hierarchies. In area 17, only rare NFT are seen in layer III, which projects to the superficial layers of area 18. This corresponds to the presence of consistent densities of SP in these layers in area 18. From the superficial layers of area 18, feedforward neurons projecting to higher hierarchical levels (in this case area 20) are involved, resulting in higher NFT densities. The terminal fields of the projections from area 18 in layers III and V–VI of area 20 contain higher densities of SP. In area 20, NFT are present in higher number not only in layer III, but also in layers V–VI, indicating that feedback projections may be involved by the degenerative process, which may be correlated to the high densities of SP in layers II–IV of area 17, where feedback projections from areas 18 and 20 terminate. Layer IV is shown by dark gray shading.

The distribution of NFT and SP in the hippocampal formation also parallels specific projections (Hyman *et al*., 1990). Thus, the perforant pathway that projects from layers II and III of the entorhinal cortex is severely and early involved in AD, and the presence of NFT in the neurons of origin of this pathway and its termination in the dentate gyrus are correlated with high densities of SP in the molecular layer of the dentate gyrus (Hyman *et al*., 1984, 1986; Senut *et al*., 1991). High densities of NFT in layer V of the entorhinal cortex are correlated with the degeneration of connections to the amygdala and to a number of limbic and association cortical areas, and projections from the hippocampus to the entorhinal cortex, amygdala, and neocortical areas, originating from the CA1 field and the subiculum, all consistently contain numerous NFT in AD, as do nuclei in the amygdala that project to the entorhinal cortex (Brady and Mufson, 1990; Hyman *et al*., 1990). Furthermore, SP are found predominantly in the mediobasal nucleus of the amygdala, which receives the entorhinal cortex projections, whereas SP are more numerous in layers I and III of the entorhinal cortex that correspond to the termination zone of amygdala and hippocampus afferents, consistent with the notion that the regional pathologic changes in the hippocampus correspond to the disconnection of intrinsic connections and of projections to other limbic and neocortical regions (Brady and Mufson, 1990, 1991; Hyman *et al*., 1990).

The degeneration of presumed corticocortical circuits within the neocortex therefore appears to be the necessary factor for the clinical expression of the dementia in AD (Morrison and Hof, 1997). Elderly individuals can maintain a high level of cognitive performance while sustaining a significant compromise of hippocampal circuits and may rely more on neocortical than on hippocampal circuits for memories essential for daily activities (Albert, 1996). In terms of cognitive performance, healthy elders may present with difficulties in learning and retrieving new information. In fact, the difficulty lies in the

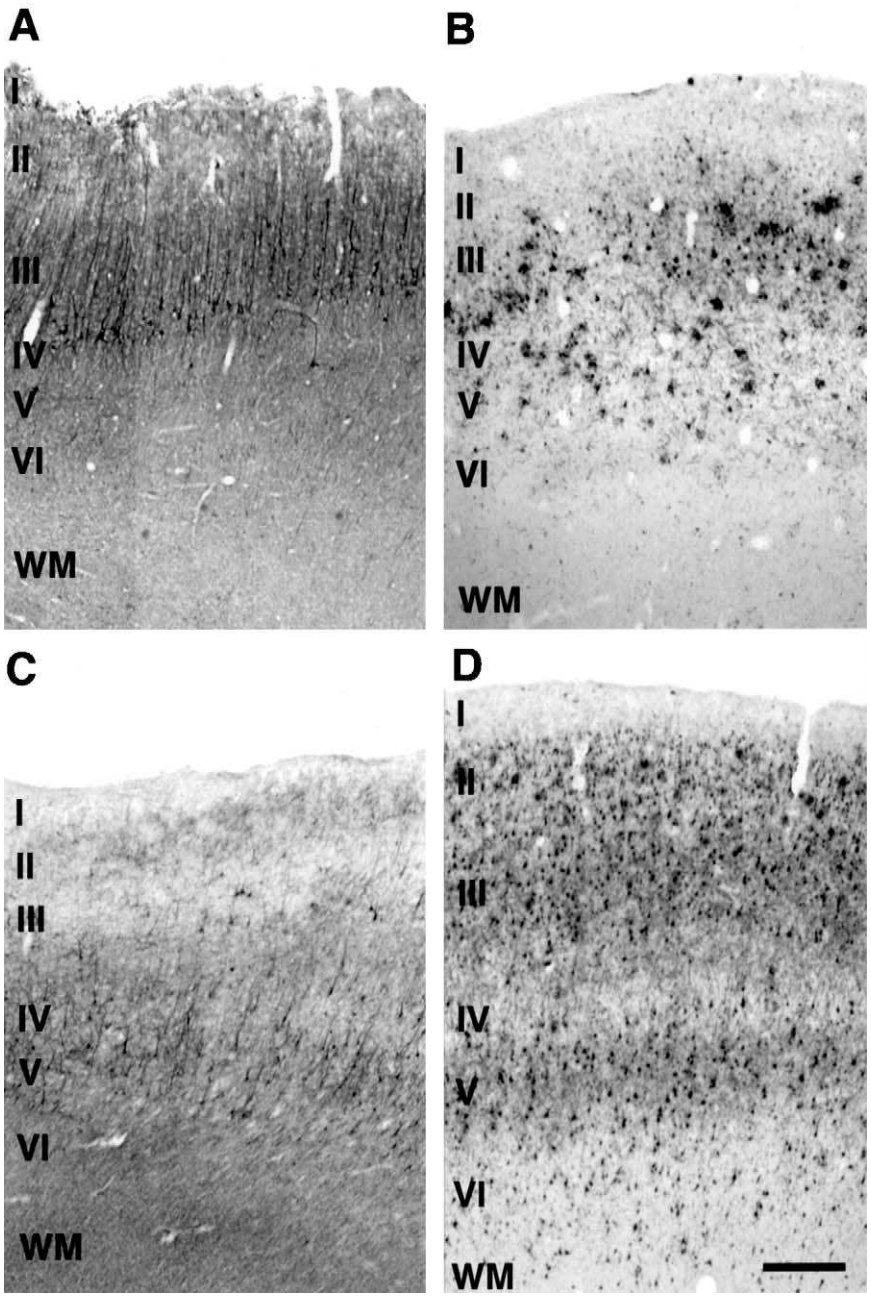

FIG. 9.4. Correlative distribution of neurofilament protein-enriched neurons (A and C) and NFT (B and D) in area 18 (A and B), and area 20 (C and D) of a severe AD case. In area 18, immunoreactive neurons are located in the deep portion of layer III and are affected moderately. Their distribution matches that of NFT (A and B). In area 20, there is a severe loss of neurofilament protein-immunoreactive neurons in layers III and V (C) that parallels the very high densities of NFT in these layers (D). Scale bar (on D): 100 μm.

amount of information they can learn within a given period of time in comparison to younger individuals. Compared to patients with early AD, healthy elders retain the new information after a delay, whereas patients with mild cognitive impairment retain little of it (Albert, 1996). This impairment in information retention that characterizes the very early stages of AD is correlated to neuronal loss in the entorhinal cortex and to volumetric changes in the temporal lobe (Albert, 1996; Gómez-Isla *et al*., 1996, 1997; Fama *et al*., 1997; Hof *et al*., 1999b). Increases in the volume of the temporal horn of the lateral ventricle may selectively reflect the loss of projections from the entorhinal cortex. Normal aging can thus be defined by intact cognitive abilities, despite the presence of scarce neurofibrillary pathology in the entorhinal cortex, which can be referred to as aging-related asymptomatic AD-like neurofibrillary pathology.

III. Morphologic and Molecular Correlates of Neuronal Vulnerability

A. Neuronal Types Prone to Neurofibrillary Tangle Formation

Not all neuron types form NFT in AD, even though NFT are distributed widely throughout the brain (Hof *et al*., 1990; Gómez-Isla *et al*., 1996, 1997; Morrison and Hof, 1997). In the neocortex, the number of NFT clearly cannot account for the total population of pyramidal neurons (Fig. 9.4). This suggests that only certain subpopulations of pyramidal neurons are selectively susceptible to paired helical filament aggregation. Specifically, large pyramidal cells in layers, III and V represent the most affected cell class, whereas smaller pyramidal neurons in layers II, VI, and in the upper part of layer III appear to be considerably more resistant to NFT formation. Also, the spiny stellate cells and small pyramidal cells in layer IV are not affected by this process as are many of the various morphological types of inhibitory interneurons. It is important to note that in terms of morphology and connectivity, all of the vulnerable neurons are efferent cells that send long projections to other cortical regions or to subcortical structures and all are large pyramidal neurons (Hof *et al*., 1990; Hof and Morrison, 1990). Similarly, in the hippocampal formation, the large pyramidal efferent neurons in layers II, III, and V of the entorhinal cortex and of the CA1 field and subiculum are all severely affected. However, other cellular characteristics than these are also linked to vulnerability in the degenerative process, as certain large efferent neurons, such as the principal cells in the CA3 field and the large neurons of the dentate hilus, are generally relatively resistant to degeneration in AD. In subcortical structures, NFT appear in several nuclei that project to the cerebral cortex. For instance, catecholaminergic cell groups in the brain stem are frequently affected as are the cholinergic neurons in the nucleus basalis of Meynert: in the thalamus, NFT are mostly restricted to relay neurons within intralaminar and limbic nuclei, whereas sensory nuclei are unaffected (Rossor *et al*., 1984; Braak and Braak, 1991; see also Chapter 19), further demonstrating the existence of a correlation between selective neuronal vulnerability and specific projection systems.

B. Neurofilament Protein Is a Marker of Neuronal Vulnerability in Alzheimer's Disease

Certain pyramidal neurons in the neocortex of both human and monkeys have been shown to be enriched in neurofilament protein (Campbell and Morrison, 1989; Hof *et al*., 1990; Hof and Morrison, 1990, 1995). Neurofilament protein immunoreactivity in the primate neocortex is restricted to the perikaryon and dendrites of a subpopulation of large pyramidal and is more concentrated in the cell body and dendrites of the largest of these neurons. Interestingly, neurofilament as well as other cytoskeletal proteins have been implicated in NFT formation (Ksiezak-Reding *et al*., 1987; Morrison *et al*., 1987; Zhang *et al*., 1989; Trojanowski *et al*., 1993; Morrison and Hof, 1997; Hof *et al*., 1999a), and pyramidal cells with a high content of nonphosphorylated neurofilament protein emerge as a neuron type highly susceptible to NFT formation.

Detailed descriptions of the cellular, laminar, and regional characteristics of neurofilament protein-containing cells in many frontal, cingulate, temporal, and occipital areas of the macaque monkey and human neocortex have revealed that extensive regional heterogeneity exists in the size, density, and laminar distribution of neurofilament protein-containing neurons (Campbell and Morrison, 1989; Hof *et al*., 1990, 1995a,b, 1996, 1997; Hof and Morrison, 1990, 1995; Campbell *et al*., 1991; Hof and Nimchinsky, 1992; Nimchinsky *et al*., 1995, 1996, 1997). The distribution of neurofilament protein-containing neurons corresponds to the distribution of corticocortically projecting cells, as demonstrated by transport studies in the macaque monkey cortex (Campbell *et al*., 1991; Hof *et al*., 1995b, 1996, 1997; Nimchinsky *et al*., 1996). For example, in the anterior cingulate cortex the labeled neurons are restricted to layer V, and most long corticocortical projections from this region originate in layer V (Barbas, 1986). Area 9 in the prefrontal cortex, as well as the superior and inferior temporal cortices, has a high density of neurofilament protein-immunoreactive neurons in layers III and V, the layers from which, in these regions, corticocortical projections originate. The correlation between origins of long corticocortical projections and neurofilament protein-containing neurons is particularly visible in area 17 of monkeys and human, where layer IVB cells and Meynert cells are the only large, strongly immunoreactive neurons, and both are known to project from area 17 to mediotemporal area V5 (Shipp and Zeki, 1989a,b; Felleman and Van Essen, 1991; Hof *et al*., 1996).

In fact, the distribution of neurofilament protein-containing cells in areas 17 and 18 differs substantially from each other and from the pattern described in the inferior temporal and prefrontal cortex (Campbell and Morrison, 1989; Hof *et al*., 1990; Hof and Morrison, 1990, 1995). In area 17, in addition to layer IVB cells and Meynert cells, smaller neurofilament protein-immunoreactive neurons occur in layers III, IVA, V, and VI. In area 18, the large, intensely labeled pyramidal neurons predominate in the deep portion of layer III, with a few in layer V. The inferior temporal and prefrontal cortex have a significantly higher density of heavily labeled cells in deep layer III than area 18 and, in addition, have a much higher density of labeled cells in layers V and VI. In contrast to the inferior temporal and prefrontal cortex, the loss of neurofilament protein-containing neurons in area 17 and 18 is minimal in AD brains and is confined to layer IVB and Meynert cells in area 17 and to deep layer III neurons in area 18 (Hof and Morrison, 1990). This indicates that the short corticocortical projections from area 17 to area 18 that originate in layer III are not affected in AD. Given the morphology and distribution of the neurofilament protein-containing neurons in area 17, the immunoreactive cells in layer IVB and Meynert cells are likely at the origin of the projections to visuomotor regions of the occipitotemporal cortex, which are the human homologue of monkey mediotemporal area V5. Studies combining retrograde transport and double-labeling immunohistochemistry in macaque monkeys indicate that virtually 100% of the neurons in layer IVB and Meynert cells in area 17 that project to area V5 contain neurofilament protein and that the projection from area 18 to area V5 originates from the neurofilament protein-containing pyramidal cells in deep layer III (Hof *et al*., 1996), where the darkly stained, large neurofilament protein-containing pyramidal cells

are found in human. Thus, although the projection from area 17 to area 18 is largely intact, and the overall neuron loss may be functionally inconsequential in area 17, it is likely that two projections to the occipitotemporal regions involved in visuomotor skills are affected in AD. These observations on visual projections demonstrate the existence of a chemically defined neuronal subpopulation that is highly vulnerable in AD and that can be correlated with a specific corticocortical projection of known function (Fig. 9.4). We have also reported in the macaque monkey that many long association corticocortical projections originate from neurofilament protein-containing neurons and that in some of them, 90–100% of the neurons of origin of the projection contain neurofilament protein (Hof *et al*., 1995b). This is particularly the case of projections from the temporal to the prefrontal and parietal neocortex that are know to be involved in networks subserving many aspects of the cognitive functions (Goldman-Rakic, 1988).

Furthermore, we have observed that neurofilament protein-containing neurons in certain neocortical and hippocampal areas in AD are affected dramatically and die through NFT formation (Morrison *et al*., 1987; Hof *et al*., 1990; Hof and Morrison, 1990; Vickers *et al*., 1992, 1994, 2000; Morrison and Hof, 1997; Vickers, 1997). The laminar distribution of neurofilament protein-containing neurons in visual association, prefrontal, and anterior cingulate in human cerebral cortex is very similar to the distribution of NFT. Furthermore, layers that have high NFT density in an AD brain no longer contain a high density of neurofilament protein-immunoreactive neurons (Hof *et al*., 1990, 1999a,b; Hof and Morrison, 1990) (Figs. 9.4 and 9.5). A comparable situation exists in the hippocampal formation, where layers II, III, and V of entorhinal cortex and the pyramidal neurons of the subiculum have a very high density of neurofilament protein-immunoreactive neurons in the normally aging human brain and present with a dramatic loss of these neurons in AD (Vickers *et al*., 1992, 1994; Morrison and Hof, 1997).

These observations demonstrate that neurofilament protein-containing neurons are highly vulnerable in AD, and quantitative analyses have demonstrated a severe loss of neurofilament protein-containing neurons in layers III and V in the inferior temporal and superior frontal cortex (Fig. 9.5). The severity of the loss correlates with the size of these neurons in that neurofilament protein-containing neurons larger than 6000 μm^3 of perikaryal volume are the most affected, with up to 60% cell loss, whereas smaller size neurons (2000–6000 μm^3 of perikaryal volume) are not affected (Hof *et al*., 1990, 1999b). In addition to regional distribution and relationship to connectivity, NFT identified by thioflavine S stain or immunohistochemistry using antibodies to neurofilament protein and microtubule-associated protein tau have revealed dynamic cellular alterations in vulnerable neuronal populations during normal aging. For instance, layer II of the entorhinal cortex

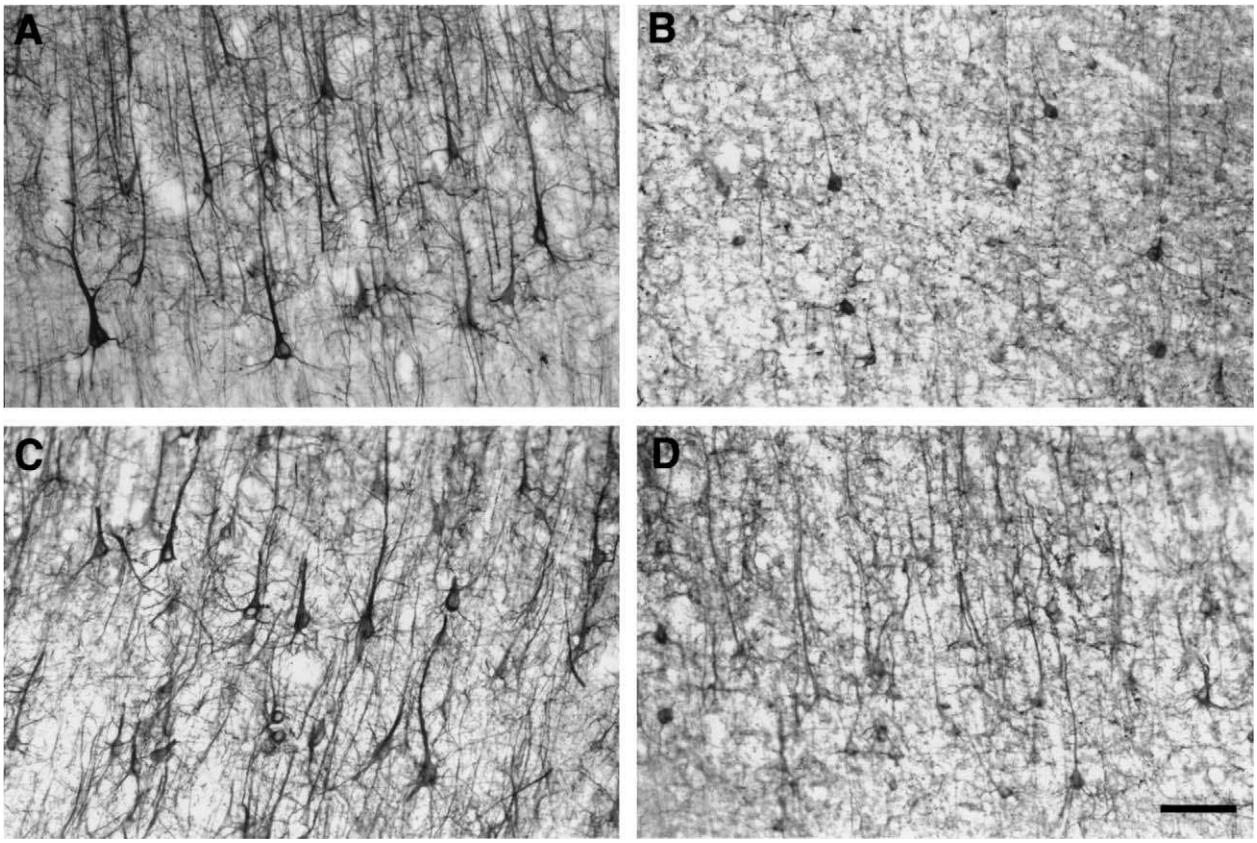

FIG. 9.5. Involvement of neurofilament protein-immunoreactive pyramidal neurons in layer III of area 9 (A and B) and area 20 (C and D), in control cases (A and C), and severe AD cases (B and D). Note the dramatic neuronal loss and changes in dendritic morphology and staining intensity in the remaining neurons in the AD cases. Scale bar (on D): 120 μm.

contains neurofilament protein-immunoreactive neurons that also display immunoreactivity to tau protein and thioflavine S-positive materials, suggesting the existence of transitional forms of NFT (Vickers *et al*., 1992; Morrison and Hof, 1997). In such cases, very rare NFT are seen in the frontal cortex, which contains preserved neurofilament protein-immunoreactive neurons in layers III and V (Fig. 9.6, see color insert). However, in AD cases, most NFT in the entorhinal cortex progress to an end stage and are no longer immunoreactive to tau and neurofilament proteins but are stained only with thioflavine S. At this stage, transitional forms of NFT are observed in the frontal cortex, indicating that a time-dependent process takes place in the formation of NFT in certain neurofilament protein-containing neurons (Vickers *et al*., 1992, 1994, 2000; Morrison and Hof, 1997; Vickers, 1997; Hof *et al*., 1999b) (Fig. 9.6). Moreover, the presence of high levels of neurofilament protein appears to be a prerequisite for the formation of NFT, as certain neurons, such as the pyramidal neurons of the CA1 field, that do not normally express detectable levels of this protein in young adults, yet are prone to NFT formation in AD, begin to show increasing levels of neurofilament protein immunoreactivity during aging (Vickers *et al*., 1994). If monkey data are considered within the context of the distribution of neurofilament protein-containing neurons and NFT in human, it is likely that human homologues of the neurofilament protein-containing, corticocortically projecting neurons of the macaque monkey are those that are highly vulnerable in AD. Thus, one of the neurochemical characteristics of the vulnerable neurons in AD is the presence of high somatic and dendritic concentrations of nonphosphorylated neurofilament proteins, although this may clearly represent only part of the neurochemical phenotype associated with selective vulnerability.

C. Other Factors Linked to Vulnerability

Disruption of glutamate metabolism and glutamate receptor-mediated excitoxicity represents one of the major mechanisms of neuron death in many neurodegenerative disorders, as well as in other pathological conditions, such as brain ischemia and epilepsy (Choi, 1988; Greenamyre and Young, 1989; Choi and Rothman, 1990; Meldrum and Garthwaite, 1991; Coyle and Puttfarcken, 1993; Blümcke *et al*., 1996; Obrenovitch and Urenjak, 1997). Glutamate receptor-mediated excitotoxicity presumably results from increased calcium flux, leading to toxic intracellular concentrations of this ion, and this mechanism is likely associated with all types of ionotropic glutamate receptors that are involved in facilitating or regulating calcium fluxes. Whereas the *N*-methyl-D-aspartate (NMDA) receptor has been the receptor subtype associated primarily with calcium flux, it is now clear that both kainate and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) kainate receptors also increase calcium flux (Gasic and Heinemann, 1992). Interestingly, neurons in culture that possess high levels of calcium-permeable AMPA/kainate and NMDA receptors are highly sensitive to exposure to calcium load, and such neurons are much more likely to express the calcium-permeable AMPA subunits GluR1 and GluR4 than the calcium-impermeable GluR2 subunit (Lu *et al*., 1996). Several studies have shown that following ischemic brain injury, there is a downregulation in the expression of the calcium-impermeable GluR2 subunit in the CA1 field of the hippocampus (Pellegrini-Giampietro *et al*., 1992, 1994; Pollard *et al*., 1993). Thus, the glutamate receptor profile of cortical neurons and related circuits has emerged as an important parameter when correlating identified neurons and circuits with susceptibility for vulnerability to degeneration through excitotoxicity because the defining characteristics of a given glutamatergic projection with respect to ion fluxes depend on the subunit composition of the receptors that dominate that system. Importantly, glutamate-containing neurons have long been known to be prone to NFT formation (Kowall and Beal, 1991), and depletion of glutamate and certain glutamate receptors has been reported in the perforant pathway in AD cases (Hyman *et al*., 1987; Greenamyre and Young, 1989; Dewar *et al*., 1991; see also Chapter 20). Glutamate toxicity mediated through calcium-activated processes has been linked to degeneration and cytoskeletal alterations comparable to those involved in NFT formation (Sautière *et al*., 1992; Sindou *et al*., 1992, 1994; Couratier *et al*., 1995, 1996). Autoradiographic, immunocytochemical, or *in situ* hybridization studies have demonstrated considerable alterations in the distribution and density of several glutamate receptor subunits in the cerebral cortex in normal aging and AD (Armstrong *et al*., 1994; Ikonomovic *et al*., 1995, 1997; Yasuda *et al*., 1995; Armstrong and Ikonomovic, 1996; Ikonomovic and Armstrong, 1996). These issues are reviewed in detail in Chapter 20. In addition, changes in the expression of specific glutamate receptor subunits leading to functional decline without neuronal degeneration are known to occur during normal aging (Gazzaley *et al*., 1996, 1997; Duan *et al*., 1999). For instance, in aged monkeys, compared to juvenile and young adult monkeys, the NMDA receptor levels decrease specifically and consistently in the outer molecular layer of the dentate gyrus where the perforant path terminates, but there are no significant differences in the expression of AMPA or kainate glutamate receptor subunits, and no morphologic reflection of degeneration of the perforant path (Gazzaley *et al*., 1996, 1997). Similar evidence from neocortical connections began to emerge in that neurons forming long corticocortical connections undergo a downregulation in the expression of NMDAR1 and the AMPA subunit GluR2 and that a certain degree of reduction in GluR2 expression also exists in certain short corticocortical pathways (Duan *et al*., 1999). Such alterations in expression of a calcium-impermeable glutamate receptor subunit may represent a cellular correlate of the toxic increase in calcium flux and define subsets of pyramidal neurons particularly prone to degeneration. These data also suggest that the intradendritic parcellation of a particular neurotransmitter receptor is modifiable in an age-related and circuit-specific manner and that such changes may provide a substrate for age-related memory impairment (Gazzaley *et al*., 1996; Morrison and Hof, 1997; Duan *et al*., 1999).

It is also interesting to note that a population of pyramidal neurons, residing principally in layer III of the neocortex and that express the calcium-binding protein calbindin, is also vulnerable in AD (Hof and Morrison, 1991) (Fig. 9.7). Studies in the macaque monkey and humans have shown that these neurons are more numerous in the association cortex and that there is a gradient in their local densities from the primary sensory areas to the secondary and higher-order cortical regions (Hof

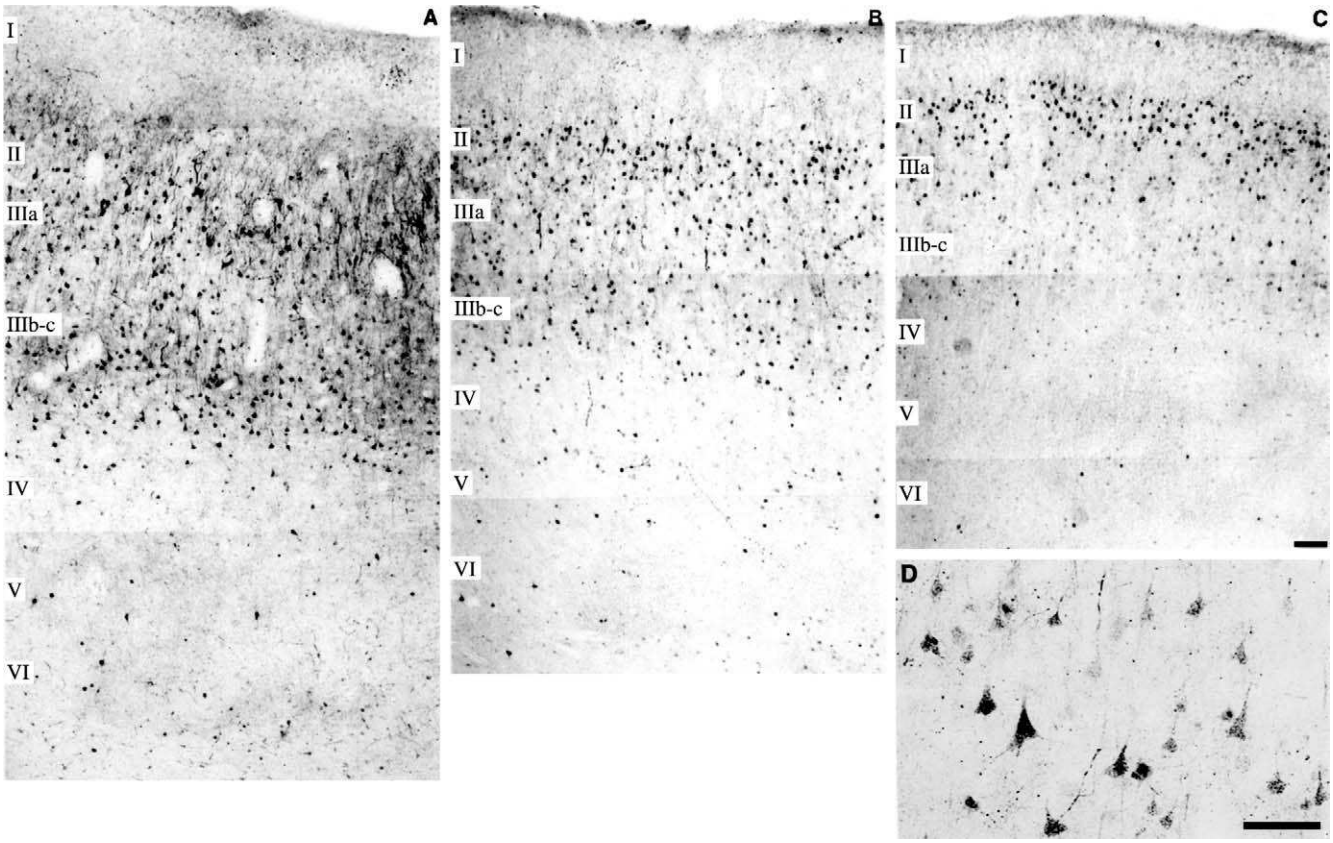

FIG. 9.7. Distribution of calbindin-immunoreactive neurons in area 9 of a control case (A), an AD case with mild cognitive impairment (B), and a severe AD case (C). There is a high number of labeled interneurons in layer II and superficial III, whereas layers V–VI have fewer neurons (A). In AD cases, there is a gradual reduction in the dendritic arborizations of these neurons, but no cell loss is apparent (B and C). In layer III there is a population of pyramidal neurons that also contain calbindin (A and D), which are affected in AD cases. Scale bars: 100 μm.

and Morrison, 1991; Kondo *et al*., 1994, 1999). These neurons have also been shown to be enriched in neurofilament proteins (Hayes and Lewis, 1992). Possible alterations in the expression of an important protein involved in intracellular calcium buffering in these pyramidal neurons might be related to their heightened vulnerability in the course of AD. Such changes combined with the decrease in aging in an AMPA receptor protein protective against calcium toxicity, such as GluR2, together with a high content of neurofilament protein could represent key factors in the dynamic definition of a neurochemical profile of cellular vulnerability in AD.

IV. Factors Conferring Resistance to the Degenerative Process

Quantitative analyses of the superior frontal (area 9) and inferior temporal (area 20) in AD have demonstrated that several classes of GABAergic interneurons containing the calcium-binding proteins parvalbumin, calbindin, and calretinin are largely resistant to the degenerative process, even in severe cases displaying very high densities of NFT and SP (Hof *et al*., 1991, 1993; Hof and Morrison, 1991; Sampson *et al*., 1997). The cellular distribution of calcium-binding proteins is known to be coextensive with that of GABA in cortical interneurons, and these proteins subdivide the GABAergic neurons into nonoverlapping interneuron populations that together account for the vast majority of the GABAergic cells (Hendry *et al*., 1989; Celio, 1990; Hof and Nimchinsky, 1992; Résibois and Rogers, 1992; Andressen *et al*., 1993; Hof *et al*., 1995a; Nimchinsky *et al*., 1997).

Parvalbumin- and calretinin-immunoreactive interneurons are resistant to degeneration (Figs. 9.8 and 9.9) and show a well-preserved morphology and staining pattern in AD, but calbindin-immunoreactive neurons display some degree of differential vulnerability (Hof and Morrison, 1991; Hof *et al*., 1991, 1993). Whereas calbindin-containing interneurons in layers II and III are strongly resistant to degeneration in AD (Fig. 9.7), those in layers V and VI are affected in AD cases with high NFT densities, possibly reflecting differential patterns of connectivity between subgroups of calbindin-containing interneurons in layer II and upper layer III and those in layer V. It is likely that layer V calbindin-immunoreactive neurons project to pyramidal cells that are more affected in AD, whereas the targets of neurons in layers II and III are less vulnerable (Hof and Morrison, 1991).

Other markers of cellular resistance in GABAergic interneurons include several neuropeptides. For example, somato-

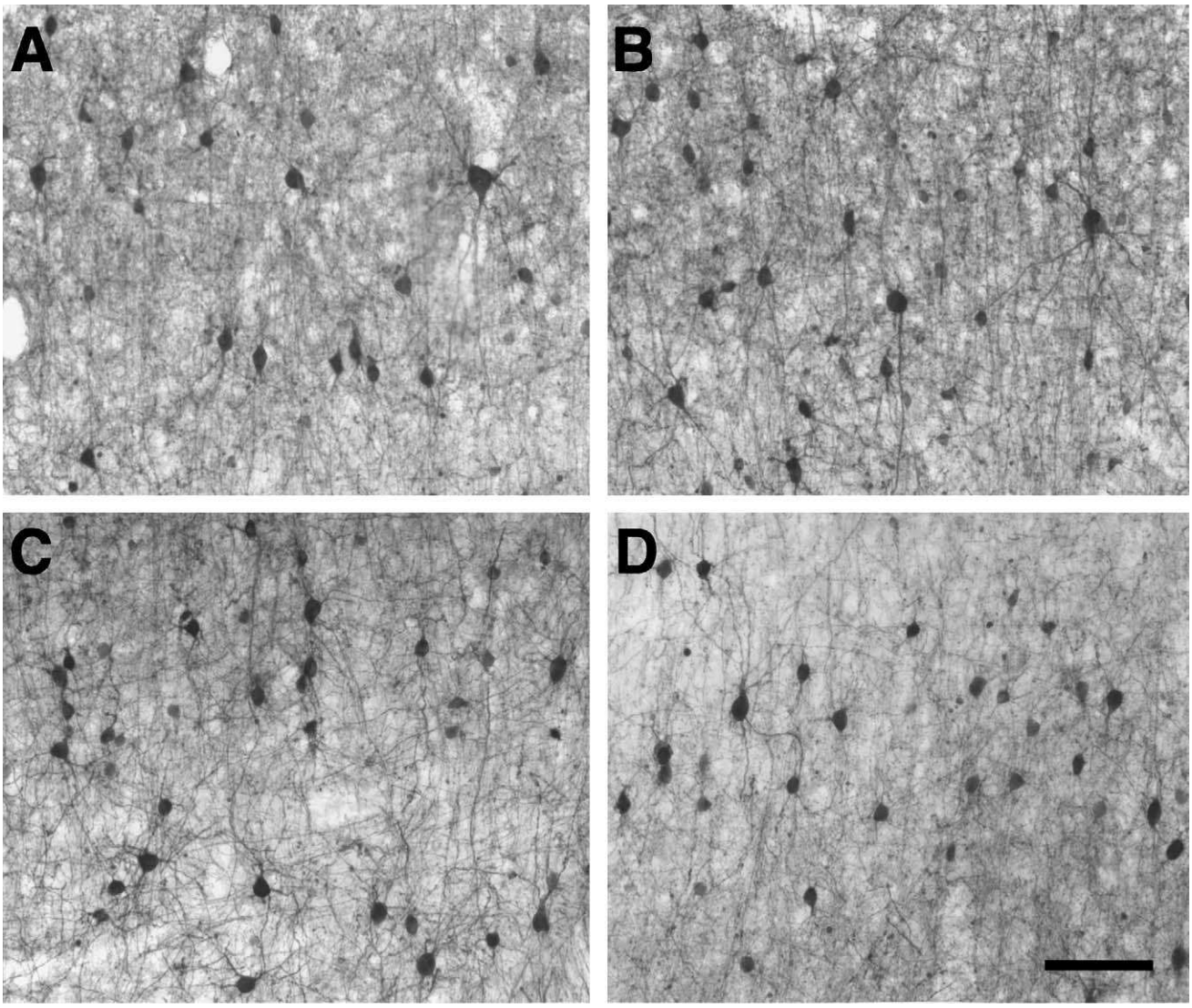

FIG. 9.8. Distribution of parvalbumin-immunoreactive neurons in areas 9 (A and B) and 20 (C and D) of a control case (A and C) and an AD case (B and D). These interneurons are present in all neocortical layers, but predominate in layers II–IV. These photomicrographs are all centered on the lower part of layer III and on layer IV. In AD cases, there is a remarkable preservation of the local density and morphology of these neurons. Scale bar: 100 μm.

statin and calbindin are colocalized in some interneurons (DeFelipe *et al*., 1989). Somatostatin-immunoreactive fibers occur in SP and thus degenerate to some degree in AD (Morrison *et al*., 1985). Interestingly, layer III pyramidal neurons are thought to be a principal target of these interneurons, suggesting that somatostatin- and calbindin-immunoreactive fibers are more vulnerable in AD than perikarya of the interneurons that contain these two markers (Gaspar *et al*., 1989; DeLima and Morrison, 1990). The differential vulnerability of the axonal portion of certain classes of interneurons may be linked to the fact that they are connecting onto a population of particularly vulnerable pyramidal neurons. Furthermore, the resistance of somatostatin neurons appears to be dependent on the colocalization of neuropeptide Y and/or NADPH diaphorase, and the density of interneurons containing neuropeptide Y, somatostatin, and NADPH diaphorase is not decreased in AD in the neocortex and hippocampus, although some of these neurons display morphologic alterations (Kowall and Beal, 1988). Corticotropin-releasing factor is found in association with parvalbumin in certain interneurons that remain unaffected in AD (Kelly and Kowall, 1989). Finally, some parvalbumin-immunoreactive interneurons in the hippocampal formation, as well as some calretinin-containing neurons, suffer dendritic alterations but are not lost and do not form NFT or associate with SP (Brion and Résibois, 1994).

Moreover, parvalbumin-immunoreactive neurons have been shown to be resistant to degeneration in the cerebral cortex of Huntington's disease, Pick's disease, Guamanian amyotrophic lateral sclerosis/parkinsonism–dementia complex, postencephalitic parkinsonism, progressive supranuclear palsy, corticobasal degeneration, and frontal lobe dementia, but not in Down syndrome and Creutzfeldt–Jakob's disease. Calbindin- and cal-

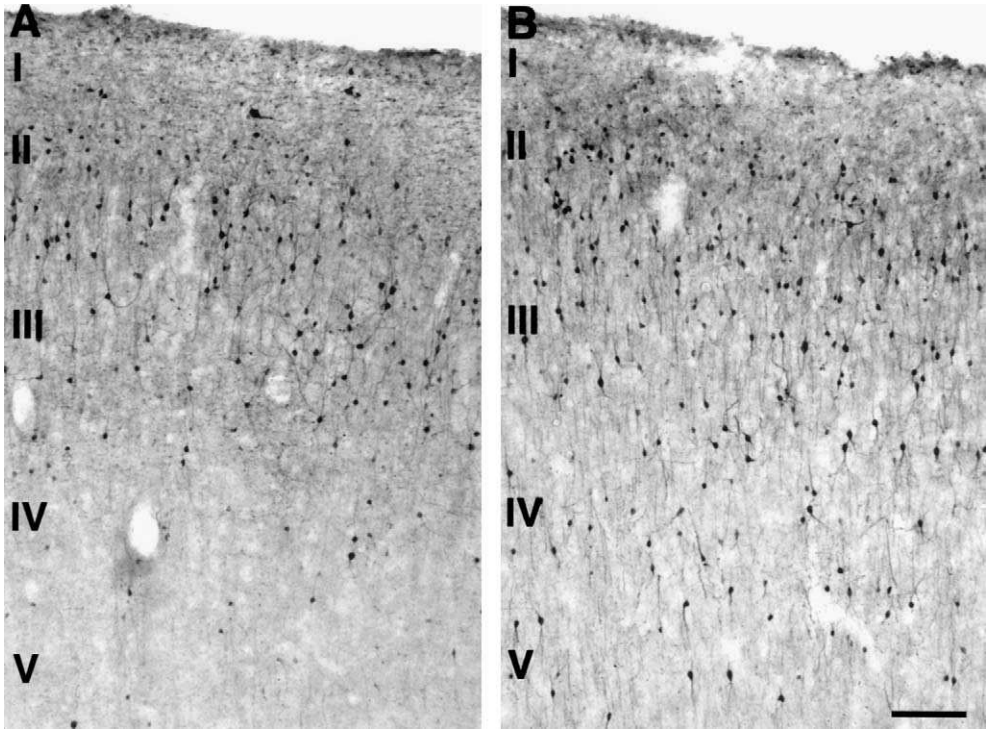

FIG. 9.9. Distribution of calretinin-immunoreactive neurons in area 9 of a control case (A) and an AD case (B). These bipolar/bitufted interneurons predominate in layers II–V. In the AD case, these neurons remain unaffected. Scale bar: 100 μm.

retinin-containing cells are also resistant in most of these disorders, except in frontal lobe dementia, where calbindin-immunoreactive neurons are vulnerable (Cudkowicz and Kowall, 1990; Kobayashi *et al*., 1990; Arai *et al*., 1991; Ferrer *et al*., 1993a,b; Hof *et al*., 1994, 1999a). The combination of factors, such as high cytoplasmic levels of calcium-binding proteins, certain neuropeptides, and GABA, with the morphological features of locally projecting interneurons may confer a heightened resistance to these neurons in a variety of neurodegenerative conditions, even at advanced stages.

V. A Synthetic Neuronal Phenotype of Vulnerability and Resistance

Selective neuronal vulnerability is a hallmark of all of the major neurodegenerative disorders and is apparent not only at the level of affected brain regions, subregions, or layers, but also at the level of specific neuron populations (Hof *et al*., 1990, 1999a; Arnold *et al*., 1991; DeLacoste and White, 1993; Morrison and Hof, 1997). The notion of differential vulnerability can be best understood in the context of a broad, yet integrative definition of neuronal typology that includes morphology, regional and laminar location, connectivity, and neurochemical phenotype. This approach is useful in delineating the cellular organization of the neocortex as well as the cellular pathologic changes in diseases such as AD, as it takes into consideration the complex relationships among these morphofunctional parameters.

In a very general sense, the most vulnerable group of cortical neurons includes large pyramidal cells and, more specifically, those providing long corticocortical projections between association neocortical areas and hippocampal projections. These systems utilize glutamate and are driven by glutamatergic inputs. However, not all corticocortical projections are equally vulnerable, as short projections from primary sensory to adjacent secondary sensory areas are resistant to degeneration. It is therefore likely that the specific neurochemical and morphologic phenotype of certain pyramidal neurons may predispose them to degeneration as well as NFT formation. In contrast to long projection neurons, GABAergic inhibitory interneurons containing the calcium-binding proteins parvalbumin, calbindin, and calretinin, as well as certain neuropeptides, are mostly resistant to degeneration in the AD neocortex. Also, the occurrence of modifications in the expression of certain glutamate receptor subunit proteins during aging or dementia, particularly the calcium-gating AMPA subunit GluR2, may determine susceptibility to calcium-mediated toxicity, which may play an important role in several aspects of the degenerative processes leading to neuronal death in AD.

The relevance and impact of pathologic changes in AD have to be understood within the context of organized systems that underlie neocortical function. For instance, integrated processing in a given sensory modality such as vision involves the simultaneous activity of numerous separable visual areas that have extensive highly ordered interconnections that establish a distributed system subserving the proper integration of the visual information. Similarly, cognition and language, which are not modality-specific functions, presumably depend strongly on the complex communication among neocortical regions provided by the corticocortical circuits, which are the particular projections that degenerate in AD, leading in turn to a

global neocortical disconnection syndrome that presents clinically as dementia (Hof *et al*., 1990, 1999a). Clearly, other degenerative processes occur in AD, and they may also contribute to the clinical characteristics of the disease, but the generalized loss of long corticocortical projections emerges functionally as the most devastating component of AD and the most directly related to dementia. Importantly, in this context, cells that provide these projections appear to be highly specialized neurons that share identifiable morphological and neurochemical features.

This interpretation of the pathological features of AD suggests that the debilitating dementia results from changes restricted to the association neocortex, whereas extensive hippocampal alterations can exist in the absence of neocortical involvement and with only minor disruptions in activities of daily living of the individual, whose memory deficits could be revealed by formal testing, but are not compatible with the diagnosis of dementing illness (see Chapter 7). As the elements of the biochemical and anatomical phenotypes, that are linked to differential cellular vulnerability in AD are increasingly recognized, it is hoped that therapeutic interventions will be developed to protect or rescue the neurons that are at higher risk in AD. The protection of these neurons appears to be an attractive strategy for the management of AD, which may have the advantage of being more achievable than the development of a cure.

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References

Albert, M. S. (1996). Cognitive and neurobiologic markers of early Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A*. **93**, 13547–13551.

Andressen, C., Blümcke, I., and Celio, M. R. (1993). Calcium-binding proteins: Selective markers of nerve cells. *Cell Tissue Res*. **271**, 181–208.

Arai, H., Noguchi, H., Makino, Y., Kosaka, K., Heizmann, C. W., and Iizuka, R. (1991). Parvalbumin-immunoreactive neurons in the cortex of Pick's disease. *J. Neurol*. **238**, 200–202.

Armstrong, D. M., and Ikonomovic, M. D. (1996). AMPA-selective glutamate receptor subtype immunoreactivity in the hippocampal dentate gyrus of patients with Alzheimer's disease—Evidence for hippocampal plasticity. *Mol. Chem. Neuropathol*. **28**, 59–64.

Armstrong, D. M., Ikonomovic, M. D., Sheffield, R., and Wenthold, R. J. (1994). AMPA-selective glutamate receptor subtype immunoreactivity in the entorhinal cortex of non-demented elderly and patients with Alzheimer's disease. *Brain Res*. **639**, 207–216.

Arnold, S. E., Hyman, B. T., Flory, J., Damasio, A. R., and Van Hoesen, G. W. (1991). The topographical and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer's disease. *Cereb. Cortex* **1**, 103–116.

Ball, M. J. (1977). Neuronal loss, neurofibrillary tangles and granulovacuolar degeneration in the hippocampus with ageing and dementia. *Acta Neuropathol*. **37**, 111–118.

Barbas, H. (1986). Pattern in the laminar origin of corticocortical connections. *J. Comp. Neurol*. **252**, 415–422.

Beach, T. G., and McGeer, E. G. (1992). Senile plaques, amyloid β-protein, and acetylcholinesterase fibres: Laminar distribution in Alzheimer's disease striate cortex. *Acta Neuropathol*. **83**, 292–299.

Blümcke, I., Beck, H., Scheffler, B., Hof, P. R., Morrison, J. H., Wolf, H. K., Schramm, J., Elger, C. E., and Wiestler, O. D. (1996). Altered distribution of α-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptor subunit GluR2(4) and *N*-methyl-D-aspartate receptor subunit NMDAR1 in the hippocampus of patients with temporal lobe epilepsy. *Acta Neuropathol*. **92**, 576–587.

Braak, H., and Braak, E. (1985). On areas of transition between entorhinal allocortex and temporal isocortex in the human brain. Normal morphology and lamina-specific pathology in Alzheimer's disease. *Acta Neuropathol*. **68**, 325–332.

Braak, H., and Braak, E. (1991). Alzheimer's disease affects limbic nuclei of the thalamus. *Acta Neuropathol.* **81**, 261–268.

Braak, H., Braak, E., and Kalus, P. (1989). Alzheimer's disease: Areal and laminar pathology in the occipital isocortex. *Acta Neuropathol*. **77**, 494–506.

Braak, H., Braak, E., and Bohl, J. (1992). Retrosplenial region involvement in Alzheimer's disease. *Neurodegeneration* **1**, 53–57.

Brady, D. R., and Mufson, E. J. (1990). Amygdaloid pathology in Alzheimer's disease: Qualitative and quantitative analysis. *Dementia* **1**, 5–17.

Brady, D. R., and Mufson, E. J. (1991). Alz-50 immunoreactive neuropil differentiates hippocampal complex subfields in Alzheimer's disease. *J. Comp. Neurol*. **305**, 489–507.

Brion, J. P. (1990). Molecular pathology of Alzheimer amyloid and neurofibrillary tangles. *Semin. Neurosci*. **2**, 89–100.

Brion, J. P., and Résibois, A. (1994). A subset of calretinin-positive neurons are abnormal in Alzheimer's disease. *Acta Neuropathol*. **88**, 33–43.

Campbell, M. J., and Morrison, J. H. (1989). A monoclonal antibody to neurofilament protein (SMI-32) labels a subpopulation of pyramidal neurons in the human and monkey neocortex. *J. Comp. Neurol.* **282**, 191–205.

Campbell, M. J., Hof, P. R., and Morrison, J. H. (1991). A subpopulation of primate corticocortical neurons is distinguished by somatodendritic distribution of neurofilament protein. *Brain Res*. **539**, 133–136.

Celio, M. R. (1990). Calbindin D-28k and parvalbumin in the rat nervous system. *Neuroscience* **35**, 375–475.

Choi, D. W. (1988). Glutamate neurotoxicity and diseases of the nervous system. *Neuron* **1**, 623–634.

Choi, D. W., and Rothman, S. M. (1990). The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. *Annu. Rev. Neurosci.* **13**, 171–182.

Couratier, P., Sindou, P., Tabaraud, F., Diop, A. G., Spencer, P. S., and Hugon, J. (1995). Modulation of tau neuronal expression induced by NMDA, non-NMDA, and metabotropic glutamate receptor agonists. *Neurodegeneration* **4**, 33–41.

Couratier, P., Lesort, M., Sindou, P., Esclaire, F., Yardin, C., and Hugon, J. (1996). Modifications of neuronal phosphorylated τ immunoreactivity induced by NMDA toxicity. *Mol. Chem. Neuropathol*. **27**, 259–264.

Coyle, J. T., and Puttfarcken P. (1993). Oxidative stress, glutamate, and neurodegenerative disorders. *Science* **262**, 689–695.

Cudkowicz, M., and Kowall, N. W. (1990). Parvalbumin immunoreactive neurons are resistant to degeneration in Huntington's disease cerebral cortex. *J. Neuropathol. Exp. Neurol*. **49**, 345.

DeFelipe, J., Hendry, S. H. C., and Jones, E. G. (1989). Synapses of double bouquet cells in monkey cerebral cortex visualized by calbindin immunoreactivity. *Brain Res*. **503**, 49–54.

De Lacoste, M. C., and White, C. L. III, (1993). The role of cortical connectivity in Alzheimer's disease pathogenesis: A review and model system. *Neurobiol. Aging* **14**, 1–16.

DeLima, A. D., and Morrison, J. H. (1990). Ultrastructural analysis of somatostatin-immunoreactive neurons and synapses in the temporal and occipital cortex of the macaque monkey. *J. Comp. Neurol*. **283**, 212–227.

Dewar, D., Chalmers, D. T., Graham, D. I., and McCulloch, J. (1991). Glutamate metabotropic and AMPA binding sites are reduced in Alzheimer's disease: An autoradiographic study of the hippocampus. *Brain Res*. **553**, 58–64.

Duan, H., He, Y., Wicinski, B., Yeung, G., Page, T. L., Janssen, W. G. M., Morrison, J. H., and Hof, P. R. (1999). Age-related changes in cortical projection neurons in macaque monkey: Dendrite morphology, spine density, and neurochemical features. *Soc. Neurosci. Abstr.* **25**, 362.

Duyckaerts, C., Hauw, J. J., Bastenaire, F., Piette, F., Poulain, C., Rainsard, V., Javoy-Agid, F., and Berthaux, P. (1986). Laminar distribution of neocortical senile plaques in senile dementia of the Alzheimer type. *Acta Neuropathol*. **70**, 249–256.

Esiri, M. M., Pearson, R. C. A., and Powell, T. P. S. (1986). The cortex of the primary auditory area in Alzheimer's disease. *Brain Res*. **366**, 385–387.

Fama, R., Sullivan, E. V., Shear P. K., Marsh, L., Yesavage, J. A., Tincklenberg, J. R., Lim, K. O., and Pfefferbaum, A. (1997). Selective cortical and hippocampal volume correlates of Mattis dementia rating scale in Alzheimer disease. *Arch. Neurol. (Chicago)* **54**, 719–728.

Felleman, D. J., and Van Essen, D. C. (1991). Distributed hierarchical processing in the primate cerebral cortex. *Cereb. Cortex* **1**, 1–47.

Ferrer, I., Casas, R., and Rivera, R. (1993a). Parvalbumin-immunoreactive cortical neurons in Creutzfeldt-Jakob disease. *Ann. Neurol*. **34**, 864–866.

Ferrer, I., Tuñon, T., Serrano, M. T., Casas, R., Alcántara, S., Zújar, M. J., and Rivera, R. M. (1993b). Calbindin D-28k and parvalbumin immunoreactivity in the frontal cortex in patients with frontal lobe dementia of non-Alzheimer type associated with amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry* **56**, 257–261.

Gasic, G. P., and Heinemann, S. (1992). Determinants of the calcium permeation of ligand-gated cation channels. *Curr. Opin. Cell Biol*. **4**, 670–677.

Gaspar, P., Duyckaerts, C., Febvret, A., Benoit, R., Beck, B., and Berger, B. (1989). Subpopulations of somatostatin 28-immunoreactive neurons display differential vulnerability in senile dementia of the Alzheimer type. *Brain Res*. **490**, 1–13.

Gazzaley, A. H., Siegel, S. J., Kordower, J. H., Mufson, E. J., and Morrison, J. H. (1996). Circuit-specific alterations of *N*-methyl-D-aspartate subunit 1 in the dentate gyrus of aged monkeys. *Proc. Natl. Acad. Sci. U.S.A*. **93**, 3121–3125.

Gazzaley, A. H., Thakker, M. M., Hof, P. R., and Morrison, J. H. (1997). Preserved number of entorhinal cortex layer II neurons in aged macaque monkeys. *Neurobiol. Aging* **18**, 549–553.

Goldman-Rakic, P. S. (1988). Topography of cognition: Parallel distributed networks in primate asociation cortex. *Annu. Rev. Neurosci*. 11, 137–156.

Gómez-Isla, T., Price, J. L., McKeel, D. W., Jr., Morris, J. C., Growdon, J. H., and Hyman, B. T. (1996). Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *J. Neurosci*. **16**, 4491–4500.

Gómez-Isla, T., Hollister, R., West, H., Mui S., Growdon, J. H., Petersen, R. C., Parisi, J. E., and Hyman, B. T. (1997). Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. *Ann. Neurol*. **41**, 17–24.

Greenamyre, J. T., and Young, A. B. (1989). Excitatory amino acids and Alzheimer's disease. *Neurobiol. Aging* **10**, 593–602.

Hayes, T. L., and Lewis, D. A. (1992). Nonphosphorylated neurofilament protein and calbindin immunoreactivity in layer III pyramidal neurons of human neocortex. *Cereb. Cortex* **2**, 56–67.

Hendry, S. H. C., Jones, E. G., Emson, P. C., Lawson, D. E. M., Heizmann, C. W., and Streit, P. (1989). Two classes of cortical GABA neurons defined by differential calcium-binding protein immunoreactivities. *Exp. Brain Res*. **76**, 467–472.

Hirano, A., and Zimmerman, H. M. (1962). Alzheimer's neurofibrillary changes—A topographic study. *Arch. Neurol. (Chicago)* **7**, 227–242.

Hof, P. R., and Morrison, J. H. (1990). Quantitative analysis of a vulnerable subset of pyramidal neurons in Alzheimer's disease: II. Primary and secondary visual cortex. *J. Comp. Neurol.* **301**, 55–64.

Hof, P. R., and Morrison, J. H. (1991). Neocortical neuronal subpopulations labeled by a monoclonal antibody to calbindin exhibit differential vulnerability in Alzheimer's disease. *Exp. Neurol*. **111**, 293–301.

Hof, P. R., and Morrison, J. H. (1995). Neurofilament protein defines regional patterns of cortical organization in the macaque monkey visual system: A quantitative immunohistochemical analysis. *J. Comp. Neurol*. **352**, 161–186.

Hof, P. R., and Nimchinsky, E. A. (1992). Regional distribution of neurofilament and calcium-binding proteins in the cingulate cortex of the macaque monkey. *Cereb. Cortex* **2**, 456–467.

Hof, P. R., Cox, K., and Morrison, J. H. (1990). Quantitative analysis of a vulnerable subset of pyramidal neurons in Alzheimer's disease: I. Superior frontal and inferior temporal cortex. *J. Comp. Neurol*. **301**, 44–54.

Hof, P. R., Cox, K., Young, W. G., Celio, M. R., Rogers, J., and Morrison, J. H. (1991). Parvalbumin-immunoreactive neurons in the neocortex are resistant to degeneration in Alzheimer's disease. *J. Neuropathol. Exp. Neurol.* **50**, 451–462.

Hof, P. R., Nimchinsky, E. A., Celio, M. R., Bouras, C., and Morrison, J. H. (1993). Calretinin-immunoreactive neocortical interneurons are unaffected in Alzheimer's disease. *Neurosci. Lett*. **152**, 145–149.

Hof, P. R., Nimchinsky, E. A., Buée-Scherrer, V., Buée, L., Nasrallah, J., Hottinger, A. F., Purohit, D. P., Loerzel, A. J., Steele, J. C., Delacourte, A., Bouras, C., Morrison, J. H., and Perl, D. P. (1994). Amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam: Quantitative neuropathology, immunohistochemical analysis of neuronal vulnerability, and comparison with related neurodegenerative disorders. *Acta Neuropathol.* **88**, 397–404.

Hof, P. R., Mufson, E. J., and Morrison, J. H. (1995a). The human orbitofrontal cortex: Cytoarchitecture and quantitative immunohistochemical parcellation. *J. Comp. Neurol*. **359**, 48–68.

Hof, P. R., Nimchinsky, E. A., and Morrison, J. H. (1995b). Neurochemical phenotype of corticocortical connections in the macaque monkey: Quantitative analysis of a subset of neurofilament protein-immunoreactive projection neurons in frontal, parietal, temporal, and cingulate cortices. *J. Comp. Neurol*. **362**, 109–133.

Hof, P. R., Ungerleider, L. G., Webster, M. J., Gattass, R., Adams, M. M., Sailstad, C. A., and Morrison, J. H. (1996). Neurofilament protein is differentially distributed in subpopulations of corticocortical projection neurons in the macaque monkey visual pathways. *J. Comp. Neurol*. **376**, 112–127.

Hof, P. R., Ungerleider, L. G., Adams, M. M., Webster, M. J., Gattass, R., Blumberg, D. M., and Morrison, J. H. (1997). Callosally-projecting neurons in the macaque monkey V1/V2 border are enriched in nonphosphorylated neurofilament protein. *Visual Neurosci*. **14**, 981–987.

Hof, P. R., Bouras, C., and Morrison, J. H. (1999a). Cortical neuropathology in aging and dementing disorders: Neuronal typology, connectivity, and selective vulnerability. *Cereb. Cortex* **14**, pp. 175–312.

Hof, P. R., Wicinski, B., Lin, G. I., Bussière, T., Giannakopoulos, P., Bouras, C., Perl, D. P., and Morrison, J. H. (1999b). Neurofilament proteins identify vulnerable neocortical neurons: Stereologic analysis in normal aging and Alzheimer's disease. *Soc. Neurosci. Abstr.* **25**, 593.

Hyman, B. T., Damasio, A. R., Van Hoesen, G. W., and Barnes, C. L. (1984). Alzheimer's disease: Cell specific pathology isolates the hippocampal formation. *Science* **225**, 1168–1170.

Hyman, B. T., Van Hoesen, G. W., Kromer, L. J., and Damasio, A. R. (1986). Perforant pathway changes and the memory impairment of Alzheimer's disease. *Ann. Neurol.* **20**, 472–481.

Hyman, B. T., Van Hoesen, G. W., and Damasio, A. R. (1987). Alzheimer's disease: Glutamate depletion in the hippocampal perforant pathway zone. *Ann. Neurol*. **22**, 37–40.

Hyman, B. T., Van Hoesen, G. W., and Damasio, A. R. (1990). Memory-related neural systems in Alzheimer's disease: An anatomic study. *Neurology* **40**, 1721–1730.

Ikonomovic, M. D., and Armstrong, D. M. (1996). Distribution of AMPA receptor subunits in the nucleus basalis of Meynert in aged humans: Implications for selective neuronal degeneration. *Brain Res*. **716**, 229–232.

Ikonomovic, M. D., Sheffield, R., and Armstrong, D. M. (1995). AMPA-selective glutamate receptor subtype immunoreactivity in the aged human hippocampal formation. *J. Comp. Neurol*. **359**, 239–252.

Ikonomovic, M. D., Mizukami, K., Davies, P., Hamilton, R., Sheffield, R., and Armstrong, D. M. (1997). The loss of GluR2(3) immunoreactivity precedes neurofibrillary tangle formation in the entorhinal cortex and hippocampus of Alzheimer brains. *J. Neuropathol. Exp. Neurol*. **56**, 1018–1027.

Kelly, M., and Kowall, N. W. (1989). Corticotropin-releasing factor immunoreactive neurons persist throughout the brain in Alzheimer's disease. *Brain Res*. **501**, 392–396.

Kobayashi, K., Emson, P. C., Mountjoy, C. Q., Thornton, S. N., Lawson, D. E. M., and Mann, D. M. A. (1990). Cerebral cortical calbindin D-28k and parvalbumin neurones in Down's syndrome. *Neurosci. Lett*. **113**, 17–22.

Kondo, H., Hashikawa, T., Tanaka, K., and Jones, E. G. (1994). Neurochemical gradient along the monkey occipito-temporal cortical pathway. *NeuroReport* **5**, 613–616.

Kondo, H., Tanaka, K., Hashikawa, T., and Jones E. G. (1999). Neurochemical gradients along monkey sensory cortical pathways: Calbindin-immunoreactive pyramidal neurons in layers II and III. *Eur. J. Neurosci*. **11**, 4197–4203.

Kovács, T., Cairns, N. J., and Lantos, P. L. (1999). β-Amyloid deposition and neurofibrillary tangle formation in the ofactory bulb in ageing and Alzheimer's disease. *Neuropathol. Appl. Neurobiol*. **25**, 481–491.

Kowall, N. W., and Beal, M. F. (1988). Cortical somatostatin, neuropeptide Y, and NADPH diaphorase neurons: Normal anatomy and alterations in Alzheimer's disease. *Ann. Neurol*. **23**, 105–114.

Kowall, N. W., and Beal, M. F. (1991). Glutamate-, glutaminase-, and taurine-immunoreactive neurons develop neurofibrillary tangles in Alzheimer's disease. *Ann. Neurol*. **29**, 162–167.

Ksiezak-Reding, H., Dickson, D. W., Davies, P., and Yen, S. H. (1987). Recognition of tau epitopes by anti-neurofilament antibodies that bind to Alzheimer neurofibrillary tangles. *Proc. Natl. Acad. Sci. U.S.A*. **84**, 3410–3414.

Lewis, D. A., Campbell, M. J., Terry, R. D., and Morrison, J. H. (1987). Laminar and regional distribution of neurofibrillary tangles and neuritic plaques in Alzheimer's disease: A quantitative study of visual and auditory cortices. *J. Neurosci*. **7**, 1799–1808.

Lu, Y. M., Yin, H. Z., Chiang, J., and Weiss, J. H. (1996). Ca^{2+}-permeable AMPA/kainate and NMDA channels: High rate of Ca^{2+} influx underlies potent induction of injury. *J. Neurosci.* **16**, 5457–5465.

Masliah, E., Terry, R. D., Alford, M., DeTeresa, R., and Hansen, L. A. (1991). Cortical and subcortical patterns of synaptophysin-like immunoreactivity in Alzheimer's disease. *Am. J. Pathol*. **138**, 235–246.

Masliah, E., Mallory, M., Hansen, L., DeTeresa, R., and Terry, R. D. (1993). Quantitative synaptic alterations in the human neocortex during normal aging. *Neurology* **43**, 192–197.

Meldrum, B., and Garthwaite, J. (1991). Excitatory amino acid neurotoxicity and neurodegenerative diseases. *Trends Pharmacol. Sci*. **11**, 54–61.

Mirra, S. S., Hart, M. N., and Terry, R. D. (1993). Making the diagnosis of Alzheimer's disease—A primer for practicing neuropathologists. *Arch. Pathol. Lab. Med*. **117**, 132–144.

Mochizuki, A., Tamaoka, A., Shimohata, A., Komatsuzaki, Y., and Shoji, S. (2000) Aβ42-positive non-pyramidal neurons around amyloid plaques in Alzheimer's disease. *Lancet* **355**, 42–43.

Morrison, J. H., and Hof, P. R. (1997). Life and death of neurons in the aging brain. *Science* **278**, 412–419.

Morrison, J. H., Rogers, J., Scherr, S., Benoit, R., and Bloom, F. E. (1985). Somatostatin immunoreactivity in neuritic plaques of Alzheimer's patients. *Nature (London)* **314**, 90–92.

Morrison, J. H., Lewis, D. A., Campbell, M. J., Huntley, G. W., Benson, D. L., and Bouras, C. (1987). A monoclonal antibody to non-phosphorylated neurofilament protein marks the vulnerable cortical neurons in Alzheimer's disease. *Brain Res*. **416**, 331–336.

Mountjoy, C. Q., Roth, M., Evans, N. J. R., and Evans, H. M. (1983). Cortical neuronal counts in normal elderly controls and demented patients. *Neurobiol. Aging* **4**, 1–11.

Nimchinsky, E. A., Vogt, B. A., Morrison, J. H., and Hof, P. R. (1995). Spindle neurons of the human anterior cingulate cortex. *J. Comp. Neurol*. **355**, 27–37.

Nimchinsky, E. A., Hof, P. R., Young, W. G., and Morrison, J. H. (1996). Neurochemical, morphologic and laminar characterization of cortical projection neurons in the cingulate motor areas of the macaque monkey. *J. Comp. Neurol*. **374**, 136–160.

Nimchinsky, E. A., Vogt, B. A., Morrison, J. H., and Hof, P. R. (1997). Neurofilament and calcium-binding proteins in the human cingulate cortex. *J. Comp. Neurol.* **384**, 597–620.

Obrenovitch, T. P., and Urenjak, J. (1997). Altered glutamatergic transmission in neurological disorders: From high extracellular glutamate to excessive synaptic efficacy. *Prog. Neurobiol*. **51**, 39–87.

Pearson, R. C. A., Esiri, M. M., Hiorns, R. W., Wilcock, G. K., and Powell, T. P. S. (1985). Anatomical correlates of the distribution of the pathological changes in the neocortex in Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4531–4534.

Pellegrini-Giampietro, D. E., Zukin, R. S., Bennett, M. V. L., Cho, S., and Pulsinelli, W. A. (1992). Switch in glutamate receptor subunit gene expression in CA1 subfield of hippocampus following global ischemia in rats. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 10499–10503.

Pellegrini-Giampietro, D. E., Pulsinelli, W. A., and Zukin, R. S. (1994). NMDA and non-NMDA receptor gene expression following global brain ischemia in rats: Effect of NMDA and non-NMDA receptor antagonists. *J. Neurochem*. **62**, 1067–1073.

Pollard, H., Héron, A., Moreau, J., Ben-Ari, Y., and Khrestchatisky, M. (1993). Alterations of the GluR-B AMPA receptor subunit flip/flop expression in kainate-induced epilepsy and ischemia. *Neuroscience* **57**, 545–554.

Résibois, A., and Rogers, J. H. (1992). Calretinin in rat brain: An immunohistochemical study. *Neuroscience* **46**, 101–134.

Rogers, J., and Morrison, J. H. (1985). Quantitative morphology and regional and laminar distributions of senile plaques in Alzheimer's disease. *J. Neurosci*. **5**, 2801–2808.

Rossor, M. N., Iversen, L. L., Reynolds, G. P., Mountjoy, C. Q., and Roth, M. (1984). Neurochemical characteristics of early and late onset types of Alzheimer's disease. *Br. Med. J*. **288**, 961–964.

Sampson, V. L., Morrison, J. H., and Vickers, J. C. (1997). The cellular basis for the relative resistance of parvalbumin and calretinin immunoreactive neocortical neurons to the pathology of Alzheimer's disease. *Exp. Neurol*. **154**, 295–302.

Sautière, P. E., Sindou, P., Couratier, P., Hugin, J., Wattez, A., and Delacourte, A. (1992). Tau antigenic changes induced by glutamate in rat primary culture model: A biochemical approach. *Neurosci. Lett*. **140**, 206–210.

Scheff, S. W., and Price, D. A. (1993). Synapse loss in the temporal lobe in Alzheimer's disease. *Ann. Neurol*. **33**, 190–199.

Senut, M. C., Roudier, M., Davous, P., Fallet-Bianco, C., and Lamour, Y. (1991). Senile dementia of the Alzheimer type: Is there a correlation between entorhinal cortex and dentate gyrus? *Acta Neuropathol*. **82**, 306–315.

Shipp, S, and Zeki, S. (1989a). The organization of connections between areas V5 and V1 in macaque visual cortex. *Eur. J. Neurosci*. **1**, 309–332.

Shipp, S., and Zeki, S. (1989b). The organization of connections between areas V5 and V2 in macaque visual cortex. *Eur. J. Neurosci*. **1**, 333–354.

Sindou, P., Couratier, P., Barthe, D., and Hugon, J. (1992). A dose-dependent increase of Tau immunostaining is produced by glutamate toxicity in primary neuronal culture. *Brain Res*. **572**, 242–246.

Sindou, P., Lesort, M., Couratier, P., Yardin, C., Esclaire, F., and Hugon, J. (1994). Glutamate increases tau phosphorylation in primary neuronal cultures from fetal rat cerebral cortex. *Brain Res*. **646**, 124–128.

Terry, R. D., Peck, A., DeTeresa, R., Schechter, R., and Horoupian, D. S. (1981). Some morphometric aspects of the brain in senile dementia of the Alzheimer's type. *Ann. Neurol*. **10**, 184–192.

Terry, R. D., Masliah, E., Salmon, D. P., Butters, N., DeTeresa, R., Hill, R., Hansen, L. A., and Katzman, R. (1991). Physical basis of cognitive alterations in Alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. *Ann. Neurol*. **30**, 572–580.

Tomlinson, B. E. (1992). Ageing and the dementias. *In* ''Greenfield's Neuropathology'' (J. H. Adams and L. W. Duchen, eds.), 5th ed., pp. 1284–1410. Oxford University Press, New York.

Trojanowski, J. Q., Schmidt, M. L., Shin, R. W., Bramblett, G. T., Rao, D., and Lee, V. M. Y. (1993). Altered tau and neurofilament proteins in neurodegenerative diseases: Diagnostic implications for Alzheimer's disease and Lewy body dementias. *Brain Pathol*. **3**, 45–54.

Vickers, J. C. (1997). Commentary: A cellular mechanism for the neuronal changes underlying Alzheimer's disease. *Neuroscience* **78**, 629–639.

Vickers, J. C., Delacourte, A., and Morrison, J. H. (1992). Progressive transformation of the cytoskeleton associated with normal aging and Alzheimer's disease. *Brain Res*. **594**, 273–278.

Vickers, J. C., Riederer, B. M., Marugg, R. A., Buée-Scherrer, V., Buée, L., Delacourte, A., and Morrison, J. H. (1994). Alterations in neurofilament protein immunoreactivity in human hippocampal neurons related to normal aging and Alzheimer's disease. *Neuroscience* **62**, 1–13.

Vickers, J. C., Dickson, T. C., Adlard, P. A., Saunders, H. L., King, C. E., and McCormack, G. (2000). The cause of neuronal degeneration in alzheimer's disease. *Prog. Neurobiol*. **60**, 139–165.

Vogt, B. A., Van Hoesen, G. W., and Vogt, L. J. (1990). Laminar distribution of neuron degeneration in posterior cingulate cortex in Alzheimer's disease. *Acta Neuropathol*. **80**, 581–589.

Vogt, B. A., Vogt, L. J., Vrana, K. E., Meadows, R. S., Challa, V. R., Hof, P. R., and Van Hoesen, G. W. (1998). Neuropathological subtypes of Alzheimer's disease: Laminar patterns of neurodegeneration in posterior cingulate cortex. *Exp. Neurol*. **153**, 8–22.

Walker, L. C., Kitt, C. A., Cork, L. C., Struble, R. G., Dellovado, T. L., and Price, D. L. (1988). Multiple transmitter systems contribute neurites to individual senile plaques. *J. Neuropathol. Exp. Neurol*. **47**, 138–144.

Wisniewski, H. M., Bancher, C., Barcikowska, M., Wen, G. Y., and Currie, J. (1989). Spectrum of morphological appearance of amyloid deposits in Alzheimer's disease. *Acta Neuropathol*. **78**, 337–347.

Yasuda, R. P., Ikonomovic, M. D., Sheffield, R., Rubin, R. T., Wolfe, B. B., and Armstrong, D. M. (1995). Reduction of AMPA-selective glutamate receptor subunits in the entorhinal cortex of patients with Alzheimer's disease pathology: A biochemical study. *Brain Res*. **678**, 161–167.

Zhang, H., Sternberger, N. H., Rubinstein, L. J., Herman, M. M., Binder, L. I., and Sternberger, L. A. (1989). Abnormal processing of multiple proteins in Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A*. **86**, 8045–8049.

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Patterns of Cortical Neurodegeneration in Alzheimer's Disease: Subgroups, Subtypes, and Implications for Staging Strategies

Normal brain aging into the ninth decade is associated with cognitive impairments that are quantitative in nature rather than forming subgroups of changes and it is not associated with appreciable neuron degeneration. In contrast, although age is a risk factor for Alzheimer's disease (AD), cognitive deficits form statistically unique subgroups and the patterns of neocortical neurodegeneration occur according to early deficits. There is substantial evidence for clincopathological subgroups and some studies suggest the possibility of subtypes. A subtype is comprised of cases with unique clinical deficits, pattern of brain lesions, and etiology. One possible AD subtype are patients with early spastic paraparesis associated with the deletion of exon 9 from the presenilin 1 gene, a unique type of senile plaque, and degeneration of the corticospinal tract. Also suggestive of subtypes are different laminar patterns of neurodegeneration in posterior cingulate cortex. These patterns of neuron death are not compatible with a linear regression model of large neuron death, but rather a multivariate model based on principal components analysis. Because AD is not composed of a single pattern of clinical, cognitive, and pathological lesions, staging at postmortem examination can only be used as a general guide to the level of neurofibrillary and amyloid deposition in the cerebral cortex. The presence of clinicopathological subgroups, the lack of a strong relationship between neuron death and neurofibrillary tangles, and the possibility of subtypes raise the specter that no current staging strategy precisely defines disease status. Thus, linear regression of population variables and simple staging need to be replaced by multivariate models and assessments of disease progression that accommodate many statistical and topographical subgroups and/or subtypes of AD.

I. Introduction

Neuron death is not a normal part of aging into the ninth decade. This has been shown for temporal cortex (Giannakopoulos *et al*., 1996; Gómez-Isla *et al*., 1996), posterior cingulate cortex (Vogt *et al*., 1995, 1998), and hippocampal sectors CA1–3 (West, 1993; West *et al*., 1994; Šimić *et al*., 1997). These latter studies demonstrate that although hippocampal formation does show losses in the subiculum and hilus of the dentate gyrus, these losses are qualitatively different from those observed in Alzheimer's disease (AD). Indeed, the loss of neurons previously thought to be associated with advancing age was probably associated with early stages of Alzheimer's and other dementing diseases. Because neuron loss in AD engages specific functional systems in the cerebral cortex, it should be straightforward to define which neurons degenerate and the topography of such a process. The pattern of neocortical neurodegeneration, however, is poorly understood. Such a task is daunting for a number of reasons. Cortical neurons are different sizes, they are organized into layers that differ in size and composition by cortical region, and there are so many of them that it is not possible to simply count all neurons in all areas. Finally, there is not a simple and established relationship between one or more neurodegenerative processes and the densities of neurofibrillary tangles (NFT) and senile plaques (SP). If such relationships existed, the density of NFT, for example, could be used as a surrogate marker for neurodegeneration.

The problem of defining neurodegeneration in AD is not a simple matter of blind counting all neurons in the brain. Even if such counts were available, they would be useless because one of the essential problems confronting the neurobiology of AD is conceptual; what model best fits the data? Is this a single disease with minor heterogeneity in clinical symptoms and neuropathology or is it a complex disease

with multiple genetic and environmental origins and risk factors, different ages at onset and rates of progression, different patterns of cortical outcomes, and fundamentally different lesion structures and patterns of neuron death? In this complicated neurological condition, how are neuron losses related to possible mechanisms of disease? One common strategy is to overlook diversity in the AD population and to seek "the" mechanism of neurodegeneration. As we will see, however, numerous clinical, neuropsychological, glucose metabolic, and neuropathological studies argue convincingly that AD is heterogeneous and composed of subgroups. The concept of clinicopathological subgroups must be a part of any neuron-counting protocol. In this context, a blind count of neurons in the entire cerebral cortex will not be adequate. Should early- and late-onset cases and/or familial and sporadic cases be included in each sample and in what proportions? What is the relation to neurofibrillary degeneration and neuron densities in any layer and area in a simple staging scheme (i.e., one that assumes a single progression)? To muddy the waters further, the hypothesis may eventually be proven that there are subtypes. A blind count of all neurons in all cortical areas will have no value outside the context of a specific disease model that takes into account a full range of variables, including disease onset and duration, genetic and other risk factors, and multiple mechanisms of neurodegeneration.

The numerous efforts to stage AD progression based on neurofibrillary and amyloid lesions, initiated by Braak and Braak (1991, 1997), have not resolved an essential contradiction; NFT and neuron densities do not correlate and the pattern of neurodegeneration in the temporal lobe and elsewhere may require mechanisms beyond that of tau phosphorylation. Moreover, the notion that neurofibrillary degeneration can be used to stage AD without reference to laminar patterns of neurodegeneration provides a limited and possibly unrepresentative view of the progression of AD and its clinical heterogeneity. Indeed, the Braak theory of staging requires the view that all cases fall into a single continuum and that conspicuous cell loss is not a predominant feature, even in fully developed AD.

This review evaluates current evidence for single or multiple laminar patterns of neocortical neuron degeneration and the extent to which cell death is correlated with NFT. It is observed that there is essentially no relationship between cell death and NFT and that a simple staging strategy cannot be applied to neurodegeneration nor will it explain clinical heterogeneity. Furthermore, studies of posterior cingulate cortex over the past decade clearly show that AD pathology does not cascade to a single and common end point as required by simple staging theories. More importantly, it does not begin with a single laminar pattern of neuron death and, once again, there is no substantive relationship between total neurodegeneration and NFT. These observations have led us to the use of nonlinear models to evaluate the characteristics of qualitatively independent subgroups of AD. These different laminar patterns of neurodegeneration are referred to as neuropathological subtypes within AD. Although subtypes of AD have not yet been identified, neurobiological studies support a continued effort to test the subtypes hypothesis. This review considers some of the compelling findings that support this viewpoint and multivariate statistical strategies that will be needed to test the subtypes hypothesis.

II. Neurofibrillary Degeneration, Clinical Symptoms, and Neurodegeneration

Densities of NFT and neuritic plaques are related to dementia severity in AD (McKee *et al*., 1991; Bierer *et al*., 1995; Nagy *et al*., 1995; Berg *et al*., 1998). The topographical distribution of NFT has been related to impaired cortical function and associated clinical symptoms in a general manner (e.g., Brun and Englund, 1981; Arnold *et al*., 1991), and studies evaluating specific clinical impairments and their association with cortical patterns of NFT confirm this expectation. Thus, patient populations with early signs of visual agnosia (Giannakopoulos *et al*., 1999), Bálint-like symptoms and posterior cortical atrophy (Hof *et al*., 1997), or various apraxias (Giannakopoulos *et al*., 1998) have differential distributions of NFT in areas that play a prominent role in each of these functions. For example, associative visual agnosia correlates with NFT in occipitotemporal visual association areas, whereas apperceptive visual agnosia is associated with diffuse rather than localized NFT (Giannokopoulos *et al*., 1999). In addition, SP densities appear to bear no specific relationship to this functional deficit. Thus, the density and topographical distribution of NFT can be related to selective and early clinical deficits.

Because dementia severity is highly correlated with large neuron degeneration in layers III and V (Neary *et al*., 1986), it is not surprising that NFT density is inversely linked to neurodegeneration (Mountjoy *et al*., 1983; Neary *et al*., 1986; Bondareff *et al*., 1993; Gómez-Isla *et al*., 1997). What is not clear, however, is the extent to which this relationship may explain total neurodegeneration. The following concerns about this relationship are worth considering. First, many non-NFT-containing neurons have DNA damage (Su *et al*., 1994; Sheng *et al*., 1998) and/or evidence for free-radical damage, as shown with nitrotyrosine immunohistochemistry (Su *et al*., 1997) suggesting that all neurodegeneration is not mediated by NFT. Second, the total number of NFT greatly understates total neuron loss in neocortex (Vogt *et al*., 1990, 1998; Gómez-Isla *et al*., 1997). Unless many NFT are transient, much neocortical cell death may not be associated with NFT. Third, longitudinal biopsy/autopsy studies report relatively stable NFT in patients with progressive cognitive decline (Mann *et al*., 1988; Bennett *et al*., 1993). Although earlier methods may not be as sensitive as current immunohistochemistry, NFT were stable in one study that reported neuron losses in frontal and temporal cortices, and neuron losses increased until death (Mann *et al*., 1988). It is likely, therefore, that NFT are only the tip of the neurodegeneration iceberg. It is possible that exploring the patterns of neurodegeneration with other methods will provide a greater range of information about the origins of cell death in AD. Finally, the extent to which NFT do not accurately reflect total neurodegeneration could raise concerns about staging AD based on the pattern and "spread" of markers in the temporal lobe and throughout neocortex, as implied and stated in various staging strategies.

III. Linear Model of Neurodegeneration: Temporal Cortex

Although an early study showed that large neurons degenerate in AD (Terry *et al*., 1981), it used extensive averaging

across many areas, quartile-depth measurements rather than specific laminar architectures of each area, and automatic counting at low magnification. Subsequent studies of cell death in AD also employed techniques that may have biased the outcomes. Some limited the analysis to layers III and V, which are known to have large neurons (Neary *et al*., 1986), whereas others used estimates of total neurons (Mountjoy *et al*., 1983; Gómez-Isla *et al*., 1997). None of these counting strategies provide a complete picture of the laminar patterns of cell death. A precise area and laminar analysis has not been performed throughout the cerebral cortex and it is still not known to what extent different sized neurons are involved in the pathogenesis of AD. In addition to restrictive *a priori* hypotheses, another problem with many studies is the underlying statistical assumptions and models used to analyze data.

Studies of cell death sometimes assume that neuron loss in a single neocortical area has an inverse association with age at death and disease duration. Because these associations could be used to explain a progressive decline in cognition, associations between neuron loss and disease progression are of particular importance. An inverse relation between total number of neurons and disease duration has been reported for the superior temporal cortex (Gómez-Isla *et al*., 1997). Some AD cases had neuron numbers that fell within the control range, and it was suggested that neuron losses in the superior temporal cortex were delayed in relation to the earliest functional impairments based on the Blessed dementia scale. It was also noted that neuronal changes were delayed in relation to the very earliest losses in the entorhinal cortex.

If the extent and progression of short-term memory impairment are dominated by the entorhinal cortex, this region should show a loss of neurons in relation to memory impairment and one would expect a progressive loss with aging. Gómez-Isla *et al*. (1996) provided information about neuron densities in entorhinal cortex, patient age at death, and clinical dementia rating scores (CDR) for 10 cases. Layer II neuron densities were closely related to disease severity measured by the CDR, although total neuron densities were not related to CDR. This suggests that defining cortical neurodegeneration with total neuron counts for an area will overlook important and laminar specific changes and changes that may underlie particular clinical symptoms.

To the extent that AD has an age risk, it is interesting to observe that total neurons in the entorhinal cortex do not change over three decades of AD and that there is a positive correlation between age at death and the number of neurons in layer II (Gómez-Isla *et al*., 1996). In this study, the three cases with earliest age of death (67–77 years), and presumably earliest onset, had significantly fewer neurons in layer II ($0.1 \times 10^6 \pm 0.026$; mean $\pm$ SEM) versus the seven cases with much later ages at death (85–95 years; $0.221 \times 10^6 \pm 0.048$). This latter observation suggests that the AD population is not uniform and a linear regression would not be appropriate to analyze data. There appears to be an important age-at-onset confound that cannot be extracted by the least-squares model. One solution to the paradox that layer II of the entorhinal cortex is involved early in AD and yet lacks an inverse relationship between neuron densities and age at death is that a nonlinear model of multiple subgroups better describes data than the assumption of a single underlying continuum.

IV. Neurodegeneration and NFT in Temporal Cortex

It is often assumed that neurodegeneration parallels NFT deposition, particularly in medial temporal areas; a view that has become widespread with efforts to stage disease severity with neurofibrillary changes. The facts, however, are open to a number of interpretations. For example, Gómez-Isla *et al*. (1996) concluded, "Neuronal number in AD was inversely proportional to NFT formation..." Total neuron density/$mm^3 \times 10^3$ and NFT densities in entorhinal cortex were reported for nine cases. The densities of NFT were rank ordered before performing the linear regression with neuron density, which produced an inverse relationship. The rank ordering, however, overweighed a few outlier cases and provided for limited statistical significance, and the inclusion of control cases should not have been used to power this relationship for AD. If data for each case are plotted without rank ordering, there is no relationship between total neuron densities and NFT. Finally, a study of entorhinal cortex, area CA1 of the hippocapmus, and area 20 in centenarians reported no correlation between NFT and neuron densities (Giannakopoulos *et al*., 1996). Thus, there is not an inverse relationship between neurodegeneration and NFT in entorhinal cortex and NFT cannot be used as a surrogate marker of overall neurodegeneration.

Another way to assess relationships between NFT and neuron densities in the temporal lobe is to consider an area that has a radically different cytoarchitecture than the entorhinal cortex, i.e., the lamina externa or granular layer, of the presubiculum. Figure 10.1A provides an example of this area with many small and medium-sized neurons in a control case stained for thionin. The remainder of Fig. 10.1 is from patient FG, who presented initially with dysexecutive syndrome and other evidence of frontal lobe involvement. Patient FG had the disease for 4.5 years and postmortem assessment confirmed bilateral, focal atrophy of the prefrontal cortex. This case has been discussed in detail previously (Vogt *et al*., 1999) and is considered again later. Figure 10.1B, of the granular layer stained with thionin, shows that there are very few neurons and many astrocytes in this layer. Figure 10.1C is immunoreacted for a monoclonal antibody to amyloid-β 42 peptide (Aβ42) in the external pyramidal layer of the presubiculum and shows a heavy deposit of this long form of amyloid. Figure 10.1D shows immunohistochemistry for hyperphosphorylated tau (AD2; Buée-Scherrer *et al*., 1996), counterstained with thionin, and it shows that there are no NFT in this layer. Although there is some nonspecific staining that is enhanced by the Nissl stain, no NFT or neuropil threads are in this layer. For comparison of the AD2 reaction, Fig. 10.1E shows NFT in layer II of the entorhinal cortex in a noncounterstained section from the same case. These preparations demonstrate a profound loss of neurons in this layer, absent NFT, and the abundant and diffuse deposition of Aβ42. Case FG was in Braak stage III of neurofibrillary degeneration and stage C of amyloid deposition. Thus, in medial temporal areas, small and medium-sized neurons degenerate, their degeneration may not be associated with NFT, and amyloid peptides could play a role in their death. This case provides an example of a strong match between neurodegeneration and Aβ42 deposition, while there is no association

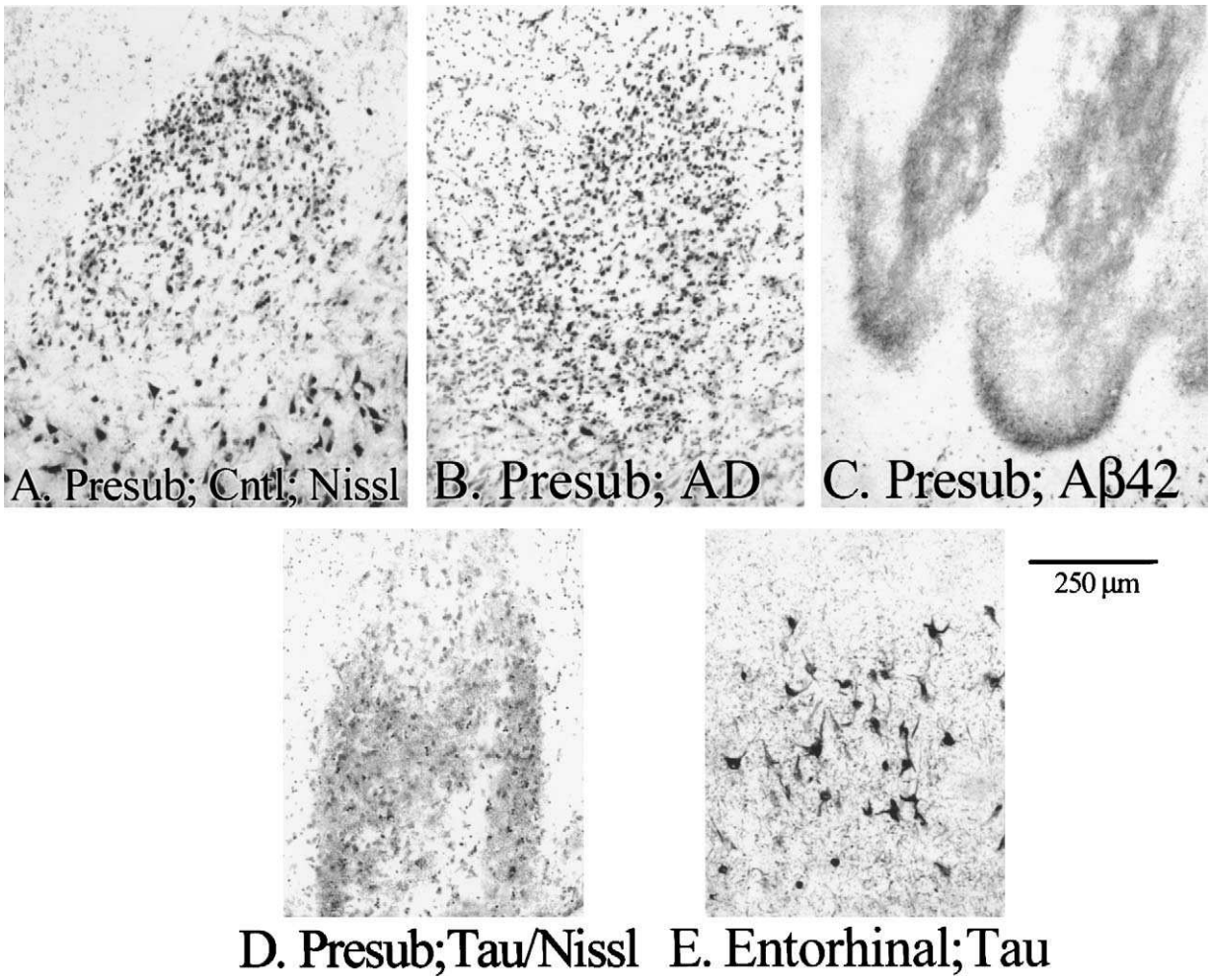

FIG. 10.1. Neurodegeneration in the presubiculum of case FG involves small and medium-sized neurons, no NFT, and substantial and diffuse deposits of Aβ42. (A) A thionin-stained section showing the external pyramidal cell layer and a few large neurons in the inner pyramidal layer from a control case. (B) A thionin-stained section from the same part of the presubiculum in case FG. Note that most neurons have degenerated along the cap of the external pyramidal layer, although some have been retained in its inner portion. (C) Aβ42 immunohistochemistry showing dense deposits in the external pyramidal layer that do not extend into the internal layer. (D) A Nissl-counterstained section of AD2 immunohistochemistry for hyperphosphorylated tau shows that no NFT are present in the external pyramidal layer. (E) The same section as in D (not counterstained) shows the level of NFT in entorhinal cortex layer II. Clearly small and medium-sized pyramidal neurons degenerate in this case without the formation of NFT as is characteristic of the entorhinal cortex.

between neuron death and NFT in the presubiculum. The only way one might make the argument for such a relationship is to assume that NFT formed early and were solubilized and cleared from the neuropil; an alternative that is cast into further doubt later where early neurodgeneration and laminar patterns of markers in posterior cingulate cortex is considered for case YW. The lack of an association between small and medium-sized neuron and neurofibrillary degeneration has consequences for global spreading and staging hypotheses of AD.

To the extent that NFT do not accurately reflect neurodegeneration, the staging scheme for neurofibrillary degeneration does not represent patterns of cell death. The possibility that there are subgroups of neurodegeneration in entorhinal cortex has important consequences for the Braak staging hypothesis. Questions have been raised as to whether clinically characterized cases express a consistent link to Braak stages. It was shown that Braak stages for CDR values of 1–3 are very similar and that the primary distinction occurs late in the disease with CDR 4 and 5 (Haroutunian *et al*., 1999). It appears that no single cortical area goes through a simple sequence of neurodegeneration, although stages can be identified with NFT. As noted earlier, even the entorhinal cortex has neurodegeneration that is greater when the disease begins earlier in life, suggesting that more than a single sequence of neuron death is at work. To the extent that neurodegeneration is (1) not substantially represented by NFT, (2) paramount to understanding the clinical syndrome, and (3) not associated with multiple and qualitatively unique patterns of NFT, the Braak staging strategy cannot provide a context for explaining clinical heteroge-

neity and it does not bear a close relationship to disease progression.

V. Posterior Cingulate Cortex: Functions and Contributions to AD Symptoms

Because our studies of posterior cingulate cortex do not support popular views of AD as a single disease that consummates in a final common pattern of neurodegeneration, we must interject here how it fits into the larger scheme of cortical connectivity, function, and dysfunction in AD. The posterior cingulate cortex is reciprocally connected with most anterior and posterior parahippocampal areas, including the entorhinal cortex and areas TF and TH (Vogt *et al*., 1979; Baleydier and Mauguière, 1980; Pandya *et al*., 1981; Insausti *et al*., 1987; Vogt and Pandya, 1987). The posterior cingulate cortex, including retrosplenial areas, is part of the medial swath of neurodegeneration that extends from the rostral and medial temporal cortex around the splenium of the corpus callosum (Brun and Englund, 1981). Furthermore, it is well documented that the cytoarchitecture and chemoarchitecture of the cingulate cortex is involved in AD, and this literature has been reviewed thoroughly (Vogt *et al*., 1997). In their topographical studies, Brun and Englund (1981) reported that the widest range of neurodegeneration (0–80%) occurred in the posterior cingulate cortex. Although this was part of their effort to grade their cases according to cell loss, gliosis, and other measures, our evaluation of this region in terms of cortical layers did not demonstrate simple relationships among large neuron degeneration, neuron losses in layers III and V, NFT and SP densities, or disease duration and age at onset (Vogt *et al*., 1990). For example, large and small neurons were lost in early or late stages of the disease, neurons were not lost in a single laminar pattern nor were they lost uniformly across all layers in all cases, and there was no simple relationship between NFT and neurodegeneration. Additionally, the progressive involvement of the cingulate cortex and its anterior thalamic afferents in AD has been considered by Braak and Braak (1991, 1993) in the broader context of their staging scheme.

Two possibilities are suggested by this large body of research. One view is that events in the posterior cingulate cortex are unique to this region alone. To the extent that area 23 is isolated from the rest of the brain, such changes can be overlooked as irrelevant to the pathophysiology and clinical symptoms of this disease. The posterior cingulate cortex, however, has massive and reciprocal connections, with many of the medial temporal regions often thought to be fundamental to the etiology of AD. Indeed, the posterior cingulate cortex shows early glucose hypometabolism in AD patients experiencing memory impairments (Minoshima *et al*., 1997). Additionally, many of our cases that were thought to have AD symptoms for 1–3 years had substantial neurodegeneration in the posterior cingulate cortex. Thus, changes in cingulate architecture, chemistry, and function occur early in the disease. *An alternative view* of the cingulate cortex in AD, therefore, forms the basis of the present assessment: the cingulate cortex is linked reciprocally to temporal cortices involved in AD, and cingulate function is impaired early and contributes significantly to the clinical syndrome. Most importantly, structural changes in this region do not fulfill the expectation that AD proceeds along a single course to a final common pathology, that there is only a weak relationship between neuron losses and NFT, and that long amyloid peptides may account for much early cell death. These views from the cingulate gyrus suggest that a reconsideration of other neocortical regions, such as entorhinal and prefrontal cortices, may lead to new insights into the one or more etiologies of this disease.

Disruption of the cingulate cortex early in AD may contribute to a number of symptoms, including visuospatial disorientation, contructional apraxias, and impairments in memory. Although all of the functions of the posterior cingulate cortex are not known, some important findings support its contributions to AD symptomatology. Single neurons in the monkey posterior cingulate cortex encode the position of the eye in the orbit, the direction and amplitude of saccadic eye movements, and large visual field patterns and visual scenes (Olson *et al*., 1993, 1996). Lesions in this region do not disrupt motor function, but they do impair spatial memory (Murray *et al*., 1989). It is possible, therefore, that neurons in the posterior cingulate cortex determine the locations of visual objects relative to the eyes and head. The convergence of eye orientation and large visual field responses is unique to the posterior cingulate cortex and belies its role in visuospatial processing and constructional praxis. Finally, the retrosplenial cortex has high basal glucose metabolism that is elevated further during a delayed alternation task associated with working memory (Matsunami *et al*., 1989).

The persistent involvement of the posterior cingulate cortex in AD likely contributes to memory and visuospatial disorientation early in the disease. In a group of patients with early memory impairment, Minoshima *et al*. (1997) reported the greatest and earliest glucose hypometabolism in the posterior cingulate cortex, and Hirono *et al*. (1998) reported a linear relationship between spatial disorientation and glucose hypometabolism in this region. Nybäck *et al*. (1991) reported substantial glucose hypometabolism in the posterior cingulate cortex, and it was significantly correlated with reduced performance on the Claesson–Dahl retention test and the similarities subtest of the Wechsler–Bellevue intelligence scale. Finally, Giannakopoulos *et al*. (1998) reported a specific correlation between constructional apraxia and the density of NFT in posterior area 23 as well as areas 7 and 19.

Before considering structural changes in the AD posterior cingulate cortex, a few comments on its organization in cognitively normal individuals serve as a basis for comparison. The structure of the human posterior cingulate and retrosplenial cortices is well known (Vogt, 1976; Braak, 1979; Zilles *et al*., 1986; Vogt *et al*., 1995, 1997). Figure 10.2 shows the cytoarchitecture of this cingulate region, including areas 29, 30, and 23, and high magnification photomicrographs of areas 29 and 23 to show the details of the cytoarchitecture for each. Area 29l is adjacent to ectosplenial area 26 and has a granular layer that is comprised to some extent of undifferentiated layers II–IV. Below this is a lamina dissecans and layers V and VI. The granular layer continues into area 29 m, where it appears to divide into layer IV and layers II and IIIab. Area 30 is dysgranular with a layer IV of variable thickness and at places the large layer IIIc and Va pyramids appear to intermingle. Also, layers II and IIIab are quite neuron dense and form a rather

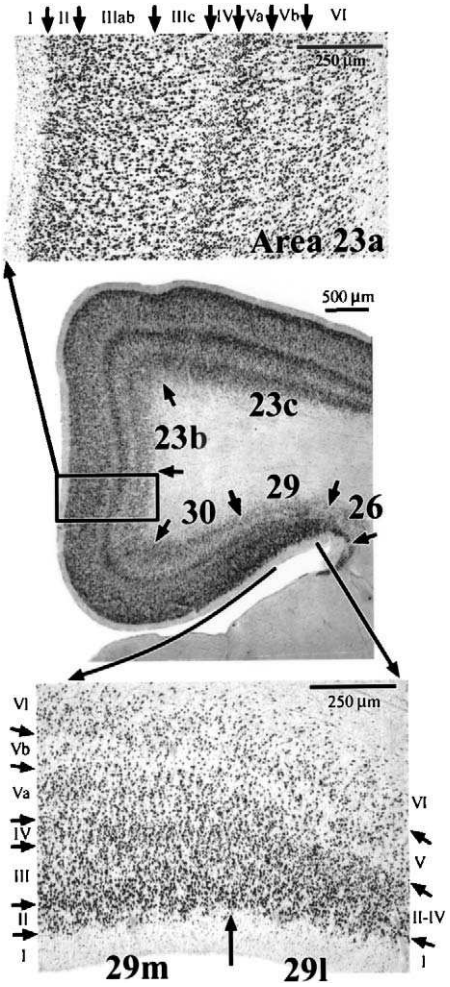

FIG. 10.2. Overview of the cytoarchitecture of the posterior cingulate gyrus with immunohistochemistry for neuron-specific, nuclear-binding protein and counterstained with thionin. One of the outstanding features of this cortex is the broad and densely stained neurons of layer V. Each area is delineated with arrows, and higher magnification photographs are provided for areas 29 m/l and 23a. The poorly differentiated granular layer II–IV is shown for area 29m as are layers IV and Va of area 23. The granular layer and layer Va in both of these areas are severely impacted early in AD, as shown in Figs. 10.5 and 10.6.

striking change at the apex of the ventral cingulate gyrus. Medial and dorsal to this is area 23a, which can be detected with the clear though thin layer IV and very large layer IIIc pyramids. These neurons contain a high proportion of intermediate neurofilaments as reported in articles cited earlier. As is true for each subdivision of area 23, layer Va in area 23a is very dense and thick. This is not true for areas 30 or 29 m, although the layer is present in these latter areas.

VI. Linear Model of Neurodegeneration in Posterior Cingulate Cortex

Although reports of large neuron losses in the neocortex are based on averaging of cell densities across many cytoarchitecturally different areas (Terry *et al*., 1981; Mountjoy *et al*., 1983; Neary *et al*., 1986), the posterior cingulate cortex should be expected to express similar changes. Therefore, we plotted the number of neurons in layer Va of area 23a, a layer with large neurons, as a function of disease duration for 69 cases of definite AD (Vogt *et al*., 1998). The number of neurons for each layer was calculated by counting perikaryal profiles in each layer through a 160-µm-wide strip of cortex. Examples of these drawings have been provided (Vogt *et al*., 1999). The counts were made from six strips and averaged to form the final mean values for control and AD cortex. As predicted, a plot of neurons in layer Va was inverse with disease duration, as shown in Fig. 10.3A (see color insert), and the correlation coefficient was significant ($r = -0.27$; $F = 6.19; p = 0.015$). Concern might be raised that two or three cases with durations of 18–25 years (circled in Fig. 10.3A) had an unusually large influence on the regression line. Indeed, removal of these three cases from the analysis reduced the correlation coefficient to nonsignificance ($r = -0.16$; $F = 1.74; p = 0.19$). Furthermore, if neurons were only lost in layers IIIc and Va, where most large neurons are located, the conclusion could have been made that neurodegeneration in the cingulate cortex occurs in an inverse pattern of primarily large neurons. However, neurons do not degenerate only in these two layers. Indeed, cases with fewer than 10 neurons in layer Va are part of a subgroup with extensive neurodegeneration in all layers. These cases are color coded with green in Fig. 10.3A and will be considered in more detail later.

The problem of ''outlier'' cases in linear regression is well recognized and has been discussed by Stevens (1984). Although all outliers do not necessarily influence regression, one or more cases can be influential and alter the results significantly. Because outlier analysis provides important clues to the composition of the population, let us consider their influence on data discussed earlier in more detail. Although it has been reported that Cook's distance is a good measure of the influence of one or more points on the regression (Kleinbaum *et al*., 1988), it is not associated with a statistical distribution that can be used for significance testing. Analysis of jacknife residuals provides a means of evaluating the veracity of a regression model and, because they are linked directly to distribution, the significance of an outlier in a least-squares model can be determined.

A residual e_i is the difference between the observed value of Y_i and its predicted value $\hat{Y}_i$ from a regression model; i.e., the discrepancy that is still present after fitting with least squares. These error terms should be independent, have a mean of zero, have a common variance, and follow a normal distribution (see Kleinbaum *et al*., 1988, for further details). The jacknife residual is calculated with the *i*th observation deleted. A graphic plot of residuals against predictor values is a way to evaluate the regression assumptions and the influence of possible outliers. A plot of jacknife residuals against disease duration for all cases is provided in Fig. 10.3B (see color insert). The dispersion of residuals plotted against the predictor variable is evidence that the basic regression assumption stating that ''the X variable must be known without error'' is not followed in this population of cases. Furthermore, the coefficient b1 has a small but nonzero value that displaces individual points from a straight line. There is a systematic divergence of the 17

members with overall severe neurodegeneration, i.e., those cases with fewer than 10 neurons sampled in layer Va. The model, therefore, appears to be inappropriate for this data set. Furthermore, the *t* value for the case with a 25-year duration is significant, confirming the expectation that it is an outlier, whereas the *t* value for the 23-year duration case was not quite significant.

A statistical rationale to delete one or more cases as outliers does not alone justify their exclusion from the regression analysis. Because this outlier in the current analysis signaled that a larger subgroup of cases share characteristics that distinguish them from the remainder of the cases, it provided an important hint that the least-squares model was invalid. The 17 cases with severe neurodegeneration are defined as those with greater than 80% neuron loss in most cortical layers. In other words, neuron losses in these cases are not only in layer Va but throughout the entire cortex. This is one rationale for considering this entire group of 17 cases as a subgroup. All of the cases with fewer than 10 neurons in layer Va were part of a larger group we refer to as neuropathological subtype severe (NSTSevere). Deleting all 17 cases from the linear regression reduces the correlation coefficient to an F value of only 0.67 ($p = 0.41$). Importantly, NSTSevere had a range of disease durations from 4.5 to 25 years, suggesting that cases with very few neurons in layer Va may be part of an independent subgroup. In this broader context, long-duration outliers are of no consequence in and of themselves. It is their membership in the larger subgroup that is important.

An assessment of jacknife residuals and outliers in this regression analysis led to the conclusion that a subgroup of cases provided all of the statistical significance for least-squares fitting of linear regression. The demonstration of at least one statistically and pathologically unique subgroup leads to the conclusion that this data set is not uniform and that regression analysis is not justified for all 69 cases together. A multivariable method is needed to define the complex relationships among laminar patterns of neurodegeneration, as well as the role of various AD risk factors, such as age at disease onset and apolipoprotein E genotype. Because multivariate analyses are well established as a means of identifying neuropsychological and clinical subgroups in AD, we begin with these observations before considering a multivariate strategy for assessing cingulate pathology.

VII. Multivariate Models of Cognitive Function: Clinical Subgroups

Aging in the normal population is associated with heterogeneous impairments of cognitive function. Valdois *et al*. (1990), for example, observed six subgroups that differed mainly in overall degree of performance. These subgroups, however, did not exhibit contrasting profiles of impaired and preserved cognitive abilities and their distinguishing features were primarily quantitative rather than qualitative in nature. In the nondemented controls evaluated by Kanne *et al*. (1998), principal component analysis (PCA) showed that only one factor accounted for the neuropsychological profiles, suggesting a functionally homogeneous group. In contrast, cognitive impairments in AD differ significantly from those in normal aging. Martin (1987) and Martin *et al*. (1986) were among the first to recognize that the cognitive onset and progression of AD do not follow a single pattern and proposed that there were neuropsychological subgroups. The claim of subgroups refers to the fact that AD patients can present with qualitatively distinct patterns of impaired and preserved cognitive abilities. These differences do not refer to disease progression and markers thereof, such as extrapyramidal signs, psychosis, and myoclonus, nor to features common to all cases, such as short-term memory disruption. The best documented subgroups consist of patients with deficits in semantic memory and word-finding ability, concurrent with normal or near normal visuospatial skills, and patients with the opposite profile (Haxby *et al*., 1985; Martin *et al*., 1986; Martin, 1987; Becker *et al*., 1988; Albert *et al*., 1990; Grady *et al*., 1990; Fisher *et al*., 1996). Kanne *et al*. (1998) observed three categories of cases with the following factors in their multivariate analysis of 407 AD patients: mental control/frontal, memory-verbal/temporal, and visuospatial/parietal. For those individuals that came to autopsy, there were high correlations with mature plaques in each cortical region and the three psychometric factors.

Disease progression in relatively focal cases is characterized by continued deterioration within the affected cognitive domain (Martin, 1987) and a widening of symptoms to other, previously intact, domains (Becker *et al*., 1988). The fact that the disease continues to progress within a single cognitive domain suggests that the progression of pathology is not random, but rather carves a course that parallels the functional organization of the affected systems. The differences are not simply quantitative and, therefore, are not related to differences in severity, disease duration, or rate of progression in any simple manner. Groups of patients like these define qualitatively distinct clinical subgroups, even though they all may have other symptoms in common, such as difficulty learning and remembering recently presented information.

The different neuropsychological profiles of clinical subgroups are associated with correspondingly different patterns of cortical dysfunction. In the first type of patient noted earlier, there was severe glucose hypometabolism of the posterior parietal cortex, especially in the right hemisphere, relative to other regions, whereas the second type of patient had most severe hypometabolism in the left temporal lobe (Martin, 1990). Cognitive and metabolic heterogeneity is also apparent in the frontal cortex. Grady *et al*. (1990) observed inappropriate behaviors in cases with greatest prefrontal glucose hypometabolism, and Mann *et al*. (1992) reported that two groups with equivalent parietal glucose hypometabolism and impaired measures of parietal function could be distinguished with frontal lobe glucose metabolism, measures of executive function, and rate of progression. Foster *et al*. (1983) observed that patients with a disproportionate failure of language function had hypometabolism in the left frontal, temporal, and parietal regions, whereas those with predominant visuocontructive dysfunction had hypometabolism mainly in the right parietal cortex. Finally, Royall *et al*. (1994) observed early impairment of executive functions in AD that could be attributed to disrupted frontal lobe function. In a review considering patient FG with dysexecutive function, we proposed that focal prefrontal atrophy may account, to some extent, for the specific neuropsychological profile (Vogt *et al*., 1999; see later).

Various multivariate models have been used to evaluate subgroup characteristics in AD. Martin *et al*. (1986), Becker *et al*. (1988), and Kanne *et al*. (1998) used principal components analysis, whereas Fisher *et al*. (1996) used a number of factor and cluster analytical techniques. In each instance, evidence for three subgroups was provided. Becker *et al*. (1988) found that the largest group had nonfocal deficits, whereas smaller subgroups had impairments in lexical/semantic function or visuoconstructional abilities. Martin *et al*. (1986) had a subgroup with equal impairment of word-finding, visuospatial, and constructional skills, a subgroup with severe word-finding concurrent with intact spatial and constructional ability, and a subgroup with the opposite profile. Fisher *et al*. (1996) observed the largest subgroup with severe anomia and constructional dyspraxia, whereas smaller subgroups had severe anomia and preserved visual-perceptual/constructional functioning and another with moderate difficulty copying overlapping figures and intact naming and nonverbal reasoning. Finally, Kanne *et al*. (1998), as reported earlier, observed three patterns of cognitive impairments that were highly correlated with mature plaques in each of the cortical regions that dominate processing in particular functional domains. These observations clearly show that cognitive impairments are not uniform, the differential patterns of dysfunction suggest selective disruption of functional subsystems, including differential impact of pathology, and there should be an equivalent heterogeneity in the cortical expression of this disease. In other words, it should be possible to identify neuropathological subgroups by applying multivariate analytical techniques.

VIII. Clinicopathological Subgroups

Very few neuropathological studies seek to assess subgroups of cases. In the context of clinicopathological subgroups, we can recast the conclusions of previous works that associated differential cortical topography of lesions with particular classes of symptoms, each of which has been cited earlier. Although most of these studies do not seek subgroups *per se*, they have identified clinical subsets of patients with unique patterns of lesions. Four striking clinicopathological subgroups include early disturbances of visuomotor and visual attention, associative visual agnosia, and ideomotor and constructional apraxias and each is associated with a different pattern of cortical lesions.

In the subgroup with early and prominent visuomotor and visual attention symptoms, there is optic ataxia and impaired visual tracking, disturbance of visual attention or simultanagnosia, and variable amounts of alexia, anomia, agraphia, and transcortical aphasia. This constellation of Bálint-like deficits was first reported in AD by Grünthal (1928) and is associated with posterior cortical atrophy as reviewed by Hof *et al*. (1997). The distribution of NFT and neuritic plaques is most prominent in the occipito-parieto-temporal junction, including areas MT and posterior cingulate cortex, whereas there is a somewhat lower lesion density in area 17 and much less in prefrontal cortex, which is relatively spared in these cases (Fig. 10.4, see color insert). In contrast, patients with early associative visual agnosia have preserved visual perception of forms, correctly copy and match items, and have preserved visual attention, but they are unable to identify objects verbally. This syndrome is quite different from the visuomotor symptoms characteristic of the Bálint-like syndrome and is correlated with a high level of NFT in areas 18, 19, and 37 (Giannakopoulos *et al*.,1999). Finally, two subgroups of AD with different apraxias have different distributions of NFT (Giannakopoulos *et al*., 1998). Visuocontructional apraxia is associated with high levels of NFT in areas 7, 19, and 23, whereas ideomotor apraxia is associated with elevated lesions in anterior cingulate area 24.

These four clinicopathological entities are subgroups because they depend on the differential distribution of a common marker, NFT and neuritic plaques. Each subgroup, therefore, could be a consequence of the same mechanism of neuron death without invoking a different etiology for each. Such subgroups are compatible with the notion that there is a stochastic expression of a single etiology of the disease. Future efforts to refine the topography of lesion distributions and neuropsychological testing will certainly uncover more examples of clinicopathological subgroups. Finally, staging the disease, particularly based on markers in the temporal lobe, is compatible with the subgroups hypothesis to the extent that all cases share a common clinical deficit(s) which is related to impairments in this region. To the extent that pathological alterations may start in other regions early in the disease in some cases, the single-model, staging strategy will not prove useful.

IX. The Subtypes Hypothesis

Jorm (1985) considered the threshold criteria for subtypes of AD. In order to demonstrate subtypes, a pattern of clinical symptoms, associated brain lesions, and different mechanisms of neurodegeneration must be identified. According to this standard, defining subtypes proceeds through three stages. Stage 1 is essentially completed and involves demonstrating statistical subgroups based on different patterns of cognitive impairments. Had all AD cases presented with the same first symptom and progressed through a uniform sequence of cognitive impairments, the subtypes hypothesis would long ago have been moot. In view of the unique presenting symptoms, differential progression of cognitive impairments, and altered patterns of cortical glucose metabolism shown with principal components analysis (e.g., Becker *et al*., 1988; Rapoport, 1991), stage 2 studies are underway. In stage 2, patients with unique and early symptoms are assessed at postmortem to determine the extent to which different patterns of neurodegeneration and disease markers can be associated with early symptoms. The observations of clinicopathological subgroups fulfill this activity. Once it is clear that there are unique patterns of cognitive impairments and associated cortical lesions, stage 3 will pursue unique etiologies for each.

According to Jorm criteria for subtypes, the report by Crook *et al*. (1998) provided evidence for a possible subtype of AD. Members of a Finnish pedigree have deletion of exon 9 of the presenilin 1 gene and present early with spastic paraparesis followed by progressive dementia. These latter symptoms include impairments in short- and long-term memory, language, and visuoconstructive and spatial abilities. Postmortem analysis shows unique SP and patterns of neurodegeneration in the cer-

ebral cortex. The SP are two to three times larger than typical for AD and there are no central cores. Although the sensorimotor cortex is not usually involved, even in late stages of AD, the unique SP are in the sensorimotor cortex, as well as other areas, and there is degeneration of the corticospinal tract in some cases. Although some cases with exon 9 deletion do not have spastic paraparesis, and other mutations, such as the splice site acceptor mutation in exon 9, also produce these symptoms, this is the first clear example of unique patterns of lesions, symptoms, and a potentially unique mechanism of neurodegeneration supporting it as a possible subtype.

In any instance in which there is a unique pattern of lesions that might require a unique mechanism of neurodegeneration, there is the possibility of a subtype. Giannakopoulos *et al*. (1996) reported that the CA1 field of the hippocampus was particularly resistant to neurodegeneration in centenarians and that there was an inverse correlation between SP and neuron densities in area 20, a finding not usually made in earlier onset cases. There was also a low rate of NFT formation in areas 20 and 9, despite neurodegeneration in these areas. Finally, a lack of association between apolipoprotein E (ApoE) $\epsilon 4$ genotype and AD over the age of 90 has been reported (Sobel *et al*., 1995; Asada *et al*., 1996). These observations suggest a differential pattern of lesions in centenarians and the possibility for different mechanisms of neurodegeneration. What is lacking to understand further the extent to which these cases form a subtype is a detailed neuropsychological profile that would indicate a unique pattern of cognitive deficits.

A preliminary case for subtypes can be made from cases with particular deletions in the presenilin 1 gene and AD in centenarians. In both instances, the pathological differences are not based solely on a differential distribution of NFT, but rather on differential patterns of neurodegeneration, some of which could be independent of NFT densities. Different laminar patterns of neurodegeneration in the posterior cingulate cortex suggest to us that it is likely that more than one mechanism of neurodegeneration will be required to explain cell death in this region. The observations discussed later, therefore, provide further evidence for neuropathological subtypes and may eventually lead to an understanding of subtypes based on Jorm criteria.

X. Multivariate Analysis of Neuron Losses and the Concept of Neuropathological Subtypes

In light of the evidence for clinical subgroups and relationships between specific clinical symptoms and the topographical distributions of NFT, it is surprising that few neuropathological studies of AD have employed multivariate strategies to address these complex and nonlinear relationships. Armstrong *et al*. (1996) entered a large number of variables into hierarchical and principal component models, including measures of brain weight, neurodegeneration in frontal cortex and the CA1 sector of the hippocampus, and NFT and amyloid deposition in many structures. Although the rationale for each measure was not provided, variables with extensive cross correlations were not removed, and the features of each subtype were not provided, these investigators suggested that five subtypes existed in their sample. Although they concluded that many of the differences underlying their subtypes were due to differential distributions of SP and NFT, neuronal losses were only measured in two cortical areas. It is possible that the multivariate strategy can be pushed further to understand the unique features of neurodegeneration and, eventually, associated patterns of clinical deficits.

The need to develop a multivariate strategy arose from our studies of the posterior cingulate cortex in postmortem cases. An objective assessment of neuron numbers in all layers of this region in cases of definite AD failed to show a single laminar pattern of neuron death and a single disease progression (Vogt *et al*., 1990). All cases with cortical or subcortical Lewy bodies were excluded from the analysis as part of the AD diagnosis to ensure that different patterns of neurodegneration were not a consequence of comorbidities with other neurological diseases. In this carefully screened series of cases, five laminar patterns of neurodegeneration were observed and each had a full range of disease durations. These findings suggested that cases with no losses in one class are not necessarily early forms of more profound cell loss in other classes. Moreover, the most severe degeneration occurred in early-onset cases and could not be the end point of neurodegeneration in classes with less severe degeneration and with a late onset. We tested the subgroup hypothesis with a sample three times as large as the earlier one (Vogt *et al*., 1998) and confirmed and extended many of the earlier predictions. Most importantly, five statistical subgroups were confirmed, the neuropathological subtype with the most severe neurodegeneration contained most of the early-onset cases and cases homozygous for the $\epsilon 4$ allele of ApoE, NFT were unrelated to the laminar patterns of neurodegeneration but did mark disease progression, and NFT were weakly related to neurodegeneration in some layers (III and V) and unrelated to cell death in others (II, IV, and VI). A reconsideration of these findings provides further statistical support for the neuropathological subtypes hypothesis and makes specific predictions for further research endeavors.

The concept of neuropathological subtypes is a hybrid concept meant to distinguish clearly different neuropathological states from those defined by the inclusive definition provided by Jorm's concept of AD subtypes. The Jorm (1985) concept correctly requires an association between unique clinical deficits and lesion patterns as well as unique mechanisms for each subtype. In contrast, a neuropathological subtype is composed of cases that are statistically unique from other cases in terms of some pattern of neuron death and the changes suggest unique mechanisms of death. It is an interim concept to allow for hypothesis testing during the decades needed to analyze this issue without being held to the currently popular view that AD is a single entity and that most cases are the same with only minor differences. As noted earlier in the discussion of neuropsychological subgroups, these differences are not simply quantitative, such as more or fewer SP or NFT. There are instances where more NFT in a region may be related to particular functional deficits; however, the differential distribution of NFT does not predict a unique mechanism for each subgroup. Rather, there might be a stochastic expression of a single mechanism in different cortical regions. In contrast, different laminar patterns of neurodegeneration suggest different mechanisms of neuron death. Selective degeneration of layer IIIab neurons, for example, likely requires a very different

mechanism of cell death than in cases where layer IIIab is intact but neurons in layers IV–V are lost. Furthermore, even when there are quantitative differences in the statistical analysis, such as moderate neuron losses in most layers or severe neuron losses in all layers, there need to be patient characteristics that distinguish qualitatively unique features between the two statistical subtypes. As noted earlier, the subtype with severe neurodegeneration is unique because it has most early-onset cases and most cases that are homozygous for the $\varepsilon 4$ allele of ApoE.

The neuropathological subtypes discussed here cannot be elevated to full Jorm subtypes until they are redefined in terms of clinical deficits and unique mechanisms of cell death have been defined for each. Although ''ST'' and the layer with greatest neurodegeneration were used previously as a short-hand way of referring to each neuropathological subtype (Vogt *et al*., 1998), we now extend this nomenclature to emphasize that a narrowly defined subtype is under consideration rather than Jorm subtypes. Thus, NSTIIIab refers to the neuropathological subtype with greatest neuron death in layer IIIab, whereas NSTSevere refers to cases with severe neurodegeneration in all layers.

Let us reconsider data used for linear regression and assessment of jacknife residuals in Fig. 10.3 in terms of a multivariate model with PCA. The PCA seeks to explain as much of the total variation in the data as possible with a few factors (Kleinbaum *et al*., 1988). Although the first few principal components explain most of the variance and are not correlated, they are difficult to interpret in terms of subsets of original variables; in this instance, numbers of neurons in layers IIIab, IIIc, IV, and Va of area 23a are calculated as discussed earlier for layer Va. A simple structure in the analysis is achieved by rotation of the data so that factor loadings for a single variable are large and those for the others close to zero. We have used orthogonal rotation of the eigenvector projections in three-dimensional graphics with the Ein*Sight statistical and pattern recognition software (InfoMetrix, Woodinville, WA). Graphic rotation was performed to identify groupings of cases that were color coded according to *a priori* hypotheses about the five laminar patterns of neurodegeneration. Figure 10.3C (see color insert) shows the optimal orientation of the three-dimensional projection where all members of each statistical subgroup segregate into a unique space. Because an objective and unbiased statistical method confirms the presence of five subgroups of laminar patterns of neurodegeneration, we can now characterize each of these subgroups by which layers have the greatest cell death and the patient characteristics for each subgroup. Moreover, this strategy can be used to evaluate other variables, such as the age of disease onset, amyloid or tau burden in one or more layers, and the contribution of particular risk factors to cell death.

The characteristics of each neuropathological subtype have been presented (Vogt *et al*., 1998, in Table 3). The independence of each can be argued from this table. There is a full range of disease durations. The only exception is NSTSevere, which has a 4- to 25-year duration because this subgroup also contains most early-onset cases, which have fewer disease comorbidities and live for a longer time with their disease. Indeed, NSTSevere is fundamentally different from all other NST with its early onset, greatest number of SP and NFT, and highest proportion of ApoE $\epsilon 4$ allele homozygotes. Another important point can be drawn from this table. The NST0 with no neurodegeneration is not simply an early stage of cell death that precedes one or more of the other subtypes because it can occur within 10 years of onset. Finally, tests were performed of the hypothesis that the ApoE genotype is associated with one or more patterns of neurodegeneration. Although there are six possible allelic associations, there was no ApoE genotype effect for each NST. This was deduced by including this risk factor in the PCA and by evaluating the proportions of the various allelic combinations in each NST. Only NSTSevere had a disproportionately high density of $\epsilon 4$ homozygotes.

The multivariate strategy is a powerful tool for analyzing complex relationships, including both patient characteristics and neuropathological endpoints. This analysis predicts that there are five laminar patterns of neurodegeneration early in the disease. Indeed, a number of cases included in the analysis discussed earlier had a short to moderate length of the disease (1–4 years). It is important that such cases remain as members in their respective NST, indicating that the same pattern of cell death occurs early in the disease.

In conclusion, multiple laminar patterns of neurodegeneration form the basis for neuropathological subtypes. This nomenclature emphasizes subtypes rather than subgroups because the neurochemical differences among different cortical layers and associated neuron death will likely require different mechanisms, as discussed briefly later. If neurodegeneration occurred according to one size class of neuron and systematically involved only one or two layers, we would have used the term subgroups as employed for evaluating clinicopathological subgroups based on differential patterns of NFT. The term neuropathological modifies the subtype designation to emphasize that, in this instance, the clinical correlates are not yet known. When the clinical and neuropsychological profiles and a unique mechanism of neuron death are identified for each neuropathological subtype, they will be graduated to the status of subtypes based on the Jorm criteria.

XI. NFT are Weakly Related to Neurodegeneration

In our first assessments of the posterior cingulate cortex using thioflavine-S-stained materials, the densities of NFT and SP were not related to the different laminar patterns of neurodegeneration (Vogt *et al*., 1990). In the expanded study there were more SP in NSTSevere, which also had the highest frequency of ApoE $\epsilon 4$ homozygotes, as noted earlier (Vogt *et al*., 1998). NFT densities, however, were about the same for all neuropathological subtypes. Most importantly, the number of NFT greatly understated total neurodegeneration. In layers III and V, there were approximately eight neurons lost for each NFT. In layers II, IV, and VI, where there are few or no NFT and often vast amounts of neuron death, there was not even a weak relationship between lost neurons and NFT.

This is not to say that the laminar distributions and densities of NFT are uninformative, they are. The distribution of NFT is altered throughout the course of the disease in NSTII–V and NSTSevere in the same manner. In early stages of the disease,

NFT are mostly in layers IIIc and Va. As the disease progresses, NFT are more prominent in layers, IIIab and Vb and, late in the disease, most are in layers II and IIIab. A multivariate model of disease duration and the density of NFT in each of three groups of layers confirmed that NFT in different layers mark early, middle, and late stages of the disease. Because this progression was observed in two neuropathological subtypes, these changes are not a feature of any one subtype but reflect a common feature of disease progression.

In conclusion, depending on the layer, there is a weak or no relationship between neuron losses and NFT. This means that the laminar patterns of neurodegeneration cannot be assessed with NFT. It also means that the clinicopathological subgroups identified with NFT may have multiple counterparts in terms of neurodegeneration that are not observable with NFT. It is still possible, therefore, that these clinicopathological subgroups represent subtypes. In order to prove this latter statement, it will be necessary to show that each subgroup has a different laminar pattern of neurodegeneration and that each are subserved by a different mechanism of neuron death.

XII. Early Changes in Posterior Cingulate Cortex

One of the tests that there are five neuropathological subtypes of AD is to evaluate early clinical stages of the disease and identify five laminar patterns of neurodegeneration that are not the result of late stage disease and are not influenced by a larger group of cases that include late-stage cases. Although our previous work included a wide range of cases, we were fortunate to have material from individuals with disease durations estimated to be 1–3 years. These short-duration cases were part of the larger subgroups reported in multivariate analyses and did not segregate into a separate statistical space in eigenvector projections. Another way to evaluate the neuropathological subtype hypothesis is to select cases with CDR of 1–1.5 and disease durations of less than 2 years. Although a complete study of this nature will require many years, we provide patient YW here to show that neurodegeneration occurs early in the posterior cingulate cortex, the pattern of cell death in this instance is compatible with NSTIV–V, this degeneration is unrelated to the formation of NFT, and, in some instances, layers with heaviest neuron loss and gliosis contain the highest amounts of long amyloid β-peptide (Aβ42).

Patient YW was 88 years old when she died of a myocardial infarction. This patient's disease evolved over a period of 2 years with an acceleration during the second year. Until the last 3 months of life, YW had a Mini Mental Status Exam of 22–23, although it was 15 at the time of death. At death there was mild cortical atrophy and NFT in entorhinal cortex and the CA1 sector of the hippocampus and only rare NFT throughout the neocortex in a pattern compatible with Braak stage II. There were a few NFT and neuritic plaques in posterior cingulate areas 29, 30, and 23, as shown with the AD2 antibody. Interestingly, there was substantial gliosis in the granular layer of area 29 (Fig. 10.5) associated with a severe loss of small and medium-sized neurons in this layer and a loss of some large, layer Va pyramids. In area 23a a similar pattern of gliosis and neuron loss was apparent (Fig. 10.6). This pattern of neuron loss fits our NSTIV–V because neurons in layers II–IIIc and Vb–VI of area 23a appear intact when compared to control cases. Note layer IIIc, for example, in Fig. 10.6.

Although there were rare NFT in posterior cingulate and other neocortices identified with the AD2 antibody, there is evidence in this case for an earlier phosphorylation of tau protein. The AT8 monoclonal antibody detects abnormal phos-

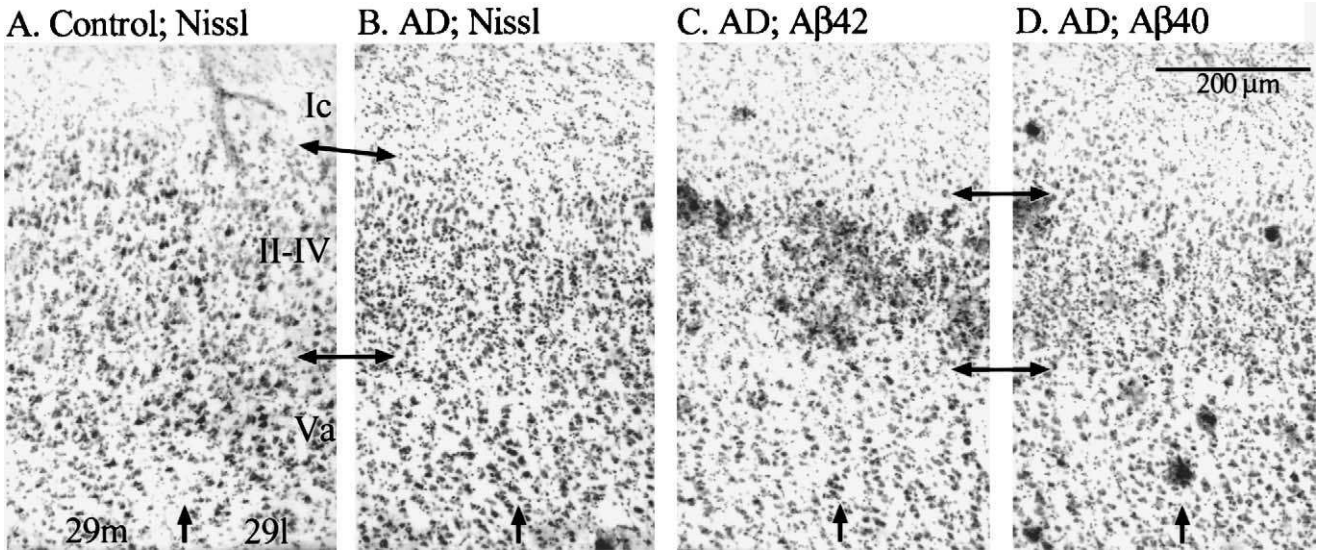

FIG. 10.5. Assessment of case YW (CDR 1.0; 2-year duration). Changes are shown for areas 29 m and 29l. Comparison with the control case shows profound gliosis and neuron losses in the granular layer and, to a lesser extent, in layer Va. There is a dense deposit of Aβ42 in granular layers II–IV, whereas Aβ40 is primarily deposited outside the limits of layers II–IV. Because there were almost no NFT in this layer (i.e., no AD2 immunoreactivity), neurodegeneration is most likely associated with the long form of the amyloid β peptide.

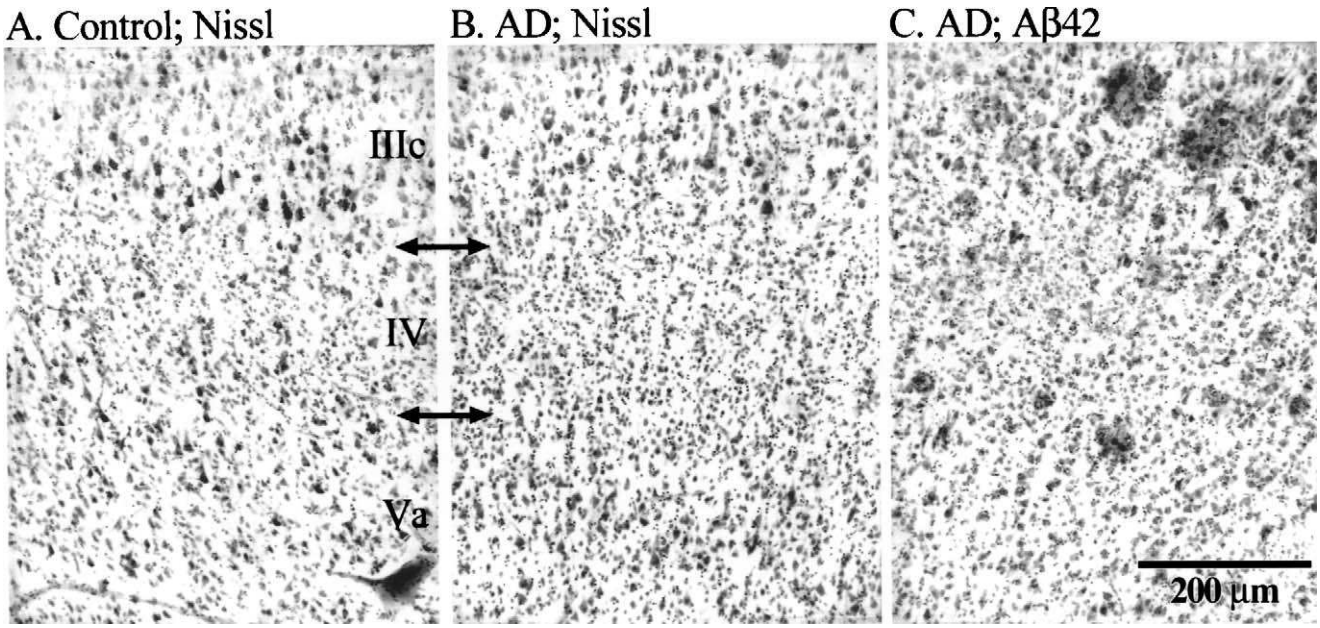

FIG. 10.6. Area 23a is compared in control and case YW. Changes is area 23a early in AD are only partially associated with large cortical neurons. In case YW, neuron losses and gliosis occur in both layers IV and Va and this laminar pattern is characteristic of NSTIV–V. Note that layer IIIc is much less impacted and that there were almost no NFT in any layer of this area (not shown). Interestingly, Aβ42 was deposited mainly in layer IIIc and, to a lesser extent, in layer Va. To the extent that Aβ42 might contribute to neuron death in the granular layer of area 29, this mechanism is not tenable in layer IV of area 23. Indeed, there is clear deposition of the peptide in layer IIIc, which is almost normal in terms of neuron densities.

phorylation of tau at Ser-202 (Goedert *et al*., 1993; DeLeys *et al*., 1996), and it has been reported that one of the earliest cytoskeletal changes in vulnerable neurons in AD might be hyperphosphorylation of neurofilaments in plaque-associated neurites (Su *et al*., 1996). In ectosplenial area 26, which is lateral to area 29, there are a large number of axons labeled with AT8. Although at a somewhat lesser density, there are also a large number of such fibers in area 29, including the granular layer as shown in Fig. 10.7. Because there are AT8-positive somata in the subiculum, it is possible that axons in the retrosplenial cortex derive from this area.

Immunohistochemistry for different length Aβ peptides shows an interesting association between deposition of Aβ42 and neuron loss in area 29. Most Aβ42 is in the granular layer where most neurons were lost (Fig. 10.5C). Although there was some loss of layer V neurons in area 29, there was almost no Aβ42 in this layer. In contrast, Aβ40 had a limited presence in the granular layer and was displaced to sites adjacent to the granular layer in layers I and V. The deposition of Aβ peptides was different in area 23 as shown in Fig. 10.6. In this instance, Aβ42 was not primarily in layer IV where maximal neurodegeneration and gliosis occurred, but rather in layer IIIc, which had a normal density of neurons, and in layer Va, where there was a clear loss of cells and even some gliosis. Interestingly, Aβ40 was rarely deposited in area 23 and had no preferential localization.

In conclusion, posterior cingulate and retrosplenial areas are damaged early in AD. Small neuron degeneration is prominent in the granular layer of area 29 and in layer IV of areas 30 and 23, yet it is not limited to layer IV. The clear involvement of large neurons in layer Va suggests that this case is an example of NSTIV–V. Furthermore, this short-duration case raises a number of important issues and questions. Neurodegeneration in the granular layer of area 29 is associated with an elevation of Aβ42 and no NFT. This is a pattern of laminar changes similar to those reported earlier for the presubiculum in

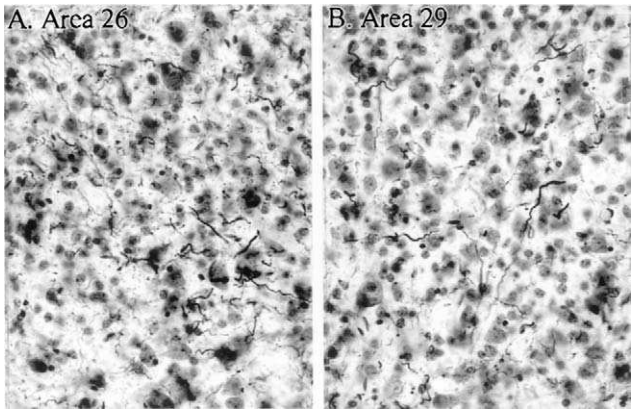

FIG. 10.7. AT8 immunoreactivity of axons in the granular layer of ectosplenial area 26 and retrosplenial area 29l. Although there were more immunoreative axons in area 26, those in area 29 are also quite dense. Because there were only rare NFT and neuritic plaques in each of these areas, it appears that the earliest phosphorylation event in this region is the cytoskeleton of axons.

another case. As suggested previously (Busciglio *et al*., 1995; Su *et al*., 1996; Tan *et al*., 1997), high levels of Aβ42 could induce early phosphorylation of tau, which may explain the AT8 immunoreactivity in area 29. Because there are no NFT in the granular layer and almost none in layer V, Aβ42 could induce cell death via protein nitration (discussed later) rather than tau phosphorylation. This direct action of the amyloid-β peptide could occur both at the somata of neurons in the granular layer and at the apical dendrites of large, layer V pyramids that pass through the granular layer.

Changes in area 23 suggest that either this and the retrosplenial cortices are involved at different time points or there may be different mechanisms of neuron death in these two areas. In layers IV–Va of area 23 there was very clear neuron death and gliosis, but no NFT, AT8-positive axons, or selective deposition of Aβ42 and Aβ40. Once again, it appears that protein nitration or some other form of free radical damage leads to neuron death in this area and that most early forms of neurodegeneration are not a consequence of tau phosphorylation. Finally, although the hypothesis that underlies staging strategies involves some form of ''spread'' of the disease, the differential involvement of the retrosplenial and posterior cingulate cortices cannot be easily understood in this framework.

XIII. Amyloid Peptides and Neurodegeneration

The case of YW suggests that, in some instances, amyloid peptides have a causal role in neurodegeneration. Although it is not possible to prove causal relationships in autopsy tissue, many findings support a preclinical and neurotoxic role for Aβ42 in AD. Autosomal-dominant mutations of the amyloid precursor protein and the presenilin genes elevate Aβ42 versus Aβ40 (Haass *et al*., 1994; Scheuner *et al*., 1996), and transgenic mice with similar mutations have elevated Aβ42 (Duff *et al*., 1996; Hsiao *et al*., 1996). Levels of Aβ42 in cerebrospinal fluid are associated with cognitive impairment in AD (Jensen *et al*., 1999) and distinguish between early- and late-onset cases, with the former having significantly lower levels (Andreasen *et al*., 1999). Because Aβ42 accumulates preclinically (Troncoso *et al*., 1998), it is a primary suspect for an early role in neurodegeneration. Finally, some transgenic systems that over-express Aβ42 have high levels of DNA damage, neuron loss, and gliosis in cortex (LaFerla *et al*., 1995).

The link between cell death and amyloid peptides was strengthened by Geula *et al*. (1998), who showed that a plaque-equivalent concentration of fibrillar, but not soluble, Aβ peptide induced microglial proliferation, tau phosphorylation, and neuronal loss in aged, but not young, rhesus monkeys. It is interesting to note that the tau phosphorylation extended beyond the area of neuron loss, raising further questions about the links between all neurodegeneration and neurofibrillary degeneration. The age dependency of this effect emphasizes the age risk factor for the role of Aβ peptides in neurodegeneration.

Concerns have been raised, however, regarding the Aβ mechanism of neuron death. Counts of SP do not correlate with neurodegeneration in entorhinal (Giannakopoulos *et al*., 1993; Gómez-Isla *et al*., 1996), superior temporal (Gómez-Isla *et al*., 1997), middle temporal (Neary *et al*., 1986), inferior temporal (Giannakopoulos *et al*., 1993), and posterior cingulate (Vogt *et al*., 1990, 1998) cortices. Biopsy studies did not show an increase in SP between the time of biopsy and death (Mann *et al*., 1988; Bennett *et al*., 1993). Finally, there does not appear to be a relationship between specific clinical symptoms, such as visual agnosia and the various apraxias, and SP densities (Giannakopoulos *et al*., 1998, 1999) nor with general measures of dementia (Neary *et al*., 1986; Mann *et al*., 1988; Bierer *et al*., 1995). Some of these studies employed methods that emphasized SP with β-pleated sheets of amyloid and structures with a high content of Aβ40, whereas the linkage to earlier events associated with Aβ42 is likely of more importance to neurodegeneration. To the extent that Aβ peptides are an early and triggering event, it need not be expected that SP correlate exactly with neurodegeneration or dementia later in the disease (Selkoe, 1997). Indeed, although Aβ42 appears in the cerebrospinal fluid of early and mild cases of AD, it declines with further disease progression (Jensen *et al*., 1999).

Although the densities of SP do not appear to correlate with neurodegeneration, there are instances in which the laminar load of Aβ42 is associated with neuron loss as in patient YW and patient FG, discussed later. Neurodegeneration in the context of laminar patterns that are not dependent on neurofibrillary mechanisms of cell death highlights the need to evaluate non-NFT pathways to neuron death. One of the problems with this approach is that there are very few compelling assessments of neurodegeneration with which to compare the laminar levels of these peptides. Realizing there need not be a 1:1 ratio of Aβ42 deposition and neurodegeneration, we present a case that has focal patterns of neurodegeneration and Aβ42 deposition and seek to characterize layers in the prefrontal, parietal, and posterior cingulate cortex where there are matches and mismatches between these markers as well as NFT.

XIV. Early Dysexecutive Syndrome and Frontotemporal Neurodegeneration

Cognitive signs of frontal lobe impairment occur early in AD. Tasks requiring concurrent manipulation of information (Lafleche and Albert, 1995) or shifting between stimulus dimensions (Sahakian *et al*., 1990) form an underlying impairment. These deficits in executive function precede language and spatial impairments in most patients (Grady *et al*., 1988; Albert and Moss, 1999) and emphasize the early and important role of prefrontal deficits in clinical symptoms.

Although most patients express some executive dysfunction, a subgroup of cases stand out as statistically unique and may have selective vulnerability of frontal cortex. A subgroup of AD patients have frontal glucose hypometabolism and a high degree of early behavioral deficits typical of frontal lobe damage. Grady *et al*. (1990) observed more inappropriate behaviors in cases with greatest prefrontal glucose hypometabolism. Mann *et al*. (1992) reported that two groups with equivalent parietal glucose hypometabolism and impaired neuropsychological measures of parietal function could be distin-

guished in terms of frontal lobe glucose metabolism, measures of executive functions, and rate of disease progression. Patients with the most rapid progression and impaired executive functions had greatest glucose hypometabolism in the prefrontal cortex. Finally, Royall *et al*. (1994) observed early impairment of executive control functions in AD that could be attributed to disrupted frontal lobe functions.

In light of the cognitive and metabolic heterogeneity of frontal lobe impairments, it is not surprising that frontal lobe pathology also is not uniform in AD. There is a wide range of neurodegeneration, with many cases having a normal density of neurons (Terry *et al*., 1981). Brun and Englund (1981) reported only modest losses of neurons in the prefrontal cortex. Furthermore, there is heterogeneity in NFT densities that are significantly different for early- vs late-onset cases (Hansen *et al*., 1988). In the context of subgroups/subtypes, the frontal cortex is a region that is differentially impacted by AD, and cases of definite AD, early behavioral deficits, and prominent frontotemporal atorphy are instructive.

Patient FG was an intelligent, right-handed man who was an electrical engineer. At the age of 85 he showed early signs of dysexecutive syndrome, including disinhibition and perseveration. Within a few years he had increased spatial disorientation and recent memory impairment. The details of this case and photographs of the focal frontotemporal atrophy at postmortem examination have been shown previously (Vogt *et al*., 1999). We also showed that this case had a prominent deposition of diffuse Aβ42 in prefrontal area 46, including highest deposits in layers II–IIIab. Although there were some NFT in superficial layers in areas 46 and 40, most were in layer V displaced from the diffuse deposits of Aβ42. Area 23 was of particular interest, not only because it is heavily interconnected with prefrontal cortex, but also because there were numerous NFT in layers II–IIIab and layer V showing a partial dissociation of Aβ42 and NFT. In layer V there was almost no Aβ42. Figure 10.8 revisits this issue in order to emphasize the relationship between heavy neurodegeneration in layers II–IIIab of prefrontal area 46. Note that there is a moderate amount of diffuse Aβ42 in layer IV, yet there is still significant neurodegeneration in this layer. Furthermore, there is almost no diffuse Aβ42 in layers V and VI, yet there is still substantial neurodegeneration in layer Va. A similar pattern appears in area 23, although at an overall lower density of diffuse Aβ42. Note also that the neurons in layer V of area 23 (thionin) are preserved more clearly than in layers II–III. Given that NFT in both areas are in both superficial and deep layers, the formation of NFT may be independent of Aβ42 deposition. Alternatively, NFT formation may be dependent on Aβ42 via the exposure of perisomatic regions to the neurotoxin in layers II–IV and the exposure of apical dendrites of layer V pyramids to the neurotoxin in layer III.

Case FG suggests that Aβ42 plays an important, although possibly nonexclusive, role in neurodegeneration. It is our impression that NFT density is related to Aβ42 deposition and that NFT do not fully account for all neurodegeneration. Substantial evidence supports the role of Aβ peptides in tau phosphorylation (Takashima *et al*., 1993; Busciglio *et al*., 1995; Le *et al*., 1997; Tan *et al*., 1997). Finally, it can be concluded from this case that focal frontotemporal atrophy in AD is due to severe neurodegeneration in layers II–IIIab and IV and, to a lesser extent, in deeper layers. To the extent that neurodegeneration in layers II–IIIab accounts for focal atrophy, as is true for Pick and other diseases that impact the frontal lobe, it is likely that the gross structural changes in this and similar cases are a consequence of early and sustained deposition of Aβ42.

XV. Free Radical Damage and Neurodegeneration in the Absence of NFT

Because NFT have a weak, and in some instances no, relationship to laminar patterns of neurodegeneration, other explanations for cell death need to be considered. This is particularly necessary in light of the significant layer IV cell death and the absence of NFT early in the disease observed in case YW. One explanation for the excess of cell death over that associated with tau phosphorylation is the direct neurotoxic actions of Aβ42 via free radical damage. Oxidative damage mediated by the nitration of proteins has been identified with immunohistochemistry for nitrotyrosine. Although nitrotyrosine occurs in NFT (Good *et al*., 1996; Smith *et al*., 1997), it is not restricted to neurons with NFT, and a number of Aβ protein-mediated mechanisms of free radical damage have been observed that do not involve NFT. These include the conjugation of lipid peroxidation products to the neuronal glucose transport protein (GLUT3) that impairs glucose transport (Mark *et al*., 1997), oxidation of RNA and DNA (Nunomura *et al*., 1999), and elevation of H_2O_2 and lipid peroxides in primary central nervous system cultures (Behl *et al*., 1994).

Having observed significant neurodegeneration in the absence of NFT in layer IV of the posterior cingulate cortex in case YW and in NSTIV–V, one wonders if this represents a wider extent of neurodegeneration in the cerebral cortex or whether this is unique to the posterior cingulate region? Su *et al*. (1997) evaluated the visual cortex in AD and found most intensely labeled neurons with nitrotyrosine in layers III and IV, the latter of which is composed of small neurons that do not usually form NFT. In addition, although many of the nitrotyrosine-labeled neurons had DNA damage, many did not. Thus, neurodegeneration outside of the context of NFT formation, including small neurons in layer IV, occurs in other posterior cortical regions, not just the cingulate cortex. The mechanisms of neuron death associated with the early deposition of Aβ42 and free radical damage may provide insight into the most common mechanisms of neurodegeneration in posterior cortical areas, including visual, cingulate, and parietal cortices.

XVI. Theories of Staging in the Context of Subgroups and Subtypes

To the extent that AD is viewed as a single entity, it is natural to stage its progression in a single continuum. An early effort at staging was made by Brun and Englund (1981), who placed their observations in the context of a graded progression in neurodegeneration and gliosis. More recently, the formal strategy of Braak and Braak (1991, 1997) provides a consistent nomenclature to evaluate the amount and distribu-

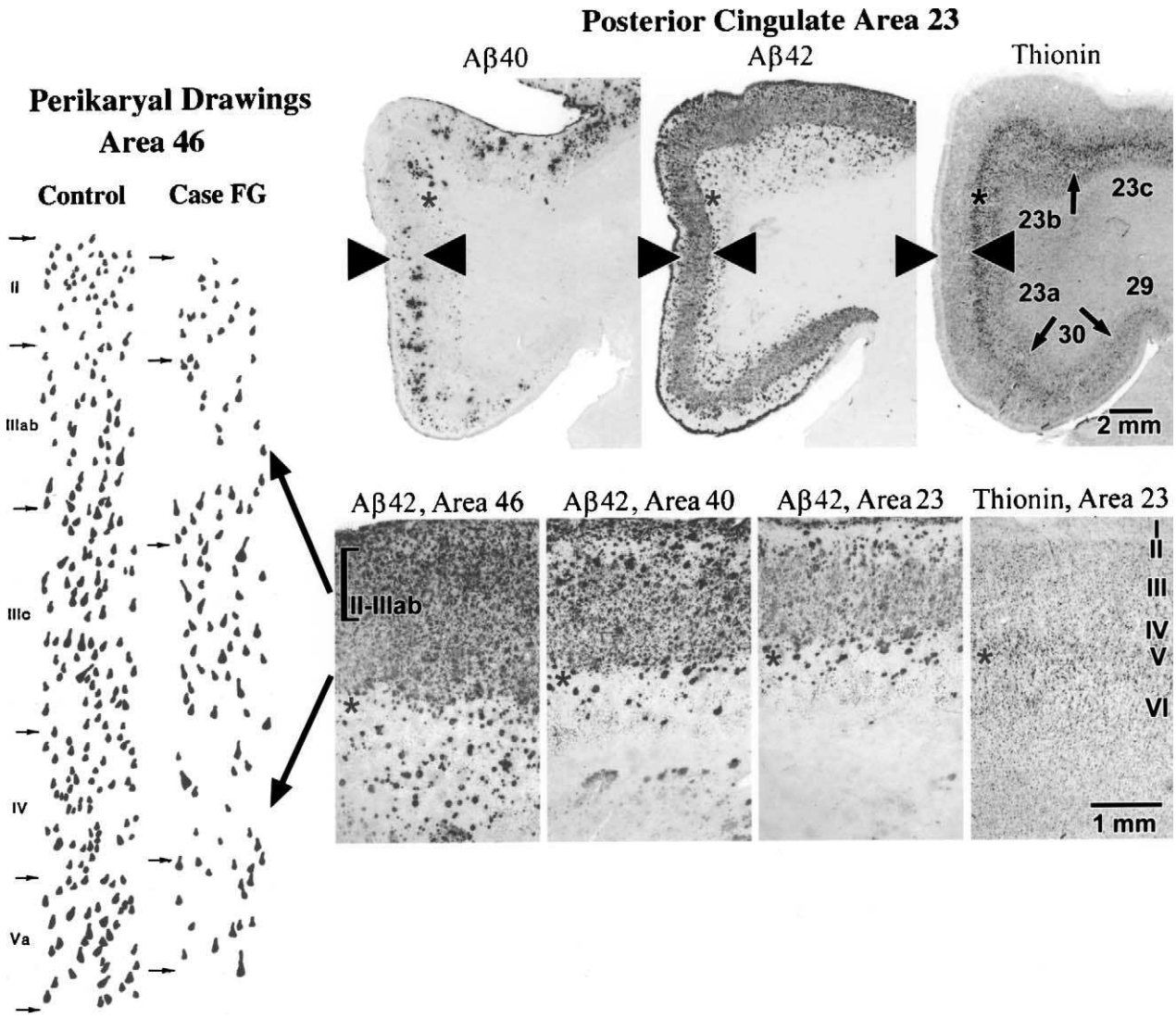

FIG. 10.8. An overview of some of the patterns of neurodegeneration in prefrontal area 46 and the posterior cingulate cortex in case FG; a case of early dysexecutive syndrome and focal frontotemporal atrophy. Comparison of perikaryal drawings with the deposition of Aβ42 shows a close relationship between both in layer IIIab, which is not the case for layer Va where there is substantial neurodegeneration but little Aβ42. Asterisks represent layer Va. Note that the greatest density of Aβ42 is in layers II–IIIab. Neurodegeneration, particularly in layers II–IIIab, may account to a large extent for the focal prefrontal atrophy in this case. In area 23, it can be seen that Aβ40 aggregates in the deep part of layer III and in layer IV, whereas Aβ42 is throughout layers II–IV. Neurodegeneration is particularly profound in layers II–IV, as shown with the thionin section between the large arrowheads. Note also in this section that layer Va is relatively spared. Once again, neurodegeneration in this case is closely associated with Aβ42 in the external layers but not in layer V.

tion of neurofibrillary and amyloid degeneration. There are instances of apparent agreement between Braak stage and a measure of the patient's disease. For example, Ohm *et al.* (1999) found that patients with one or two ApoE ϵ4 alleles were associated with a higher Braak stage for both neurofibrillary and amyloid changes. However, there is substantial variance in the data and overlap among measures of brain pathology that do not support a precise relationship. Because it is likely that the ApoE genotype does not account for most laminar patterns of neurodegeneration (Vogt *et al.*, 1998), apparent relationships between Braak stage and ApoE genotype may be misleading.

Gold *et al.* (2000) considered the Braak stages for patients over 90 years of age at the time of death in relation to CDR scores. They conclude, ''Braak staging represents a broad concept of the evolution of NFT rather than a precise hierarchical model associated with a stepwise deterioration of cognitive abilities near the upper limit of life.'' Although stages III and IV have an impact on cingulate cortex (Braak and Braak, 1991, 1997), the observations discussed earlier of a case with CDR 1 and previous work (Vogt *et al.*, 1998) show that neurodegeneration appears earlier than predicted by the Braak stages and that neurodegeneration is not closely related to neurofibrillary changes. It is unlikely, therefore, that a single series of

stages accounts for multiple laminar and topographical patterns of neurodegeneration. There are a number of reasons why staging strategies based on the hypothesis that AD is homogeneous do not work.

1. *Lack of a close relationship with disease severity–CDR*. The Braak stages explicitly attempt to relate stages of brain pathology to ''preclinical'' and clinical impairments, but studies that relate CDR scores with the Braak stage fail to demonstrate clear relationships. Perl *et al*. (1997) did not observe a close relationship between Braak stages and CDR scores for either cognitively normal individuals or AD cases with relatively mild cognitive impairments. As noted earlier, Gold *et al*. (2000) were unable to show anything more than a loose relationship between CDR scores, and NFT in cases over 90 years of age.

2. *Clinicopathological subgroups; multiple topographic lesion patterns cannot match a single pattern of "spreading" pathology.* Clinicopathological subgroups have different patterns of NFT in cases of posterior cortical atrophy and early impairments in visuomotor function and visual attention, early signs of associational visual agnosia, ideomotor and constructional apraxias, or early dysexecutive syndrome. The notion of a single ''spreading'' process arising in transentorhinal cortex and systematically penetrating neocortical areas is misleading. Some neocortical areas will be affected differentially and these clinicopathological subgroups cannot be explained by a staging scheme that requires one pathway to one end point. After all, there is no single pattern of NFT or SP deposition in neocortex.

3. *Lack of relationship between NFT and neurodegeneration*. It is well established that the loss of neurons is closely related to cognitive impairment. However, the logic of current staging theories is that neurofibrillary and amyloid changes can be used as a surrogate to mark the course of cell death, and no studies of NFT in the neocortex show anything more than a weak relationship between neurodegeneration and NFT. Thus, a single staging of NFT cannot be used to evaluate complex patterns of neurodegeneration in neocortex.

4. *Free radical damage to small, layer IV neurons*. Because there is extensive small neuron damage mediated by free radicals and not associated with NFT, staging strategies that rely on traditional markers overlook this type of cell death. To the extent that layer IV neurodegeneration occurs early in the disease, early ''stages'' such as Braak stages I and II will diagnose the primary lesions incorrectly. To the extent that defined staging strategies are sought, it may be necessary to describe the progression of neuron death with markers of free radical damage such as nitrotyrosine immunohistochemistry.

5. *Neurodegeneration in early- and late-onset disease*. Early-onset cases often have the most severe neurodegeneration (Vogt *et al*., 1998). Even when cases are matched for disease duration, early-onset disease is associated with more extensive neurodegeneration than late-onset disease. Thus, staging of cases into a single progression cannot accommodate overall differences in neurodegeneration. Indeed, different risk factors for early-onset cases, such as the ApoE $\epsilon 4$ risk, will not fit into a single pattern of neurodegeneration to the extent that it is related to early onset.

6. *Neuropathological subtypes: the ultimate test for staging strategies*. We provide two arguments for the possibility of subtypes in AD: spastic paraparesis early in the disease and its associated lesions and presenilin 1 mutation and the presence of laminar patterns of neurodegeneration in the posterior cingulate cortex that imply different mechanisms of cell death. If Jorm criteria for AD subtypes are eventually met, it will not be possible to stage the disease according to a single continuum. Rather, it will be necessary to first diagnose each subtype and then apply different criteria for progression within a subtype.

Even in the absence of proof for subtypes *per se,* the single continuum staging strategy fails to explain differential clinical outcomes associated with regional differences in cortical function and it has not been applied to patterns of neurodegeneration outside of NFT and SP. An alternate approach is to diagnose the disease according to standard practice and then devise a multigroup, staging strategy for assessing neurodegeneration directly rather than with surrogate markers. By multigroup, we mean providing stages for qualitatively different topographical and laminar patterns of neurodegeneration and different patterns of cognitive impairment. Needless to say, devising such a multigroup strategy for staging the disease will require a more thorough description of clinicopathological subgroups/subtypes with multivariate models.

XVII. The Model Matters

If first symptoms and disease progression were the same for all patients, the goal of achieving a simple staging of neuropathological variables would be valid. It would also be reasonable to expect that least-square models of neurodegeneration in one or more cortical areas would follow disease progression and provide explanations of progressive cognitive impairments. The well-documented neuropsychological and glucose metabolic subgroups based on multivariate models assure that this expectation cannot be fulfilled. Moreover, observations that there are systematic topographical differences in the expression of disease markers in association with specific cognitive deficits further assures that the simple staging strategy does not describe disease progression adequately.

In the context of clinical and neuropathological heterogeneity, linear models of changes associated with entire populations of AD patients are not justified. Although staging the disease into a single continuum may be useful for characterizing the general level of pathology, there is no *a priori* rationale for using such descriptive observations for assessing disease progression and etiology. Some would argue that it does not matter whether a linear model is used because it is a common theme in science to reduce paired variables to this simple format. Indeed it is; however, we have shown that such efforts do not support data. Rank ordering of critical variables and other efforts are used frequently to avoid the conclusion that many relationships that should hold actually do not.

Importantly, heterogeneity in cognitive impairments and cortical pathology of AD require multivariate models to characterize it. Neuron, NFT, and SP densities do not fit a simple linear model for the entire population and cannot be used as the sole analytical tool for statistical analysis. Once pure cog-

nitive and pathological subgroups have been identified, it will be possible to use least-square models to assess linear changes associated with disease progression within each subgroup.

The more difficult issue, of course, is whether statistical subgroups represent subtypes of the disease. Jorm (1985) appropriately set the bar high on this question. If there are two or more subtypes, they must differ in terms of clinical outcomes and progression, patterns of lesions in the brain, and etiology. Problems attaining these goals are obvious. The etiology(ies) of AD is/are not known. Because it has been only recently that the presence of clinical and neuropathological subgroups has been demonstrated, no studies exist that seek to address the possible differences in etiology. This does not mean, however, that there are not qualitatively different mechanisms of neurodegeneration. Although NFT and SP are the primary measures for diagnosis, the focus of mechanistic studies should be on the complete pattern of neurodegeneration early in the disease without reference to late-stage cases.

References

Albert, M. S., and Moss, M. B. (1999). Early features of Alzheimer's disease. *Cereb. Cortex* **14**, 461–474.

Albert, M. S., Duffy, F. H., and McAnulty, G. B. (1990). Electrophysiologic comparisons between two groups of patients with Alzheimer's disease. *Arch. Neurol. (Chicago)* **47**, 857–863.

Andreasen, N., Hesse, C., Davidson, P., Minthon, L., Wallin, A., Winblad, B., Vanderstichele, H., Vanmwchelen, E., and Blennow, K. (1999). Cerebrospinal fluid β-amyloid(1–42) in Alzheimer disease. *Arch. Neurol. (Chicago)* **56**, 673–680.

Armstrong, R. A., Wood, L., Myers, D., and Smith, C. U. M. (1996). The use of multivariate methods in the identification of subtypes of Alzheimer's disease: A comparison of principal components and cluster analysis. *Dementia* **7**, 215–220.

Arnold, S. E., Hyman, B. T., Flory, J., Damasio, A. R., and Van Hoesen, G. W. (1991). The topographical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer's disease. *Cereb. Cortex* **1**, 103–116.

Asada, T., Yamagata, Z., Kinoshita, T., Kinoshita, A., Kariya, T., Asaka, A., and Kakuma, T. (1996). Prevalence of dementia and distribution of APOE alleles in Japanese centenarians: An almost-complete survey in Yamanashi prefecture, Japan. *J. Am. Geriatr. Soc*. **44**, 151–155.

Baleydier, C., and Mauguière, F. (1980). The duality of the cingulate gyrus in monkey. Neuroanatomical study and functional hypotheses. *Brain* **103**, 525–554.

Becker, J. T., Huff, F. J., Nebes, R. D., Holland, A., and Boller, F. (1988). Neuropsychological function in Alzheimer's disease: Pattern of impairment and rate of progression. *Arch. Neurol. (Chicago)* **45**, 263–268.

Behl, C., Davis, J. B., Lesley, R., and Schubert, D. (1994). Hydrogen peroxide mediates amyloid β protein toxicity. *Cell (Cambridge, Mass.)* **77**, 817–827.

Bennett, D. A., Cochran, E. J., Saper, C. B., Leverenz, J. B., Gilley, D. W., and Wilson, R. S. (1993). Pathological changes in frontal cortex from biopsy and autopsy in Alzheimer's disease. *Neurobiol. Aging* **14**, 589–596.

Berg, L., McKeel, D. W., Miller, Jr., J. P., Storandt, M., Rubin, E. H., Morris, J. C., Baty, J., Coats, M., Norton, J., Goate, A. M., Price, J. L., Gearing, M., Mirra, S. S., and Saunders, A. M. (1998). Clinicopathologic studies in cognitively healthy aging and Alzheimer disease. *Ann. Neurol*. **55**, 326–335.

Bierer, L. M., Hof, P. R., Purohit, D. P., Carlin, L., Schmeidler, J., Davis, K. L., and Perl, D. P. (1995). Neocortical neurofibrillary tangles correlate with dementia severity in Alzheimer's disease. *Arch. Neurol. (Chicago)* **52**, 81–88.

Bondareff, W., Mountjoy, C. Q., Wischik, C. M., Hauser, D. L., LaBree, L. D., and Roth, M. (1993). Evidence of subtypes of Alzheimer's disease and implications for etiology. *Arch. Gen. Psychiatry* **50**, 350–356.

Braak, H. (1979). Pigment architecture of the human telencephalic cortex. IV. *Regio retrosplenialis. Cell Tissue Res*. **204**, 431–440.

Braak, H., and Braak, E. (1991). Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol*. **82**, 239–259.

Braak, H., and Braak, E. (1993). Alzheimer neuropathology and limbic circuits. *In* "Neurobiology of Cingulate Cortex and Limbgic Thalamus" (B. A. Vogt and M. Gabriel, eds.), pp. 606–6626. Birkhäuser, Boston.

Braak, H., and Braak, E. (1997). Frequency of stages of Alzheimer-related lesions in different age categories. *Neurobiol. Aging* **18**, 351–357.

Brun, A., and Englund, E. (1981). Regional pattern of degeneration in Alzheimer's disease: Neuronal loss and histopathological grading. *Histopathology* **5**, 549–564.

Buée-Scherrer, V., Condamines, O., Mourton-Gilles, C., Jakes, R., Goedert, M., Pau, B., and Delacourte, A. (1996). AD2, a phosphorylation-dependent monoclonal antibody directed against tau proteins found in Alzheimer's disease. *Mol. Brain Res*. **39**, 79–88.

Busciglio, J., Lorenzo, A., Yeh, J., and Yankner, B. A. (1995). β-amyloid fibrils induce tau phosphorylation and loss of microtubule binding. *Neuron* **14**, 879–888.

Crook, R., Verkkoniemi, A., Perez-Tur, J., Mehita, N., Baker, M., Houlden, H., Farrer, M., Hutton, M., Lincoln, S., Hardy, J., Gwinn, K., Somer, M., Paetau, A., Kalimo, H., Ylikoski, R., Poyhonen, M., Kucera, S., and Haltia, M. (1998). A variant of Alzheimer's disease with spastic paraparesis and unusual plaques due to deletion of exon 9 of presenilin 1. *Nat. Med.* **4**, 452–456.

DeLeys, R., Henderickx, G., Dekeyser, F., Demol, H., Raymackers, J., Brasseur, R., Borremans, F., Vanmechelen, E., and Van de Voorde, A. (1996). Mapping and sequence requirements of the phosphorylation-sensitive epitopes recognized by the monoclonal antibodies Tau1, BT2, and AT8. *In* "Peptides in Immunology" (C. H. Schneider, ed.), pp. 239–244. Wiley, New York.

Duff, K., Eckman, C., Zehr, C., Yu, X., Prada, C.-M., Perez-Tur, J., Hutton, M., Buee, L., Harigaya, Y., Yager, D., Morgan, D., Gordon, M. N., Holcomb, L., Refolo, L., Zenk, B., Hardy, J., and Younkin, S. (1996). Increased amyloid-β42(43) in brains of mice expressing mutant presenilin 1. *Nature (London)* **383**, 710–713.

Fisher, N. J., Rourke, B. P., Bieliauskas, L., Giordani, B., Berent, S., and Foster, N. L. (1996). Neuropsychological subgroups of patients with Alzheimer's disease. *J. Clin. Exp. Neuropsychol*. **18**, 349–370.

Foster, N. L., Chase, T. N., Fedio, P., Petronsas, N. J., Brooks, R. A., and DiChiro, G. (1983). Alzheimer's disease: Focal cortical changes shown by positron emission tomography. *Neurology* **33**, 961–965.

Geula, C., Wu, C.-K., Saroff, D., Lorenzo, A., Yuan, M., and Yankner, B. A. (1998). Aging renders the brain vulnerable to amyloid β-protein neurotoxicity. *Nat. Med*. **4**, 827–831.

Giannakopoulos, P., Hof, P. R., Surini, M., Michel, J. P., and Bouras, C. (1993). Quantitative immunohistochemical analysis of the distribution of neurofibrillary tangles and senile plaques in the cerebral cortex of nonagenarians and centagenarians. *Acta Neuropathol*. **85**, 602–610.

Giannakopoulos, P., Hof, P. R., Kövari, E., Vallet, P. G., Herrmann, F. R., and Bouras, C. (1996). Distinct patterns of neuronal loss and Alzheimer's disease lesion distribution in elderly individuals older than 90 years. *J. Neuropathol. Exp. Neurol*. **55**, 1210–1220.

Giannakopoulos, P., Duc, M., Gold, G., Hof, P. R., Michel, J.-P., and Bouras, C. (1998). Pathologic correlates of apraxia in Alzheimer disease. *Arch. Neurol. (Chicago)* **55**, 689–695.

Giannakopoulos, P., Gold, G., Duc, M., Michel, J.-P., Hof, P. R., and Bouras, C. (1999). Neuroanatomic correlates of visual agnosia in Alzheimer's disease. *Neurology* **52**, 71–77.

Goedert, M., Jakes, R., Croether, R. A., Six, J., Lubke, U., Vandermeeken, M., Cras, P., Trojanowski, J. Q., and Lee, V. M.-Y. (1993). The

abnormal phosphorylation of tau protein at serine 202 in Alzheimer's disease recapitulates phosphorylation during development. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 5066–5070.

Gold, G., Bouras, C., Kövari, E., Canuto, A., Glara, G., Malky, A., Hof, P. R., Michel, J.-P., and Giannakopoulos, P. (2000). Clinical validity of Braak neuropathological staging in the oldest-old. *Acta Neuropathol.* (in press).

Gómez-Isla, T., Price, J. L., McKeel, D. W., Jr., Morris, J. C., Growden, J. H., and Hyman, B. T. (1996). Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *J. Neurosci.* **16**, 4491–4500.

Gómez-Isla, T., Hollister, R., West, H., Mui, S., Growdon, J. H., Peterson, R. C., Parisi, J. E., and Hyman, B. T. (1997). Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. *Ann. Neurol.* **41**, 17–24.

Good, P. F., Werner, P., Hsu, A., Olanow, C. W., and Perl, D. P. (1996). Evidence for neuronal oxidative damage in Alzheimer's disease. *Am. J. Pathol.* **149**, 21–28.

Grady, C. L., Haxby, J. V., Howitz, B., Sundaram, M., Berg, G., Schapiro, M., Friedland, R. P., and Rapoport, S. I. (1988). Longitudinal study of the early neuropsychological and cerebral metabolic changes in dementia of the Alzheimer Type. *J. Clin. Exp. Neuropsychol.* **10**, 576–596.

Grady, C. L., Haxby, J. V., Schapiro, M. B., Kumar, A., Ball, M. J., Heston, L., and Rapoport, S. I. (1990). Subgroups in dementia of the Alzheimer type identified using positron emission tomography. *J. Neuropsychiatry* **2**, 373–384.

Grünthal, E. (1928). Zür Hirnpathologischen Analyse der Alzheimerschen Krankheit. *Psychiatr.-Neurol. Wochenschr.* **36**, 401–407.

Haass, C. Hung, A., Selkoe, D., and Teplow, D. (1994). Mutations associated with a locus of familial Alzheimer's disease result in alternative processing of amyloid β-protein precursor. *J. Biol. Chem.* **269**, 17741–17748.

Hansen, L. A., DeTeresa, R., Davies, P., Terry, R. D. (1988). Neocortical morphometry, lesion counts, and choline acetyltransferase levels in the age spectrum of Alzheimer's disease. *Ann. Med.* **28**, 255–258.

Haroutunian, V., Purohit, D. P., Perl, D. P., Martin, D., Khan, H., Lantz, M., Davis, K. L., and Mohs, R. C. (1999). Neurofibrillary tangles in nondemented elderly subjects and mild Alzheimer's disease. *Arch. Neurol. (Chicago)* **56**, 713–718.

Haxby, J. V., Duara, R., Grady, C. L., Culter, N. R., and Rapoport, S. I. (1985). Relations between neuropsychological and cerebral metabolic asymmetries in early Alzheimer's disease. *J. Cereb. Blood Flow Metab.* **5**, 193–200.

Hirono, N., Mori, E., Ishii, K., Ikejiri, Y., Imamura, T., Shimomura, T., Hashimoto, M., Yamashita, H., and Sasaki, M. (1998). Hypofunction in the posterior cingulate gyrus correlates with disorientation for time and place in Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* **64**, 552–554.

Hof, P. R., Vogt, B. A., Bouras, C., and Morrison, J. H. (1997). Atypical form of Alzheimer's disease with prominent posterior cortical atrophy: A review of lesion distribution and circuit disconnection in cortical visual pathways. *Vision Res.* **37**, 3609–3625.

Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F., and Cole, G. (1996). Correlative memory deficits, Aβ elevation and amyloid plaques in transgenic mice. *Science* **274**, 99– 102.

Insausti, R., Amaral, D. G., and Cowan, W. M. (1987). The entorhinal cortex of the monkey. II. Cortical afferents. *J. Comp. Neurol.* **264**, 356–395.

Jensen, M., Schroder, J., Blomberg, M., Engvall, B., Pantel, J., Ida, N., Basun, H., Wahlund, L. O., Werle, E., Jauss, M., Beyreuther, K., Lannfelt, L., and Hartmann, T. (1999). Cerebrospinal fluid Aβ42 is increased early in sporadic Alzheimer's disease and declines with disease progression. *Ann. Neurol.* **45**, 504–511.

Jorm, A. F. (1985). Subtypes of Alzheimer's dementia: A conceptual analysis and critical review. *Psychol. Med.* **15**, 543–553.

Kanne, S. M., Balota, D. A., Storandt, M., McKeel, D. W., and Morris, J. C. (1998). Relating anatomy to function in Alzheimer's disease. *Neurology* **50**, 979–985.

Kleinbaum, D. G., Kupper, L. L., and Muller, K. E. (1988). ''Applied Regression Analysis and Other Multivariate Methods,'' pp. 181–208. PWS-Kent, Boston.

LaFerla, F. M., Tinkle, B. T., Bieberich, C. J., Haudenschild, C. C., and Jay, G. (1995). The Alzheimer's Aβ peptide induces neurodegeneration and apoptotic cell death in transgenic mice. *Nat. Genet.* **9**, 21–29.

Lafleche, G., and Albert, M. S. (1995). Executive function deficits in mild Alzheimer's disease. *Neuropsychology* **9**, 313–320.

Le, W., Xie, W. J., Kong, R., and Appel, S. H. (1997). β-amyloid-induced neurotoxicity of a hybrid septal cell line associated with increased tau phosphorylation and expression of β-amyloid precursor protein. *J. Neurochem.* **69**, 978–985.

Mann, D. M. A., Marchniuk, B., Yates, P. O., Neary, D., and Snowden, J. S. (1988). The progression of the pathological changes of Alzheimer's disease in frontal and temporal neocortex examined both at biopsy and at autopsy. *Neuropathol. Appl. Neurobiol.* **14**, 177–195.

Mann, U., Mohr, E., Gearing, M., and Chase, T. N. (1992). Heterogeneity in Alzheimer's disease: progression rate segregated by distinct neuropsychological and cerebral metabolic profiles. *J. Neurol., Neurosurg. Psychiatry* **55**, 956–959.

Mark, R. J., Pang, Z., Geddes, J. W., Uchida, K., and Mattson, M. P. (1997). Amyloid β-peptide impairs glucose transport in hippocampal and cortical neurons: Involvement of membrane lipid metabolism. *J. Neurosci.* **17**, 1046–1054.

Martin, A. (1987). Representation of semantic and spatial knowledge in Alzheimer's patients: Implications for models of preserved learning and amnesia. *J. Clin. Exp. Neuropsychol.* **9**, 191–224.

Martin, A. (1990). Neuropsychology of Alzheimer's disease: The case for subgroups. *In* ''Modular Deficits in Alzheimer-type Dementia'' (M. F. Schwartz, ed.), pp. 145–175. MIT Press, Cambridge, MA.

Martin, A., Brouwers, P., Lalonde, F., Cox, C., Teleska, P., Fedio, P., Foster, N. L., and Chase, T. N. (1986). Towards a behavioral typology of Alzheimer's patients. *J. Clin. Exp. Neuropsychol.* **8**, 594–610.

Matsunami, K. I., Kawashima, T., and Satake, H. (1989). Mode of [^{14}C]2-deoxy-D-glucose uptake into retrosplenial cortex and other memory-related structures of the monkey during a delayed response. *Brain Res. Bull.* **22**, 829–838.

McKee, A. C., Kosik, K. S., and Kowall, N. W. (1991). Neuritic pathology and dementia in Alzheimer's disease. *Ann. Neurol.* **30**, 156–165.

Minoshima, S., Giordani, B., Berent, S., Frey, K. A., Foster, N. L., and Kuhl, D. E. (1997). Metabolic reduction in the posterior cirgulate cortex in very early Alzheimer's disease. *Ann. Neurol.* **42**, 85–94.

Mountjoy, C. Q., Roth, M., Evans, N. J. R., and Evans, H. M. (1983). Cortical neuronal counts in normal elderly and demented patients. *Neurobiol. Aging* **4**, 1–11.

Murray, E. A., Davidson, M., Gaffan, D., Olton, D. S., and Suomi, S. (1989). Effects of fornix transection and cingulate cortical ablation on spatial memory in rhesus monkeys. *Exp. Brain Res.* **74**, 173–186.

Nagy, Z., Esiri, M. M., Jobst, K. A., Morris, J. H., King, E. M.-F., McDonald, B., Litchfield, S., Smith, A., Barnetson, L., and Smith, A. D. (1995). Relative roles of plaques and tangles in the dementia of Alzheimer's disease: Correlations using three sets of neuropathological criteria. *Dementia* **6**, 21–31.

Neary, D., Snowden, J. S., Mann, D. M. A., Bowen, D. M., Sims, N. R., Northen, B., Yates, P. O., and Davison, A. N. (1986). Alzheimer's disease: A correlative study. *J. Neurol. Neurosurg. Psychiatry* **49**, 229–237.

Nunomura, A., Perry, G., Pappolla, M. A., Wade, R., Hirai, K., Chiba, S., and Smith, M. A. (1999). RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease. *J. Neurosci.* **19**, 1959–1964.

Nybäck, H., Nyman, H., Blomqvist, G., Sjögren, I., and Stone-Elander, S. (1991). Brain metabolism in Alzheimer's dementia: Studies of ^{11}C-

deoxyglucose accumulation, CSF monoamine metabolites and neuropsychological test performance in patients and healthy subjects. *J. Neurol. Neurosurg. Psychiatry* **54**, 672–678.

Ohm, T. G., Scharnagl, H., März, W., and Bohl, J. (1999). Apolipoprotein E isoforms and the development of low and high Braak stages of Alzheimer's disease-related lesions. *Acta Neuropathol.* **98**, 273–280.

Olson, C. R., Musil, S. Y., and Goldberg, M. E. (1993). Posterior cingulate cortex and visuospatial cognition: Properties of single neurons in the behaving monkey. *In* "Neurobiology of Cingulate Cortex and Limbic Thalamus" (B. A. Vogt and M. Gabriel, eds.), pp. 366–380. Birkhäuser, Boston.

Olson, C. R., Musil, S. Y., and Goldberg, M. E. (1996). Single neurons in posterior cingulate cortex in behaving macaque: Eye movement signals. *J. Neurophysiol.* **76**, 3285–3300.

Pandya, D. N., Van Hoesen, G. W., and Mesulam, M.-M. (1981). Efferent connections of the cingulate gyrus in the rhesus monkey. *Exp. Brain Res.* **42**, 319–330.

Perl, D. P., Purohit, D. P., Haroutunian, V. (1997). Clinicopathologic correlations of the Alzheimer's disease staging system introduced by Braak and Braak. *J. Neuropthol. Exper. Neurol.* **56**, 577.

Rapoport, S. I. (1991). Positron emission tomography in Alzheimer's disease pathogenesis: A critical review. *Cerebrovasc. Brain Metab. Rev.* **3**, 297–335.

Royall, D. R., Mahurin, R. K., and Cornell, J. (1994). Bedside assessment of frontal degeneration: Distinguishing Alzheimer's disease from non-Alzheimer's cortical dementia. *Exp. Aging Res.* **20**, 95–103.

Sahakian, B. J., Downes, J. J., Eagger, S., Evenden, J. L., Levy, R., Philpot, M. P., Roberts, A. C., and Robbins, T. W. (1990). Sparing of attentional relative to mnemonic function in a subgroup of patients with dementia of the Alzheimer type. *Neuropsychologia* **28**, 1197–1213.

Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T. D., Hardy, J., Hutton, M., Kukull, W., Larson, E., Levy-Lahad, E., Viitanen, M., Peskind, E., Poorkaj, P., Schellenberg, G., Tanzi, R., Wasco, W., Lannfelt, L., Selkoe, D., and Younkin, S. (1996). Secreted amyloid β-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and *APP* mutations linked to familial Alzheimer's disease. *Nat. Med.* **2**, 864–870.

Selkoe, D. J. (1997). Alzheimer's disease: Genotypes, phenotype, and treatment. *Science* **275**, 630–631.

Sheng, J. G., Mrak, R. E., and Griffin, W. S. T. (1998). Progressive neuronal DNA damage associated with neurofibrillary tangle formation in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* **57**, 323– 328.

Šimić, G., Kostović, I., Winblad, B., and Bogdanović, N. (1997). Volume and number of neurons of the human hippocampal formation in normal aging and Alzheimer's disease. *J. Comp. Neurol.* **379**, 482–494.

Smith, M. A., Harris, P. L. R., Sayre, L. M., Beckman, J. S., and Perry, G. (1997). Widespread peroxynitrite-mediated damage in Alzheimer's disease. *J. Neurosci.* **17**, 2653–2657.

Sobel, E., Louhija, J., Sulkava, R., Davanipour, Z., Kontula, K., Miettinen, H., Tikkanen, M., Kaiulainen, K., and Tilvis, R. (1995). Lack of association of apolipoprotein E allele epsilon 4 with late-onset Alzheimer's disease among Finnish centenarians. *Neurology* **45**, 903–907.

Stevens, J. P. (1984). Outliers and influential data points in regression analysis. *Psychol. Bull.* **95**, 334–344.

Su, J. H., Anderson, A. J., Cummings, B. J., and Cotman, C. W. (1994). Immunohistochemical evidence for apoptosis in Alzheimer's disease. *NeuroReport* **5**, 2529–2533.

Su, J. H., Cummings, B. J., and Cotman, C. W. (1996). Plaque biogenesis in brain aging and Alzheimer's disease. I. Progressive changes in phosphorylation states of paired helical filaments and neurofilaments. *Brain Res.* **739**, 79–87.

Su, J. H., Deng, G., and Cotman, C. W. (1997). Neuronal DNA damage precedes tangle formation and is associated with up-regulation of nitrotyrosine in Alzheimer's disease brain. *Brain Res.* **774**, 193–199.

Takashima, A., Noguchi, K., Sato, K., Hoshino, T., and Imahori, K. (1993). Tau protein kinase I is essential for amyloid β-protein-induced neurotoxicity. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 7789–7793.

Tan, S., Maher, P., and Schubert, D. (1997). The role of protein phosphorylation in beta amyloid toxicity. *Brain Res.* **765**, 159–163.

Terry, R. D., Peck, A., DeTeresa, R., Schechter, R., and Horoupian, D. S. (1981). Some morphometric aspects of the brain in senile dementia of the Alzheimer type. *Ann. Neurol.* **10**, 184–192.

Troncoso, J. C., Cataldo, A. M., Nixon, R. A., Barett, J. L., Lee, M. K., Checkler, F., Fowler, D. R., Smialek, J. E., Crain, B., Martin, L. J., and Kawas, C. H. (1998). Neuropathology of preclinical and clinical late-onset Alzheimer's disease. *Ann. Neurol.* **43**, 673–676.

Valdois, S., Joannette, Y., Poissant, A., Ska, B., and Dehaut, F. (1990). Heterogeneity in the cognitive profile of normal elderly. *J. Clin. Exp. Neuropsychol.* **12**, 587–596.

Vogt, B. A. (1976). Retrosplenial cortex in the rhesus monkey: A cytoarchitectonic and Golgi study. *J. Comp. Neurol.* **169**, 63–98.

Vogt, B. A., and Pandya, D. N. (1987). Cingulate cortex in the rhesus monkey. II. Cortical afferents. *J. Comp. Neurol.* **262**, 271–289.

Vogt, B. A., Rosene, D. L., and Pandya, D. N. (1979). Thalamic and cortical afferents differentiate anterior from posterior cingulate cortex in the monkey. *Science* **204**, 205–207.

Vogt, B. A., Van Hoesen, G. W., and Vogt, L. J. (1990). Laminar distribution of neuron degeneration in posterior cingulate cortex in Alzheimer's disease. *Acta Neuropathol.* **80**, 581–589.

Vogt, B. A., Nimchinsky, E. A., Vogt, L. J., and Hof, P. R. (1995). Human cingulate cortex: Surface features, flat maps, and cytoarchitecture. *J. Comp. Neurol.* **359**, 490–506.

Vogt, B. A., Vogt, L. J., Nimchinsky, E. A., and Hof, P. R. (1997). Primate cingulate cortex chemoarchitecture and its disruption in Alzheimer's disease. *In* "Handbook of Chemical Neruoanatomy" (F. E. Bloom, A. Björklund, and T. Hökfelt, eds.), Vol. 13, Part I, pp. 455–528. Elsevier, Amsterdam.

Vogt, B. A., Vogt, L. J., Vrana, K. E., Meadows, R. S., Challa, V. R., Hof, P. R., and Van Hoesen, G. W. (1998). Multivariate analysis of laminar patterns of neurodegeneration in posterior cingulate cortex in Alzheimer's disease. *Exp. Neurol.* **153**, 8–22.

Vogt, B. A., Martin, A., Vrana, K. E., Absher, J. R., Vogt, L. J., and Hof, P. R. (1999). Multifocal cortical neurodegeneration in Alzheimer's disease. *In* "Cerebral Cortex" (A. Peters and J. H. Morrison, eds.), Vol. 14, pp. 553–601. Kluwer-Plenum, New York.

West, M. J. (1993). Regionally specific loss of neurons in the aging human hippocampus. *Neurobiol. Aging* **14**, 287–293.

West, M. J., Coleman, P. D., Flood, D. G., and Troncoso, J. C. (1994). Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. *Lancet* **344**, 769–772.

Zilles, K., Armstrong, E., Schlaug, G., and Schleicher, A. (1986). Quantitative cytoarchitectonics of the posterior cingulate cortex in primates. *J. Comp. Neurol.* **253**, 514–524.

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11

Vascular Dementia

Atherosclerotic dementia was described in the late 19th century by Binswanger (1894) and Alzheimer (1895) at a time when syphilis was a leading cause of dementia and mental illness. For most of the 20th century, senile dementia was thought to result from decreased cerebral perfusion due to vascular disease. However, in the mid-1970s, Alzheimer's disease (AD) became recognized as the main cause of dementia, and interest in dementia of vascular origin waned. Since then, the concept has broadened and newer criteria have been proposed that have taken advantage of evolving neuroimaging techniques. At the dawn of the 21st century, vascular dementia (VaD) remains an important cause of dementia and a diagnostic challenge (Bowler and Hachinski, 1996; Desmond, 1996; Gold *et al*., 1999; Chiu *et al*., 2000). Although preventive strategies are likely to be highly beneficial, effective therapeutic interventions have yet to developed (Hachinski, 1992).

I. Dementia of Vascular Origin: An Evolving Concept

Alzheimer and Binswanger believed that VaD was secondary to chronic ischemia (del Ser *et al*., 1990). They described an arterisoclerotic brain degeneration that could be distinguished easily from syphilitic disease, as it was a focal as opposed to a diffuse process. They also reported other forms of the disease, including a chronic diffuse subcortical encephalitis where subcortical fiber loss was secondary to arteriosclerosis and senile cortical atrophy also caused by arteriosclerosis (Binswanger, 1894; Alzheimer, 1895, 1898; Mast *et al*., 1995). This clinicopathological spectrum has been felt to include multiple lacunar strokes, granular atrophy, and what is currently termed Binswanger's disease (Roman, 1999). Tomlinson *et al*. (1968) believed that loss of brain tissue secondary to stroke was the underlying pathophysiological mechanism behind dementia of vascular origin and suggested that cognitive symptoms were related to the volume of affected cerebral matter. Cerebral softening of 50 ml or more occurred in one-third of demented individuals, but only rarely in nondemented individuals; cerebral softening greater than 100 ml was only encountered in demented individuals tissue (Tomlinson *et al*., 1968). However, more recent clinicopathological studies have shown a significant overlap between affected volumes in demented and nondemented elderly (Erkinjunti *et al*., 1988; del Ser *et al*., 1990). Hachinski proposed the term multi-infarct dementia to describe dementia resulting from multiple strokes of thromboembolic origin (Hachinski *et al*., 1974). The number and location of strokes were felt to be more important than the actual volume of damaged tissue. More recently, the concept has evolved to include multiple physiopathological mechanisms related to deficiencies in cerebral blood supply and the various types of brain pathology encountered in cases of VaD. These include multiple infarcts, a single strategic infarct, small vessel disease, hypoperfusion, and hemorrhage (Roman *et al*., 1993; Desmond, 1996; Olsson *et al*., 1996). This expanded concept has prompted the use of the broader term vascular dementia. However, there is still no consensus regarding the type, location, and extent of lesions required for VaD (Fig. 11.1).

II. Epidemiology

A. Prevalence and Incidence

Use of different diagnostic systems may markedly influence the results of incidence and prevalence studies of dementia in general and VaD in particular (Erkinjuntti *et al*., 1997; Kay, 1999; Chui *et al*., 2000). Inclusion of mixed dementia can significantly increase the number of reported cases of VaD. Epidemiological data regarding dementia and VaD have been reviewed in detail (Hebert and Brayne, 1995; Jorm and Jolley, 1998; Nyenhuis and Gorelick, 1998; Fratiglioni *et al*., 1999; Kay, 1999). In more recent studies, prevalence rates of dementia in individuals aged 65 years or more range from 41 to 111 per 1000 and are generally lower in Asia than in Europe or North America (Kay, 1999). The prevalence of VaD ranges from 5 to 31 per 1000 with the highest rates reported in Asia and Italy (Rocca *et al*., 1991; Prencipe *et al*., 1996; Ferini-Strambi *et al*., 1997; Rajkumar *et al*., 1997; Chiu *et al*., 1998; Woo *et al*., 1998), and the lowest rates in France (Leten-

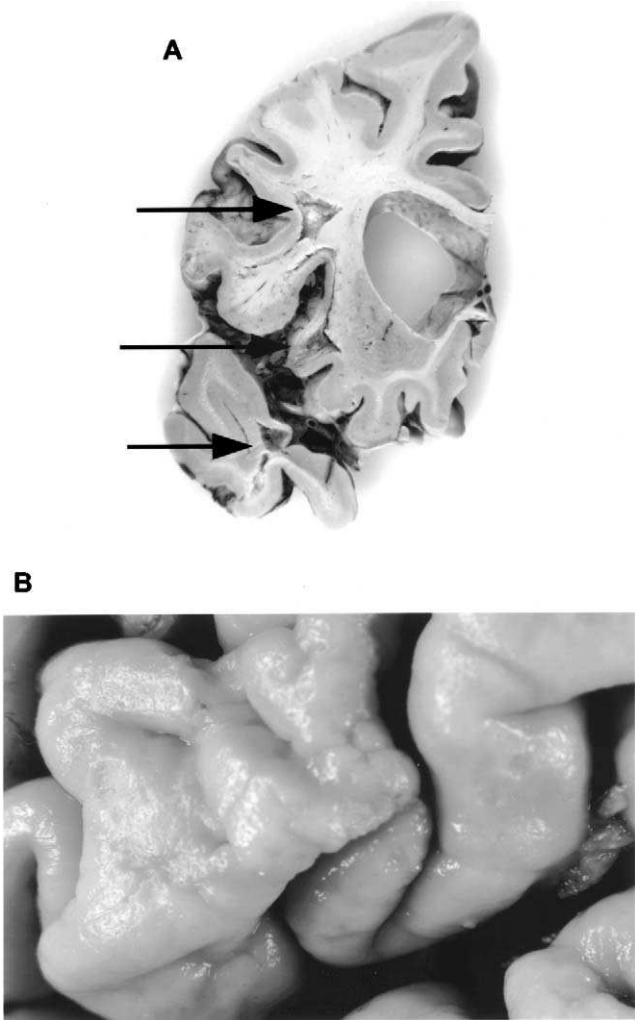

FIG. 11.1. (A) Multiple subcortical infarcts and lacunes (arrows) in the inferior frontal gyrus, anterior insula, and temporal pole of a VaD case. Large softenings are visible in the white matter of the inferior frontal gyrus. (B) Close-up of a frontal gyrus showing multiple small cortical infarcts, appearing as a surface granular atrophy due to inadequate blood flow in the terminal fields of the cortical microvasculature.

neur *et al*., 1994). However, there are marked regional variations with some Asian studies reporting particularly low rates in rural populations (H. C. Liu *et al*., 1994, 1998). Among the larger studies, the Canadian Study of Health and Aging reported a prevalence of 15 per 1000 and EURODEM a prevalence of 24 per 1000 (Rocca *et al*., 1991; Canadian Study of Health and Aging Working Group, 1994). VaD prevalence increases steeply with age, reaching 30 to 110 per 1000 in 85-year-old individuals. In most studies, AD is more prevalent than VaD; however, the proportion of dementia cases classified as VaD shows significant geographical variation and appears to be higher in Asia (38%) than in Europe (28%) and North America (10%) (Fratiglioni *et al*., 1999). While rates of AD tend to be higher in women, especially at older ages (Fratiglioni *et al*., 1997; Obadia *et al*., 1997), the opposite is true for VaD, with higher rates reported in men, although a gender effect is not always present (Hebert and Brayne, 1995; Kay, 1999).

The incidence of dementia ranges from 8 to 40 per 1000 in individuals aged 60 to 64 years, but has been reported to reach 50 to 136 per 1000 above 95 years of age (Fratiglioni *et al*., 1999). The average annual rate per 1000 of vascular dementia ranges from 11 to 16 in individuals aged 65 years or more, 19 to 43 in those aged 75 years or more, and 84 or greater in the oldest old aged 85 years or more (Kay, 1999). Despite the dearth of incidence data, it appears that new cases of VaD occur significantly less often than new AD cases in Europe and in Taiwan (Letenneur *et al*., 1994; Brayne *et al*., 1995; Aevarsson and Skoog, 1996; C. K. Liu *et al*., 1998), whereas in Japan the incidence of these two types of dementia is more similar, with VaD occurring slightly more often than AD (Yoshitake *et al*., 1995). There are conflicting data for individuals over the age of 85 years. In this age group, there was no new case of VaD in a French study (Letenneur *et al*., 1994), whereas in Sweden the incidence of VaD, in which mixed dementia was included, was greater than that of AD (Aevarsson and Skoog, 1996). In a review of pooled studies, the proportion of incident dementia cases diagnosed as VaD was 10% in North America, 28% in Europe, and 38% in Asia (Fratiglioni *et al*., 1999). However, further studies are needed to determine whether geographical variations represent true differences or whether they are related to methodological issues.

B. Risk Factors for VaD

1. Vascular Factors and Stroke

Hypertension is one of the most potent risk factors for the development of VaD, as demonstrated in several studies (Babikian and Ropper, 1987; Meyer *et al*., 1988; Gorelick *et al*., 1993, 1994; Gorelick, 1997; Rockwood *et al*., 1997; Skoog, 1998). In the Canadian Study of Health and Aging, a history of hypertension was twice as common in individuals diagnosed with VaD than in nondemented controls (Lindsay *et al*., 1997). In Japan, where the incidence of VaD is particularly high, an increase of one standard deviation in systolic blood pressure was associated with a 60% increase in the risk of VaD in older individuals (Yoshitake *et al*., 1995). A U.S. study reported that hypertension was present in 66% of patients with multi-infarct dementia as opposed to 44% of aged-matched controls (Meyer *et al*., 1988). Data on hypertension and VaD risk must nevertheless be interpreted with caution, as the presence of hypertension is included in several of the clinical criteria used to make the diagnosis of VaD. Furthermore, clinical criteria used in most research studies to establish the diagnosis of AD specifically exclude cases with symptoms or signs of cerebrovascular disease. This introduces a selection bias that tends to prevent the inclusion of subjects with vascular risk factors in AD samples. Despite this fact, several studies have suggested that hypertension may also play a role in the development of AD. Skoog and others (1996) reported that elderly individuals who developed dementia (either AD or VaD) had higher blood pressures 10 and 15 years earlier than those whose cognitive function remained unimpaired. Data from the Syst-Eur trial suggest that the treatment of isolated systolic hypertension could reduce the incidence of dementia, both VaD and AD, by 50% (Forette *et al*., 1998).

Further studies are needed, using clinical criteria that do not include the presence of vascular risk factors, in order to better delineate the relationship between hypertension and both VaD and AD. The presence of vascular risk factors is not sufficient to establish the vascular origin of a dementia.

Stroke has been closely related to dementia, particularly VaD. In the Framingham study cohort, mean Mini Mental State Examination scores decreased from 28.3 to 23.6 before and after stroke (Kase *et al*., 1998). In the Canadian Study of Health and Aging, 79.5% of patients with VaD had a history of stroke (Lindsay *et al*., 1997). Kokmen *et al*. (1996), in a population-based study, reported that the incidence of dementia was nine times higher than expected in the first year following a stroke, and Censori *et al*. (1996) found that one-quarter of a hospital-based population aged 40 to 79 years developed a dementia 3 months after an ischemic stroke. Other authors have also reported a marked increase in the occurrence of dementia following stroke (Tatemichi *et al*., 1990, 1994; Loeb *et al*., 1992; Skoog *et al*., 1993; Andersen *et al*., 1996; Inzitari *et al*., 1998). The relationship between stroke and dementia is perhaps underestimated, as asymptomatic stroke may be a significant factor in the development of dementia; patient attrition may also be a factor (Yoshitake *et al*., 1995; Desmond *et al*., 1998). However, the presence of previously unrecognized degenerative dementia may lead to an exaggerated estimate of the frequency of so-called ''poststroke'' dementia (Hénon *et al*., 1997).

Why certain individuals with stroke develop dementia while others do not remains poorly understood. Several authors have addressed this issue (Ji *et al*., 1988; Gorelick *et al*., 1993; Frisoni *et al*., 1994; Skoog, 1994; Hebert and Brayne, 1995; Prencipe *et al*., 1997; Slooter *et al*., 1997; Chapman *et al*., 1998; Nyenhuis and Gorelick, 1998; Pohjasvaara *et al*., 1998). Left-sided and recurrent lesions are associated with a higher risk of dementia, whereas infarct volume seems less important. The presence of cerebral atrophy is also a potentiating risk factor. Certain patient characteristics, such as male gender, an increasing age, a low level of education, or a low level of cognitive performance before stroke, have been shown to be predictive of dementia. Finally, the presence of vascular risk factors, including diabetes mellitus, hypertension, tobacco use, cardiac arrhythmias, and heart disease, may also increase the risk of poststroke dementia.

It is unclear whether all poststroke dementias are in fact VaD. Several cases of poststroke dementia have a progressive course more suggestive of a degenerative process. It has been reported that the incidence of AD is 50% higher in a poststroke patients compared to the general age-matched population (Kokmen *et al*., 1996). This suggests that vascular lesions may either participate in the development of AD or potentiate the deleterious cognitive effects of the AD degenerative process. The latter is consistent with the concept of mixed dementia in which cognitive impairment is due to a combination of vascular pathology and cerebral degenerative changes. This may also explain why clinical criteria for VaD based mainly on evidence of stroke or focal neurological findings are unable to satisfactorily distinguish VaD from mixed dementia.

Other vascular risk factors have been associated with VaD, including smoking (Kokmen *et al*., 1996; Galanis *et al*., 1997), diabetes mellitus (Desmond *et al*., 1993; Gorelick *et al*., 1994), increased 1 hr postprandial glucose (Ross *et al*., 1999), a history of heart disease (Yoshitake *et al*., 1995) myocardial infarction (Aronson *et al*., 1990), coronary heart disease (Ross *et al*., 1999), and atrial fibrillation (Ratcliffe and Wilcock, 1985; Gorelick *et al*., 1994). In a community-based study of 828 nondemented elderly that were followed over 7 years, alcohol consumption was associated with a two- to threefold increase in the risk of vascular dementia (Yoshitake *et al*., 1995); similar findings have been reported in a population-based study of elderly Canadians (Lindsay *et al*., 1997). However, in a French study, moderate alcohol consumption was associated with higher cognitive scores in women, whereas in another study from the same country, moderate wine drinkers had a lower incidence of dementia than nondrinkers (Du Fouil *et al*., 1997; Orgogozo *et al*., 1997).

2. Genetic Factors

The presence of an APOE $\epsilon 4$ allele is associated with an increased risk of developing AD, but its association with VaD remains controversial. Slooter *et al*. (1997) described a striking sevenfold increase in the risk of dementia with stroke in individuals homozygous for APOE $\epsilon 4$ and a twofold increase in this risk in individuals who are heterozygous for this allele compared to APOE $\epsilon 3$ homozygous individuals. However, this does not imply an association with VaD, as a significant proportion of individuals with stroke and dementia may actually have AD and concomitant cerebrovascular disease. Although many studies have explored the possible relationship between APOE alleles and VaD, they have conflicting results and this issue remains unresolved (Ji *et al*., 1988; Bétard *et al*., 1994; Frisoni *et al*., 1994; Kawamata *et al*., 1994; Stengard *et al*., 1995; Higuchi *et al*., 1996; Pirttila *et al*., 1996; Sulkava *et al*., 1996; Treves *et al*., 1996; Hofman *et al*., 1997; Katzman *et al*., 1997; Palumbo *et al*., 1997; Slooter *et al*., 1997; Wieringa *et al*., 1997; Chapman *et al*., 1998; Marin *et al*., 1998; Skoog *et al*., 1998; Tilvis *et al*., 1998). Discrepancies may, in part, be explained by the fact that these studies, with a rare exception, did not include autopsied cases and used different clinical criteria to identify dementia subtypes.

Cerebral autosomal dominant arteriopathy with subcortical infarct and leukoencephalopathy (CADASIL) and several forms of cerebral amyloid angiopathy lead to inherited VaD, but these diseases are very rare. It is likely that, in most cases, VaD is not strongly related to genetic factors. This is supported by twin studies suggesting that, contrary to AD, the environment is a more important causal factor than heredity for the development of VaD (Bergem *et al*., 1997).

III. Neuropsychological Profile of VaD

Several studies have attempted to identify neuropsychological patterns typical of VaD. It has been assumed that VaD follows a stepwise deterioration and a fluctuating course as opposed to the gradual and progressive cognitive decline of AD (Hachinski *et al*., 1974; Loeb *et al*., 1992). This is consistent with the multi-infarct model, but would not be expected within the broader modern construct of VaD. In fact, over

half of VaD patients may present with an insidious onset and follow a slowly progressive course (Fischer *et al*., 1990). It has been proposed that, as opposed to AD, VaD is associated with more marked deficits in attention, concentration, and executive function and with less pronounced memory impairment (Libon *et al*., 1990, 1996, 1998; Kertesz and Clydesdale, 1994; McPherson and Cummings, 1996; Lamar *et al*., 1997; Desmond *et al*., 1999). Individuals with VaD may perform more poorly on verbal fluency tests compared to AD cases and also exhibit more perseverations, particularly during tasks that assess frontal lobe functions (Padovani *et al*., 1995; Lamar *et al*., 1997). In an analysis of 27 studies of neuropsychological testing, VaD patients tended to have greater preservation of long-term memory and greater deficits in executive functioning than AD patients; however, language, constructional abilities, memory registration, conceptual function, and attention and tracking were similar in both types of dementia (Looi and Sachdev, 1999). In the absence of clinicopathological correlations, these proposed patterns suffer from current limitations in the clinical diagnosis of VaD and have been difficult to replicate. Furthermore, the pathophysiological heterogeneity of VaD suggests that a single neuropsychological pattern of VaD is unlikely. Further studies, including careful neuropsychological assessment and neuroimaging, as well as neuropathological examination, are needed to correlate better clinical deficits with specific cerebrovascular lesions.

IV. Clinical Criteria

Ideally, clinical criteria for dementia should apply universally to all subtypes and should be able to identify initial stages as well as established cases. However, existing criteria for dementia do not always agree. In a study of 1879 elderly subjects, the proportion of subjects with dementia varied from 3.1% with ICD-10 criteria to 29.1% with DSM-III criteria (Erkinjuntti *et al*., 1997). Generally accepted definitions of dementia are based strongly on the clinical presentation of AD and must include memory deficits. This requirement may not be appropriate for other types of dementia where behavioral disorders and marked impairment in other cognitive domains, such as executive functions and language, may precede memory impairment. Severity requirements are also an issue, particularly for VaD. This has led Hachinski (1994) to suggest that the term vascular dementia should be replaced by vascular cognitive impairment to stress early identification of affected patients across the whole spectrum of cognitive performance.

Differentiating VaD from AD is a particularly challenging endeavor. Binswanger and Alzheimer's first descriptions of arteriosclerotic brain degeneration stressed several clinical findings that were important in the differentiation between dementia of vascular origin and general paralysis. These include a relative preservation of personality, insight, and judgment and the presence of focal neurologic findings, such as aphasia, hemianopsia, hemiparesis, and hemisensory loss, as well as a fluctuating course (Binswanger, 1894; Alzheimer, 1895, 1898; Roman, 1999). Several of these findings have been incorporated into clinical criteria, some of which are still in use today.

TABLE 11.1 Hachinski's Ischemic Score

Item	Score[a]
Abrupt onset	2
Stepwise progression	1
Fluctuating course	2
Nocturnal confusion	1
Relative preservation of personality	1
Depression	1
Somatic complaints	1
Emotional incontinence	1
History of hypertension	1
History of strokes	2
Evidence of associated atherosclerosis	1
Focal neurologic symptoms	2
Focal neurologic signs	2

[a] Alzheimer's disease: $\leqslant 4$; mixed dementia: 5 or 6; and vascular dementia: $\geqslant 7$.

The Hachinski ischemic score (HIS), introduced in 1974, is the traditionally used clinical tool for the diagnosis of VaD (Hachinski *et al*., 1974) (Table 11.1). It is meant to be applied to patients with dementia in an effort to separate VaD from AD. It does not contain criteria for the diagnosis of dementia per se. It is based largely on the multi-infarct concept of VaD and may not perform as well in detecting other subtypes of VaD. Furthermore, because hypertension is one of the items favoring the diagnosis of VaD, this precludes using the HIS in epidemiological studies of the relationship between VaD and hypertension. Several modifications have sought to improve upon the HIS (Rosen *et al*., 1980; Loeb and Gandolfo, 1983). Rosen *et al*. (1980) suggested that several features of the HIS were of primary importance, such as abrupt onset, stepwise deterioration, history of stroke, and focal neurological signs and symptoms, and that others were of secondary importance, such as a history or presence of hypertension; features such as somatic complaints and emotional incontinence were deemed of questionable diagnostic value. Loeb and Gandolfo (1983) proposed a shorter version containing only four of the original HIS items (abrupt onset, focal neurological symptoms, focal neurological signs, and history of strokes), but incorporating CT scan findings. None of these modified versions have been shown conclusively to be superior to the initial HIS.

Whereas the just-described criteria are based on the simultaneous presence of cognitive impairment and evidence of cerebrovascular disease, ICD-10 and DSM-IV criteria require the presence of significant cerebrovascular disease, which may reasonably be judged to be related etiologically to the dementia (Rosen *et al*., 1980; American Psychiatric Association, 1994) (Tables 11.2 and 11.3). However, there are no instructions as to how focal neurological findings or neuroimaging data should be interpreted regarding a potential vascular etiology. Each investigator is left to make this judgment on his own. The DSM-IV is largely based on criteria for AD (criteria A and B are virtually identical in AD and VaD) and stresses memory loss and severity of disease. As stated earlier this may not be

TABLE 11.2 Summary of ICD-10 Research Criteria for VaD

Dementia as defined by
A decline in memory and other cognitive abilities that has been present for at least 6 months
Intact consciousness
A decline in emotional control or motivation or a change in social behavior manifest at least one of the following: emotional lability, irritability, apathy, coarsening of social behavior
Uneven distribution of deficits in higher cognitive functions
Focal brain damage, manifest as at least one of the following: unilateral spastic weakness of the limbs, unilaterally increased tendon reflexes, an extensor plantar response, or pseudobulbar palsy
Evidence from the history, examination, or tests of significant cerebrovascular disease, which may reasonably be judged to be etiologically related to the dementia

appropriate for VaD and may prevent early recognition of vascular cognitive impairment, thus precluding early intervention. There are several versions of the ICD-10, and the research criteria discussed here differ from the clinical descriptions and diagnostic guidelines (Wetterling *et al*., 1994). Research criteria have unusually precise requirements. The decline in cognitive abilites must include memory impairment and deterioration in judgement and thinking, such as planning and organizing; emotional changes are required and clearly outlined, and focal neurological findings are restricted to unilateral spastic weakness of the limbs, unilaterally increased tendon reflexes, an extensor plantar response, or pseudobulbar palsy. The narrow spectrum of these requirements is not consistent with the current broad view of the various pathophysiological mechanisms that may lead to VaD. The requirement for an unequal distribution in higher cognitive functions is not operationalized and thus remains investigator dependent.

Other criteria were proposed in 1992 by the State of California Alzheimer's Disease Diagnostic and Treatment Centers (ADDTC) (Chui *et al*., 1992, 2000) (Table 11.4) and in 1993 by the National Institute for Neurological Disorders and Stroke (NINDS) with support from the Association Internationale pour la Recherche et l'Enseignement en Neurosciences (AIREN) (Roman *et al*., 1993) (Table 11.5). They both require the presence of dementia, evidence for cerebrovascular disease, and, in most cases, a relationship between the two. The ADDTC definition of dementia requires two impaired cognitive domains but does not emphasize memory deficits. There must be a clear temporal relationship between the cerebral event and the onset of dementia if only one stroke has occurred, but this is not necessary if there is evidence of two or more strokes. This makes it possible to include cases with a more variable course, but leads to a diagnosis of probable VaD in any demented individual who has suffered two strokes. The ADDTC possible VaD category includes cases with a single stroke and no clear temporal relationship with the onset of dementia, as well as individuals with clinical and neuroimaging evidence for Binswanger's disease. The NINDS-AIREN definition of dementia requires impairment of memory and at least two other cognitive domains; this is more restrictive than most other criteria, including DSM-IV. The NINDS-AIREN probable criteria require clinical and radiological evidence of cerebrovascular disease, as well as a clear temporal relationship between dementia onset and stroke, which is arbitrarily set at a maximum of 3 months. However, abrupt deterioration or a stepwise course without a temporal relationship is also included in the probable VaD category. The possible VaD category includes cases with no neuroimaging, no clear temporal relationship, and an atypical course. These criteria still await prospective neuropathological validation.

Several clinical studies have compared the criteria just discussed. Verhey *et al*. (1996) applied seven different sets of VaD criteria to a sample of 124 demented patients. The frequency of VaD ranged from 6 to 32%, depending on which criteria were used; only 8 patients were diagnosed as having VaD by all criteria (Verhey *et al*., 1996). Frequencies of VaD were highest for the NINDS-AIREN criteria for possible VaD and the HIS in its initial form or as modified by Rosen; frequencies were lowest for the NINDS-AIREN and ADDTC criteria for probable VaD. However, in a study of 40 patients presenting with suspected VaD, Amar *et al*. found the ADDTC to be more sensitive than the HIS. Wetterling compared four different sets of criteria in 167 individuals who were referred for evaluation of possible dementia. While 45 cases were diagnosed as VaD according to DSM-IV, only 5 cases met all of the different sets of criteria for VaD (Wetterling *et al*., 1996). Another clin-

TABLE 11.3 Summary of DSM-IV Criteria for Vascular Dementia

Multiple cognitive deficits manifested by both
impaired memory
at least one of apraxia, agnosia, aphasia, or disturbance in executive functions
Significant impairment in social or occupational functioning as a result of the just-described cognitive deficits, representing a decline from a previous level of functioning
Focal neurological signs and symptoms or laboratory evidence of cerebrovascular disease that is judged to be etiologically related to the disturbance
The deficits do not occur exclusively during the course of a delirium

TABLE 11.4 Summary of ADDTC Clinical Criteria

Dementia definition	Deterioration in intellectual function sufficient to interfere with customary affairs of life, which is not isolated to a single category of intellectual performance and is independent of the level of consciousness
Probable VaD	Requires all the following 1. Dementia 2. Evidence of two or more strokes by history, neurological signs, and/or neuroimaging or a single stroke with a clear temporal relationship to the onset of dementia 3. Evidence of at least one infarct outside the cerebellum by CT or T1-weighted MRI The diagnosis of probable VaD is supported by 1. Evidence of multiple infarcts in brain regions known to affect cognition 2. A history of multiple transient ischemic attacks 3. History of vascular risk factors (e.g., hypertension, heart disease, diabetes mellitus 4. Elevated Hachinski ischemia scale
Possible VaD	1. Dementia and one or more of the following 2a. History or evidence of a single stroke without a clear temporal relationship with dementia onset 2b. Binswanger's disease that includes all the following: early onset of urinary incontinence or gait disturbance, vascular risk factors, and extensive white matter changes on neuroimaging
Mixed dementia	A diagnosis of mixed dementia should be made in the presence of one or more other systemic or brain disorders that are thought to be related causally to the dementia

ical study of 72 demented patients using the ICD-10 revealed that only 25% of patients fulfilling the criteria for dementia and showing vascular lesions on CT scan met the criteria for VaD, suggesting that the ICD-10 criteria may suffer from low sensitivity (Wetterling *et al*., 1994). Although clinical comparison studies are useful, the performance of different criteria is best evaluated in comparison to neuropathological findings.

In an article that pooled the results of five clinicopathological studies (174 patients for the five studies combined), the sensitivity of the HIS for the detection of VaD versus non-VaD forms of dementia was 0.42 and the specificity was

TABLE 11.5 Summary of NINDS-AIREN Clinical Criteria

Dementia definition	Decline in intellectual function affecting memory plus at least two other cognitive domains sufficient to interfere with the activities of daily living and not due to physical effects of stroke alone. Cases with disturbed consciousness and delirium are excluded
Probable VaD	Requires all the following 1. Dementia 2. Cerebrovascular disease defined by Focal signs on neurologic examination (such as hemiparesis, lower facial weakness, Babinski sign, sensory deficit, hemianopia, and dysarthria Evidence of relevant cerebrovascular disease by CT or MRI Multiple large-vessel infarcts A single strategically placed infarct (angular gyrus, thalamus, basal forebrain, or posterior or anterior cerebral artery territories) Multiple basal ganglia and white matter lacunes, or extensive periventricular white matter lesions, or combinations thereof 3. A relationship between the two disorders just described, manifested by (a) dementia onset within 3 months of a stroke or (b) abrupt deterioration in cognitive functions or fluctuating stepwise course Clinical features consistent with the diagnosis of probable vascular dementia include Early presence of a gait disturbance History of unsteadiness or frequent unprovoked falls Early urinary symptoms not explained by urologic disease Pseudobulbar palsy Personality and mood changes, abulia, depression, emotional incontinence, psychomotor retardation, and abnormal executive function
Possible VaD	May be made in the presence of dementia and focal neurological signs when 1. No neuroimaging studies exist 2. In the absence of clear temporal relationship between stroke and dementia 3. There is subtle onset and variable course of cognitive deficit and evidence of cerebrovascular disease
Alzheimer's disease with cerebrovascular disease	The term ''Alzheimer's disease with cerebrovascular disease'' should be used to classify patients fulfilling the clinical criteria for possible AD and who also present with clinical or brain imaging evidence of relevant cerebrovascular disease

0.84 (Pantoni and Inzitari, 1993). However, a meta-analysis of the HIS in 312 cases of pathologically verified dementias reported a much higher sensitivity of 0.84 and a similar specificity of 0.82 (Moroney *et al*., 1997). In another clinicopathological retrospective series of 114 autopsied cases of dementia, the sensitivity of the HIS for VaD was 0.43 and the specificity was 0.88 (Gold *et al*., 1997). These observations suggest that although the HIS performs well in excluding other causes of dementia, it remains insufficient as a screening test to detect VaD cases. Reasons for this discrepancy are unclear, but may be related to variations in HIS scoring methodology, differences in the pathological criteria used to establish the diagnosis of vascular dementia, and potential publication and selection biases. One study that included neuropathological findings as the gold standard reported relatively low sensitivities of the ADDTC (0.63) and the NINDS-AIREN criteria for possible VaD, although both were found to be more sensitive than the HIS (Gold *et al*., 1997). Mixed dementia, the simultaneous occurrence of AD and VaD, has a significant impact on the accuracy of clinical criteria. In the study described earlier, the HIS, the NINDS-AIREN, and the ADDTC behaved differently regarding mixed dementia. The HIS tended to exclude most cases of mixed dementia but failed to identify many of the VaD cases. The ADDTC and NINDS-AIREN were more sensitive for the detection of VaD, but were less able to differentiate between VaD and mixed dementia. The relatively poor performance of clinical criteria for VaD and the lack of an international consensus suggest a need for improving current diagnostic methodology. VaD can result from several pathophysiological mechanisms, leading to multiple forms of the disease, such as multiple infarcts secondary to large vessel atherosclerosis or cardioembolism, a single strategic infarct, lacunes and white matter changes secondary to small vessel disease, hypoperfusion, and hemorrhage. It is unlikely that a single set of clinical criteria can apply equally to all VaD subtypes. In this light, specific research criteria for subcortical vascular dementia have been proposed; however, they have yet to be validated (Erkinjuntti, 2000).

V. Neuroimaging

Advances in neuroimaging techniques may improve the performance of current clinical criteria. Skoog *et al*. (1993) reported a significant increase in the prevalence of vascular dementia in a population of community-dwelling elderly from 40 to 48% once CT scan findings were taken into consideration. However, in the absence of neuropathological validation, it is unclear whether this increased prevalence corresponds entirely to true cases of VaD. CT and MRI findings described in VaD include hyperintense foci in the basal ganglia and thalamus, infarctions, and white matter and irregular periventricular hyperintensities (Schmidt, 1992; Charletta *et al*., 1995; Frisoni *et al*., 1995; Fornarelli *et al*., 1996; Meyer *et al*., 1996). Although criteria that separate CT scans into grades of increasing support for VaD have been proposed, their clinical use is limited (Pullicino *et al*., 1996). In magnetic resonance imaging (MRI) and CT studies of poststroke patients, dementia has been related to multiplicity of lesions, bilaterality (Ladurner *et al*., 1982; Schmidt *et al*., 1992), atrophy (Tatemichi *et al*., 1990; Figueroa *et al*., 1992), (Liu *et al*., 1992; Schmidt *et al*., 1992; Pohjasvaara *et al*., 1998), and location in the thalamus, temporoparietal, and frontal areas. In individuals with multiple infarcts and dementia or patients with clinically defined VaD, CT or MRI findings suggestive of VaD include infarct location in the left hemisphere, thalamus and limbic structures, extensive white matter lesions, and atrophy (Erkinjuntti *et al*., 1987, 1999; Loeb *et al*., 1988; Gorelick *et al*., 1992; Charletta *et al*., 1995; Meyer *et al*., 1995a; Pullicino *et al*., 1996). Structural imaging has been proposed as a method to identify and document cerebral vascular pathology and thus help differentiate VaD from AD. However, white matter and periventricular lesions increase with age and are twice as prevalent in all dementing disorders, including both VaD and AD, compared to younger individuals (Englund *et al*., 1988; Meyer *et al*., 1992; Skoog *et al*., 1994; Frisoni *et al*., 1995; O'Brien *et al*., 1996; Englund, 1998). Furthermore, typical neuroimaging findings of AD, such as hippocampal atrophy, may also occur in VaD (Laakso *et al*., 1996).

Functionally, VaD cases usually exhibit a diffuse and asymmetric decrease of cerebral blood flow in the cerebral cortex and subcortical nuclei (Fornarelli *et al*., 1996). Furthermore, a loss of cerebral vasomotor responsiveness, a biological marker of cerebrovascular disease, is found consistently in cases with VaD or chronic hypertension (Judd *et al*., 1986; Johansson, 1992; Tamaki *et al*., 1995). In contrast, AD cases exhibit reduced CBF in temporal and parietal areas and show preserved ability to vasodilate and increase CBF in response to various stimuli (Judd *et al*., 1986; Obara *et al*., 1994; Meyer *et al*., 1995b, 1996). However, despite these differences, distinction between VaD and AD cases on the basis of SPECT CBF data remains difficult (Deutsch and Tweedy, 1987; Mielke *et al*., 1994; Starkstein *et al*., 1996; Masterman *et al*., 1997). Positron emission tomography (PET) reveals multiple focal metabolic defects in VaD as opposed to reduced regional metabolic rates in the temporal lobes in early AD (McGeer *et al*., 1986; Polinsky *et al*., 1987; Jagust *et al*., 1988, 1993; Heiss *et al*., 1991). However, a substantial decrease in glucose utilization and oxygen consumption is present in both AD and VaD, and the distinction between VaD and AD may be very difficult, particularly in severe cases (Frackowiak *et al*., 1981; Herholz, 1986; Hoyer, 1986; Heiss *et al*., 1991; Rapport, 1991; Mielke *et al*., 1994). The role of functional brain imaging in the evaluation of VaD has been reviewed: it may be limited for cortical infarcts, but it can be very valuable in assessing the impact of small subcortical infarcts on cortical function (Mori *et al*., 1999).

VI. Treatment Strategies in Vascular Dementia

The increasing body of knowledge regarding risk factors for the development of vascular dementia has led to potential preventive strategies (Gorelick *et al*., 1999). These include the treatment of hypertension, smoking cessation, control of diabetes, anticoagulation when atrial fibrillation is present, carotid endarterectomy for symptomatic patients with 70–99% carotid stenosis, and aspirin for patients at high primary vascular risk. Treatment of isolated systolic hypertension in individuals over

the age of 60 years led to a reduction in the incidence of strokes of 36% in the SHEP study (SHEP Cooperative Research Group, 1991) and 42% in the SYST-EUR trial (Staessen *et al*., 1997). In the latter trial, treatment with the calcium channel blocker nitrendipine and, as a second step, the ACE inhibitor enalapril reduced the incidence of dementia by 50% from 7.7 to 3.8 cases per 1000 patient-years when compared to placebo (Forette *et al*., 1998). The investigators estimated that 19 cases of dementia might be prevented if 1000 patients with isolated systolic hypertension were treated for 5 years. Interestingly, this reduction applied to both AD and VaD.

Once dementia has begun, the objective is to slow its progress, maximize cognitive function, and prevent new infarcts. A variety of treatments for vascular dementia have been tested. Meyer *et al*. (1989) reported encouraging results from a randomized clinical trial of 325 mg of aspirin per day in 70 patients with vascular dementia. Daily aspirin treatment improved cognitive performance and reduced or stabilized declines in cerebral perfusion in this group of patients. The authors stated that this treatment also improved quality of life and independence in activities of daily living. Nimodipine, a dihydropyridine calcium antagonist, has been proposed for the treatment of vascular dementia. This drug is reported to exert vasoactive effects by dilating predominantly small and collateral cerebral vessels and improving the blood supply to underperfused areas. In an open trial (Pantoni *et al*., 1996), 31 patients were treated with a daily dose of 90 mg nimodipine for up to 1 year and their cognitive function was shown to stabilize. Results from ongoing larger randomized placebo-controlled studies will be needed to confirm these findings (Rossi *et al*., 1999). Nicardipine, another calcium channel blocker, may also delay cognitive decline in VaD patients. Nicergoline, a thrombolytic, vasoactive ergot alkaloid, improved vigilance and information processing in patients with degenerative and vascular dementia in a double-blind, placebo-controlled study (Saletu *et al*., 1995) of 112 patients. Pentoxifylline, which has been approved for use in peripheral vascular disease (intermittent claudication), has been the subject of several studies suggesting a possible beneficial effect (Black *et al*., 1992; European Pentoxifylline Multi-Infarct Dementia Study Group, 1996). The proposed mechanism of action is through increased capillary blood flow, thereby improving tissue oxygenation. However, differences between treated patients and those given placebo were small and did not always reach statistical significance. Propentofylline, a phosphodiesterase and adenosine reuptake inhibitor, inhibits potentially neurotoxic functions of activated microglia and may have a beneficial effect on glucose metabolism in several cortical areas. It has been suggested that this drug could potentially slow the progression of dementia, but this has yet to be confirmed in large randomized, double-blind, placebo-controlled trials (Kittner *et al*., 1997; Marcusson *et al*., 1997; Kittner, 1999). A review of four phase III propentofylline trials that used DSMIIIR criteria and HIS to diagnose VaD and one phase III trial using NINDS-AIREN criteria reported a beneficial effect of unspecified magnitude on cognition but no effect on functional status, as measured by several activities of daily living scales (Kittner, 1999). In a randomized control trial of another phosphodiesterase inhibitor, denbufylline, cognitive improvements were not statistically significant compared to placebo in 110 patients with VaD or MD (Treves and Korczyn, 1999). Memantine, a *N*-methyl-D-aspartate (NMDA) receptor antagonist, is purported to provide neuroprotection from ischemia-induced apoptosis and and necrosis. Several phase III trial are ongoing (Möbius, 1999). Consumption of supplementary vitamin E was associated with a decreased likelihood of developing VaD in the Honolulu–Asia aging study; putative mechanisms include an antioxidant effect, decreased platelet adhesion, decreased carotid atherosclerosis, and neuronal protection from ischemic damage (Ross *et al*., 1999). EGb 761, an extract of the *Ginkgo biloba* tree, is prescribed in some countries for the treatment of dementia. A randomized, placebo-controlled trial carried out on 309 patients with dementia (either AD or VaD) showed a very modest effect after 52 weeks that could be measured objectively on neuropsychological testing, but could not be recognized by caregivers (Le Bars *et al*., 1997).

VII. Conclusion

The concept of vascular dementia has evolved significantly over the past century, leading to a better understanding of its underlying pathophysiological mechanisms. New clinical criteria have been developed that take into account the time relationship between vascular events and the occurrence of dementia and incorporate modern neuroimaging technology. However, further studies, including neuropsychological and functional evaluations, as well as neuroimaging and clinicopathological correlations, are needed to develop and validate better performing criteria, which could lead to a broader consensus on the clinical diagnosis of VaD and its various subtypes. Although curative therapy remains generally disappointing, epidemiological studies have led to a greater awareness of risk factors for VaD and potential preventive interventions.

References

Aevarsson, O., and Skoog, I. (1996). A population-based study on the incidence of dementia disorders between 85 and 88 years of age. *J. Am. Geriatr. Soc*. **44**, 1455–1460.

Alzheimer, A. (1895). Die arteriosklerotische Atrophie des Gehirns. *Allg. Z. Psychiatr. Psych. Gerichtl. Med*. **51**, 1809–1812.

Alzheimer, A. (1898). Neuere Arbeiten über die Dementia senilis und die auf atheromatöser Gefässerkrankung basierenden Gehirnkrankheiten. *Monatsschr. Psychiatr. Neurol* **3**, 101–115.

American Psychiatric Association. (1994). "Diagnostic and Statistical Manual of Mental Disorders." 4th ed. American Psychiatric Association, Washington, DC.

Andersen, G., Vestergaard, K., Ostergaard Riis, J., and Ingeman-Nielsen, M. (1996). Intellectual impairment in the first year following stroke, compared to an age-matched population sample. *Cerebrovasc. Dis*. **6**, 363–369.

Aronson, M. D., Ooi, W. L., Morgenstern, H., Hafner, A., Masur, D., Frishman, C. H., Fisher, D., and Katzman, R. (1990). Women, myocardial infarction, and dementia in the very old. *Neurology* **40**, 1102–1106.

Babikian, U., and Ropper, A. H. (1987). Binzwanger's disease: A review. *Stroke* **18**, 1–12.

Bergem, A. L., Engedal, K., and Kringlen, E. (1997). The role of heredity in late-onset Alzheimer disease and vascular dementia. A twin study. *Arch. Gen. Psychiatry* **54**, 264–270.

Betard, C., Robitaille, Y., Gee, M., Tiberghien, D., Larrivée, D., Roy, P., Mortimer, J. A., and Gauvreau, D. (1994). Apo E allele frequencies in

Alzheimer's disease, Lewy body dementia, Alzheimer's disease with cerebrovascular disease and vascular dementia. *NeuroReport* **5**, 1893–1896.

Binswanger, O. (1984). Die Abgrenzung der allgemeinen progressiven paralyse. *Berl. Klin. Wochenschr.* **31**, 1103–1186.

Black, R. S., Barclay, L. L., Nolan, K. A., Thaler, H. T., Hardiman, S. T., and Blass, J. P. (1992). Pentoxifylline in cerebrovascular dementia. *J. Am. Geriatr. Soc*. **40**, 237–244.

Bowler, J., and Hachinski, V. (1996). History of the concept of vascular dementia: Two opposing views on current definitions and criteria for vascular dementia. *In* ''Vascular Dementia'' (I. Prohovnik, J. Wade, S. Knezevic, T. Tatemichi, and T. Erkinjuntti, eds.), pp. 1–28. Wiley, Chichester.

Brayne, C., Gill, C., Huppert, F. A., Barkley, C., Gehlhaar, E., Girling, D. M., O'Connor, D. W., and Paykel, E. S. (1995). Incidence of clinically diagnosed subtypes of dementia in an elderly population. Cambridge Project for Later Life. *Br. J. Psychiatry* **167**, 255–262.

Canadian Study of Health and Aging Working Group. (1994). Canadian study of health and aging: Study methods and prevalence of dementia. *Can. Med. Assoc. J*. **150**, 899–913.

Censori, B., Manara, O., Agostinis, C., Camerlingo, M., Casto, L., Galavotti, B., Partziguian, T., Servalli, M. C., Cesana, B., Belloni, G., and Mamoli, A. (1996). Dementia after first stroke. *Stroke* **27**, 1205–1210.

Chapman, J., Wang, N., Treves, T. A., Korczyn, A. D., and Bornstein, N. M. (1998). ACE, MTHFR, factor V Leiden, and APOE polymorphisms in patients with vascular and Alzheimer's dementia. *Stroke* **29**, 1401–1404.

Charletta, D., Gorelick, P. B., Dollear, T. J., Freels, S., and Harris, Y. (1995). CT and MRI findings among African-Americans with Alzheimer's disease, vascular dementia, and stroke without dementia. *Neurology* **45**, 1456–1461.

Chiu, H. F., Lam, L. C., Chi, I., Leung, T., Li, S. W., Law, W. T., Chung, D. W., Fung, H. H., Kan, P. S., Lum, C. M., Ng, J., and Lau, J. (1998). Prevalence of dementia in Chinese elderly in Hong Kong. *Neurology* **50**, 1002–1009.

Chui, H. C., Victoroff, J. I., Margolin, D., Jagust, W., Shankle, R., and Katzman, R. (1992). Criteria for the diagnosis of ischemic vascular dementia proposed by the State of California Alzheimer's Disease Diagnostic and Treatment Centers. *Neurology* **42**, 473–480.

Chiu, H. C., Mack, W., Jackson, J. E., Runges, D., Reed, B. R., Tinklenberg, J., Chang, F. L., Skinner, K., Tasaki, C., and Jagust, W. J. (2000). Clinical criteria for the diagnosis for vascular dementia. A multicenter study of comparability and interrater reliability. *Arch. Neurol. (Chicago)* **57**, 191–196.

del Ser, T., Bermejo, F., Portera, A., Arredondo, J. M., Bouras, C., and Constantinidis, J. (1990). Vascular dementia. A clinicopathological study. *J. Neurol. Sci*. **96**, 1–17.

Desmond, D. W. (1996). Vascular dementia: A construct in evolution. *Cerebrovasc. Brain Metab. Rev.* **8**, 296–325.

Desmond, D. W., Tatemichi, T. K., Paik, M., and Stern, Y. (1993). Risk factors for cerebrovascular disease as correlates of cognitive function in a stroke-free cohort. *Arch. Neurol. (Chicago)* **50**, 162–166.

Desmond, D. W., Bagiella, E., Moroney, J. T., and Stern, Y. (1998). The effect of patient attrition on estimates of the frequency of dementia following stroke. *Arch. Neurol. (Chicago)* **55**, 164–168.

Desmond, D. W., Erkinjuntti, T., Sano, M., Cummings, J. L., Bowler, J. V., Pasquier, F., Moroney, J. T., Ferris, S. H., Stern, Y., Sachdev, P. S., and Hachinski, V. C. (1999). The Cognitive Syndrome of Vascular Dementia: Implications for clinical trials. *Alzheimer Dis. Assoc. Disord.* **13**(Suppl. 3), S21–S29.

Deutsch, G., and Tweedy, J. R. (1987). Cerebral blood flow in severity matched Alzheimer and multi-infarct patients. *Neurology* **37**, 431–438.

Du Fouil, C., Ducimetière, P., and Alpérovitch, A. (1997). Sex differences in the association between alcohol consumption and cognitive performance. *Am. J. Epidemiol*. **146**, 405–412.

Englund, E. (1998). Neuropathology of white matter changes in Alzheimer's disease and vascular dementia. *Dement. Geriatr. Cogn. Disord*. **9**(Suppl. 1), 6–12.

Englund, E., Brun, A., and Alling, C. (1988). White matter changes in dementia of Alzheimer's type. *Brain* **111**, 1425–1439.

Erkinjuntti, T. (2000). Research criteria for subcortical vascular dementia in clinical trials. *J. Neural Transm*. (in press).

Erkinjuntti, T., Ketonen, L., Sulkava, R., Sipponen, J., Vuorialho, M., and Palo, J. (1987). CT in the differential diagnosis between Alzheimer's disease and vascular dementia. *Acta Neurol. Scand*. **75**, 262–270.

Erkinjunti, T., Haltia, M., Palo, J., Sulkava, R., and Paetau, A. (1988). Accuacy of the clinical diagnosis of vascular dementia: A prospective clinical and post-mortem neuropathological study. *J. Neurol. Neurosurg. Psychiatry* **51**, 1037–1044.

Erkinjuntti, T., Ostbye, T., Steenhuis, R., and Hachinski, V. (1997). The effect of different diagnostic criteria on the prevalence of dementia. *N. Engl. J. Med*. **337**, 1667–1674.

Erkinjuntti, T., Bowler, J. V., DeCarli, C. S., Fazekas, F., Inzitari, D., O'Brien, J. T., Pantoni, L., Rockwood, K., Scheltens, P., Wahlund, L. O., and Desmond, D. W. (1999). Imaging of static brain lesions in vascular dementia: Implications for clinical trials. *Alzheimer Dis. Assoc. Disord*. **13**(Suppl. 3), S81–S90.

European Pentoxifylline Multi-Infarct Dementia Study Group. (1996). European Pentoxifylline Multi-Infarct Dementia Study. *Eur. Neurol.* **36**, 315–321.

Ferini-Strambi, L., Marcone, A., Garancini, P., Danelon, F., Zamboni, M., Massussi, P., Tedesi, B., and Smirne, S. (1997). Dementing disorders in north Italy: Prevalence study in Vescovato, Cremona Province. *Eur. J. Epidemiol*. **13**, 201–204.

Figueroa, M., Tatemichi, T. K., Cross, D. T., and Desmond, D. W. (1992). CT correlates of dementia after stroke. *Neurology* **42**, 176.

Fischer, P., Gatterer, G., Marterer, A., Simanyi, M., and Danielczyk, W. (1990). Course characteristics in the differentiation of dementia of the Alzheimer type and multi-infarct dementia. *Acta Psychiatr. Scand*. **81**, 551–553.

Forette, F., Seux, M. L., Staessen, J. A., Thijs, L., Birkenhager, W. H., Babarskiene, M. R., Babeanu, S., Bossini, A., Gil-Extremera, B., Girerd, X., Laks, T., Lilov, E., Moisseyev, V., Tuomilehto, J., Vanhanen, H., Webster, J., Yodfat, Y., and Fagard, R. (1998). Prevention of dementia in randomized double-blind placebo-controlled Systolic Hypertension in Europe (Syst-Eur) trial. *Lancet* **352**, 1347–1351.

Fornarelli, D., Ascoli, G., Rossi, R., Manca, A., Lanza, R., Moretti, V., Scarpino, O., and Guidi, M. (1996). Clinical and instrumental diagnosis of Alzheimer's and multi-infarct dementia. *Radiol. Med*. **92**, 22–27.

Frackowiak, R. S. J., Pozzilli, C., Legg, N. J., Du Boulay, G. H., Marshall, J., Lenzi, G. L., and Jones, T. (1981). Regional cerebral oxygen supply and utilization in dementia. A clinical and physiological study with oxygen-15 and positron tomography. *Brain* **104**, 753–778.

Fratiglioni, L., Viitanen, M., von Strauss, E., Tontodonati, V., Herlitz, A., and Winblad, B. (1997). Very old women at highest risk of dementia and Alzheimer's disease: Incidence data from the Kungsholmen Project, Stockholm. *Neurology* **48**, 132–138.

Fratiglioni, L., De Ronchi, D., and Agüero-Torres, H. (1999). Worldwide prevalence and incidence of dementia. *Drugs Aging* **15**, 365–375.

Frisoni, G. B., Calabresi, L., Geroldi, C., Bianchetti, A., D'Acquarica, A. L., Govoni, S., Sirtori, C. R., Trabucchi, M., and Franceschini, G. (1994). Apolipoprotein E $\epsilon 4$ allele in Alzheimer's disease and vascular dementia. *Dementia* **5**, 240–242.

Frisoni, G. B., Beltramello, A., Binetti, G., Bianchetti, A., Weiss, C., Scuratti, A., and Trabucchi, M. (1995). Computed tomography in the detection of the vascular component in dementia. *Gerontology* **41**, 121–128.

Galanis, D. J., Petrovitch, H., Launer, L. J., Harris, T. B., Foley, D. J., and White, L. R. (1997). Smoking history in middle age and subsequent

cognitive performance in elderly Japanese-American men. The Honolulu-Asia aging study. *Am. J. Epidemiol*. **145**, 507-515.

Gold, G., Giannakopoulos, P., Montes-Paixao, C., Jr., Herrmann, F., Mulligan, R., Michel, J. P., and Bouras, C. (1997). Sensitivity and specificity of newly proposed criteria for the diagnosis of possible vascular dementia. *Neurology* **49**, 690–694.

Gold, G., Di Pollina, L., Giannakopoulos, P., Bouras, C., and Michel, J. P. (1999). Vascular dementia: A diagnostic challenge. *Interdiscipl. Top. Gerontol*. **30**, 101–112.

Gorelick, P. B. (1997). Status of risk factors for dementia associated with stroke. *Stroke* **28**, 459–463.

Gorelick, P. B., Chatterjee, A., Patel, D., Flowerdew, G., Dollear, W., Taber, J., and Harris, Y. (1992). Cranial computed tomographic observations in multi-infarct dementia. A controlled study. *Stroke* **23**, 804–811.

Gorelick, P. B., Brody, J., Cohen, D., Freels, S., Levy, P., Dollear, W., Forman, H., and Harris, Y. (1993). Risk factors for dementia associated with multiple cerebral infarcts. A case-control analysis in predominantly African American hospital-based patients. *Arch. Neurol. (Chicago)* **50**, 714–720.

Gorelick, P. B., Freels, S., Harris, Y., Dollear, T., Billingsley, M., and Brown, N. (1994). Epidemiology of vascular and Alzheimer's dementia among African Americans in Chicago, IL: Baseline frequency and comparison of risk factors. *Neurology* **44**, 1391–1396.

Gorelick, P. B., Erkinjuntti, T., Hofman, A., Rocca, W. A., Skoog, I., and Winblad, B. (1999). Prevention of vascular dementia. *Alzheimer Dis. Assoc. Disord*. **13**(Suppl. 3), S131–S139

Hachinski, V. (1992). Preventable senility: A call for action against the vascular dementias. *Lancet* **340**, 645–648.

Hachinski, V. (1994). Vascular dementia: A radical redefinition. *Dementia* **5**, 130–132.

Hachinski, V. D., Lassen, N. A., and Marshall, J. (1974). Multi-infarct dementia. A cause of mental deterioration in the elderly. *Lancet* **2**, 207–210.

Hebert, R., and Brayne, C. (1995). Epidemiology of vascular dementia. *Neuroepidemiology* **14**, 240–257.

Heiss, W. D., Kessler, J., Szelies, B., Grond, M., Fink, G., and Herholz, K. (1991). Positron emission tomography in the differential diagnosis of organic dementias. *J. Neural Transm., Suppl*. **33**, 13–19.

Hénon, H., Pasquier, F., Durieu, I., Godefroy, O., Lucas, C., Lebert, F., and Leys, D. (1997). Preexisting dementia in stroke patients. Baseline frequency, associated factors, and outcome. *Stroke* **28**, 2429–2436.

Herholz, K. (1986). FDG PET and differential diagnosis of dementia. *Alzheimer Dis. Assoc. Disord*. **9**, 6–16.

Higuchi, S., Arai, H., Nakagawa, T., Muramatsu, T., Sasaki, H., and Trojanowski, J. Q. (1996). The apolipoprotein E gene in Binswanger's disease and vascular dementia. *Clin. Genet*. **50**, 459–461.

Hofman, A., Ott, A., Breteler, M. M., Bots, M. L., Slooter, A. J., van Harskamp, F., van Duijn, C. N., Van Broeckhoven, C., and Grobbee, D. E. (1997). Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam Study. *Lancet* **349**, 151–154.

Hoyer, S. (1986). Senile dementia and Alzheimer's disease, brain blood flow and metabolism. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **10**, 447–478.

Inzitari, D., Di Carlo, A., Pracucci, G., Lamassa, M., Vanni, P., Romanelli, M., Spolveri, S., Adriani, P., Meucci, I., Landini, G., and Ghetti, A. (1998). Incidence and determinants of poststroke dementia as defined by an informant interview method in a hospital-based stroke registry. *Stroke* **29**, 2087–2093.

Jagust, W. J., Friedland, R. P., and Budinger, T. F. (1988). Longitudinal studies of regional cerebral metabolism in Alzheimer's disease. *Neurology* **38**, 909–912.

Jagust, W. J., Eberling, J. L., Richardson, B. C., Reed, B. R., Baker, M. G., Nordahl, T. E., and Budinger, T. F. (1993). The cortical topography of temporal lobe hypometabolism in early Alzheimer's disease. *Brain Res*. **629**, 189–198.

Ji, Y., Urakami, K., Adachi, Y., Maeda, M., Isoe, K., and Nakashima, K. (1988). Apolipoprotein E polymorphism in patients with Alzheimer's disease, vascular dementia and ischemic cerebrovascular disease. *Dement. Geriatr. Cogn. Disord*. **9**, 243–245.

Johansson, B. (1992). Vascular mechanisms in hypertensive cerebrovascular disease. *Cardiovasc. Pharmacol*. **19**, S11–S15.

Jorm, A. F., and Jolley, D. (1998). The incidence of dementia: A meta-analysis. *Neurology* **51**, 728–733.

Judd, B. W., Meyer, J. S., Rogers, R. L., Gandhi, S., Tanahashi, N., Mortel, K. F., and Tawaklna, T. (1986). Cognitive perfromance correlates with cerebrovascular impairments in multi-infarct dementia. *J. Am. Geriatr. Soc*. **34**, 355–360.

Kase, C. S., Wolf, P. A., Kelly-Hayes, M., Kannel, W. B., Beiser, A., and D'Agostino, R. B. (1998). Intellectual decline after stroke. The Framingham study. *Stroke* **29**, 805–812.

Katzman, R., Zhang, M. Y., Chen, P. J., Gu, N., Jiang, S., Saitoh, T., Chen, X., Klauber, M., Thomas, R. G., Liu, W. T., and Yu, E. S. (1997). Effects of apolipoprotein E on dementia and aging in the Shanghai Survey of Dementia. *Neurology* **49**, 779–785.

Kawamata, J., Tanaka, S., Shimohama, S., Ueda, K., and Kimura, J. (1994). Apolipoprotein E polymorphism in Japanese patients with Alzheimer's disease or vascular dementia. *J. Neurol. Neurosurg. Psychiatry* **57**, 1414–1416.

Kay, D. W. K. (1999). New knowledge on the epidemiology of dementia. *Rev. Clin. Gerontol*. **9**, 163–182.

Kertesz, A., and Clydesdale, S. (1994). Neuropsychological deficits in vascular dementia vs Alzheimer's disease. Frontal lobe deficits prominent in vascular dementia. *Arch. Neurol. (Chicago)* **51**, 1226–1231.

Kittner, B. (1999). Clinical trials of propentofylline in vascular dementia. *Alzheimer Dis. Assoc. Disord.* **13**(Suppl. 3), S166–S171.

Kittner, B., Rossner, M., and Rother, M. (1997). Clinical trials in dementia with propentofylline. *Ann. N. Y. Acad. Sci.* **826**, 307–316.

Kokmen, E., Whisnant, J. P., O'Fallon, W. M., Chu, C. P., and Beard, C. M. (1996). Dementia after ischemic stroke: A population-based study in Rochester, Minnesota (1960–1984). *Neurology* **46**, 154–159.

Laakso, M. P., Partanen, K., Riekkinen, P., Lehtovirta, M., Helkala, E. L., Hallikainen, M., Hanninen, T., Vainio, P., and Soininen, H. (1996). Hippocampal volumes in Alzheimer's disease, Parkinson's disease with and without dementia, and in vascular dementia: An MRI study. *Neurology* **46**, 678–681.

Ladurner, G., Iliff, L. D., and Lechner, H. (1982). Clinical factors associated with dementia in ischaemic stroke. *J. Neurol. Neurosurg. Psychiatry* **45**, 97–101.

Lamar, M., Podell, K., Carew, T. G., Cloud, B. S., Resh, R., Kennedy, C., Goldberg, E., Kaplan, E., and Libon, D. J. (1997). Perseverative behavior in Alzheimer's disease and subcortical ischemic vascular dementia. *Neuropsychology* **11**, 523–534.

Le Bars, P. L., Katz, M. M., Berman, N., Itil, T. M., Freedman, A. M., and Schatzberg, A. F. (1997). A placebo-controlled, double-blind, randomized trial of an extract of Ginkgo biloba for dementia. North American EGb Study Group. *JAMA, J. Am. Med. Assoc*. **278**, 1327–1332.

Letenneur, L., Commenges, D., Dartigues, J. F., and Barberger-Gateau, P. (1994). Incidence of dementia and Alzheimer's disease in elderly community residents of south-western France. *Int. J. Epidemiol*. **23**, 1256–1261.

Libon, D. J., Scanlon, M., Swenson, R., and Coslet, H. B. (1990). Binzwanger's disease: Some neuropsychological considerations. *J. Geriatr. Psychiatry Neurol*. **3**, 31–40.

Libon, D. J., Mattson, R. E., Glosser, G., and Kaplan, E. (1996). A nine word dementia version of the California Verbal Learning Test. *Clin. Neuropsychol*. **10**, 237–244.

Libon, D. J., Bogdanoff, B., Cloud, B. S., Skalina, S., Giovannetti, T., Gitlin, H. L., and Bonavita, J. (1998). Declarative and procedural learning, quantitative measures of the hippocampus, and subcortical white alterations in Alzheimer's disease and ischaemic vascular dementia. *J. Clin. Exp. Neuropsychol*. **20**, 30–41.

Lindsay, J., Hebert, R., and Rockwood, K. (1997). The Canadian Study of Health and Aging: Risk factors for vascular dementia. *Stroke* **28**, 526–530.

Liu, C. K., Miller, B. L., Cummings, J. L., Mehringer, C. M., Goldberg, M. A., Howng, S. L., and Benson, D. F. (1992). A quantitative MRI study of vascular dementia. *Neurology* **42**, 138–143.

Liu, C. K., Lai, C. L., Tai, C. T., Lin, R. T., Yen, Y. Y., and Howng, S. L. (1998). Incidence and subtypes of dementia in southern Taiwan: Impact of socio- demographic factors. *Neurology* **50**, 1572–1579.

Liu, H. C., Chou, P., Lin, K. N., Wang, S. J., Fuh, J. L., Lin, H. C., Liu, C. Y., Wu, G. S., Larson, E. B., White, L. R., Graves, A. B., and Teng, E. L. (1994). Assessing cognitive abilities and dementia in a predominantly illiterate population of older individuals in Kinmen. *Psychol. Med.* **24**, 763–770.

Liu, H. C., Fuh, J. L., Wang, S. J., Liu, C. Y., Larson, E. B., Lin, K. N., Wang, H. C., Chou, P., Wu, Z. A., Lin, C. H., Wang, P. N., and Teng, E. L. (1998). Prevalence and subtypes of dementia in a rural Chinese population. *Alzheimer Dis. Assoc. Disord.* **12**, 127–134.

Loeb, C., and Gandolfo, C. (1983). Diagnostic evaluation of degenerative and vascular dementia. *Stroke* **14**, 399–401.

Loeb, C., Gandolfo, C., and Bino, G. (1988). Intellectual impairment and cerebral lesions in multiple cerebral infarcts. A clinical computed tomography study. *Stroke* **19**, 560–565.

Loeb, C., Gandolfo, C., Croce, R., and Conti, M. (1992). Dementia associated with lacunar infarction. *Stroke* **23**, 1225–1229.

Looi, J. C. L., and Sachdev, P. S. (1999). Differentiation of vascular dementia from AD on neuropsychological tests. *Neurology* **53**, 670–678.

Marcusson, J., Rother, M., Kittner, B., Rossner, M., Smith, R. J., Babic, T., Folnegovic-Smalc, V., Moller, H. J., and Labs, K. H. (1997). A 12-month, randomized, placebo-controlled trial of propentofylline (HWA 285) in patients with dementia according to DSM III-R. The European Propentofylline Study Group. *Dement. Geriatr. Cogn. Disord.* **8**, 320–328.

Marin, D. B., Breuer, B., Marin, M. L., Silverman, J., Schmeidler, J., Greenberg, D., Flynn, S., Mare, M., Lantz, M., Libow, L., Neufeld, R., Altstiel, L., Davis, K. L., and Mohs, R. C. (1998). The relationship between apolipoprotein E, dementia, and vascular illness. *Atherosclerosis* **140**, 173–180.

Mast, H., Tatemichi, T. K., and Mohr, J. P. (1995). Chronic brain ischemia: The contributions of Otto Binswanger and Alois Alzheimer to the mechanisms of vascular dementia. *J. Neurol. Sci.* **132**, 4–10.

Masterman, D. L., Mendez, M. F., Fairbanks, L. A., and Cummings, J. L. (1997). Sensitivity, specificity, and positive predictive value of technetium 99-HMPAO SPECT in discriminating Alzheimer's disease from other dementias. *J. Geriatr. Psychiatry Neurol.* **10**, 15–21.

McGeer, P. L., Kamo, H., Harrop, P., McGeer, E. G., Martin, W. R. W., Pate, B. D., and Li, D. K. B. (1986). Comparison of PET, MRI and CT with pathology in a proven case of Alzheimer's disease. *Neurology* **36**, 1569–1574.

McPherson, S. E., and Cummings, J. L. (1996). Neuropsychological aspects of vascular dementia. *Brain Cogn.* **31**, 269–282.

Meyer, J., McClintic, K., Rogers, R., Sims, P., and Mortel, K. (1988). Aetiological considerations and risk factors for multi-infarct dementia. *J. Neurol. Neurosurg. Psychiatry* **51**, 1489–1487.

Meyer, J. S., Rogers, R. L., McClintic, K., Mortel, K. F., and Lotfi, J. (1989). Randomized clinical trial of daily aspirin therapy in multi-infarct dementia: A pilot study. *J. Am. Geriatr. Soc.* **37**, 549–555.

Meyer, J. S., Kawamura, J., and Terayama, Y. (1992). White matter lesions in the elderly. *J. Neurol. Sci.* **110**, 1–7.

Meyer, J. S., Muramatsu, K., Mortel, K. F., Obara, K., and Shirai, T. (1995a). Prospective CT confirms differences between vascular and Alzheimer's dementia. *Stroke* **26**, 735–742.

Meyer, J. S., Obara, K., Muramatsu, K., Mortel, K. F., and Shirai, T. (1995b). Cognitive performance after small strokes correlates with ischemia, not atrophy of the brain. *Dementia* **6**, 312–322.

Meyer, J. S., Shirai, T., and Akiyama, H. (1996). Neuroimaging for differentiating vascular from Alzheimer's dementias. *Cerebrovasc. Brain Metab. Rev.* **8**, 1–10.

Mielke, R., Pietrzyk, U., Jacobs, A., Fink, G. R., Ichimiya, A., Kessler, J., Herholz, K., and Heiss, W. D. (1994). HMPAO SPET and FDG PET in Alzheimer's disease and vascular dementia: Comparison of perfusion and metabolic pattern. *Eur. J. Nucl. Med.* **21**, 1052–1060.

Möbius, H. J. (1999). Pharmacologic rationale for memantine in chronic cerebral hypoperfusion, especially vascular dementia. *Alzheimer Dis. Assoc. Disord.* **13**(Suppl. 3), S172–S178.

Mori, E., Kazunari, I., Hashimoto, M., Imamura, T., Hirono, N., and Kitagaki, H. (1999). Role of functional brain imaging in the evaluation of vascular dementia. *Alzheimer Dis. Assoc. Disord.* **13**(Suppl. 3), S91–S101.

Moroney, J. T., Bagiella, E., Desmond, D. W., Hachinski, V., Molsa, P. K., Gustafson, L., Brun, A., Fischer, P., Erkinjuntti, T., Rosen, W., Paik, M. C., and Tatemitchi, T. K. (1997). Meta-analysis of the Hachinski Ischemic Score in pathologically verified dementias. *Neurology* **49**, 1096–1105.

Nyenhuis, D. L., and Gorelick, P. B. (1998). Vascular dementia: A contemporary review of epidemiology, diagnosis, prevention, and treatment. *J. Am. Geriatr. Soc.* **46**, 1437–1448.

Obadia, Y., Rotily, M., and Degrand-Guillaud, A. (1997). The PREMAP study: Prevalence and risk factors of dementia and clinically diagnosed Alzheimer's disease in Provence, France. *Eur. J. Epidemiol.* **13**, 247–253.

Obara, K., Meyer, J. S., Mortel, K. F., and Muramatsu, K. (1994). Cognitive declines correlated with decreased cortical volume and perfusion in dementia of Alzheimer's type. *J. Neurol. Sci.* **127**, 96–102.

O'Brien, J., Desmond, P., Ames, D., Schweitzer, I., Harrigan, S., and Tress, B. (1996). A magnetic resonance imaging study of white matter lesions in depression and Alzheimer's disease. *Br. J. Psychiatry* **168**, 477–485.

Olsson, Y., Brun, A., and Englund, E. (1996). Fundamental pathological lesions in vascular dementia. *Acta Neurol. Scand. Suppl.* **168**, 31–38.

Orgogozo, J. M., Dartigues, M. F., Lafont, S., Letenneur, L., Commenges, D., Salamon, R., Renand, S., and Breteler, M. B. (1997). Wine consumption and dementia in the elderly: A prospective community study in the Bordeaux area. *Rev. Neurol.* **153**, 185–192.

Padovani, A., Di Piero, V., Bragoni, M., Iacoboni, M., Gualdi, G. F., and Lenzi, G. L. (1995). Patterns of neuropsychological impairment in mild dementia: A comparison between Alzheimer's disease and multi-infarct dementia. *Acta Neurol. Scand.* **92**, 433–442.

Palumbo, B., Parnetti, L., Nocentini, G., Cardinali, L., Brancorsini, S., Riccardi, C., and Senin, U. (1997). Apolipoprotein-E genotype in normal aging, age-associated memory impairment, Alzheimer's disease and vascular dementia patients. *Neurosci. Lett.* **231**, 59–61.

Pantoni, L., and Inzitari, D. (1993). Hachinski's ischemic score and the diagnosis of vascular dementia: A review. *Ital. J. Neurol. Sci.* **14**, 539–546.

Pantoni, L., Carosi, M., Amigoni, S., Mascalchi, M., and Inzitari, D. (1996). A preliminary open trial with nimodipine in patients with cognitive impairment and leukoaraiosis. *Clin. Neuropharmacol.* **19**, 497–506.

Pirttila, T., Lehtimaki, T., Rinne, J., Mattila, K., Frey, H., and Nikkari, T. (1996). The frequency of apolipoprotein E4 allele is not increased in patients with probable vascular dementia. *Acta Neurol. Scand.* **93**, 352–354.

Pohjasvaara, T., Erkinjuntti, T., Ylikoski, R., Hietanen, M., Vataja, R., and Kaste, M. (1998). Clinical determinants of poststroke dementia. *Stroke* **29**, 75–81.

Polinsky, R. J., Noble, H., and DiChiro, G. (1987). Dominantly inherited Alzheimer's disease: cerebral glucose metabolism. *J. Neurol., Neurosurg. Psychiatry* **50**, 752–757.

Prencipe, M., Casini, A. R., Ferretti, C., Lattanzio, M. T., Fiorelli, M., and Culasso, F. (1996). Prevalence of dementia in an elderly rural population: Effects of age, sex, and education. *J. Neurol., Neurosurg. Psychiatry* **60**, 628–633.

Prencipe, M., Ferretti, C., Casini, A. R., Santini, M., Giubilei, F., and Culasso, F. (1997). Stroke and dementia: Results of a population survey. *Stroke* **28**, 531–536.

Pullicino, P., Benedict, R. H., Capruso, D. X., Vella, N., Withiam-Leitch, S., and Kwen, P. L. (1996). Neuroimaging criteria for vascular dementia. *Arch. Neurol. (Chicago)* **53**, 723–728.

Rajkumar, S., Kumar, S., and Thara, R. (1997). Prevalence of dementia in a rural setting: A report from India. *Int. J. Geriatr. Psychiatry* **12**, 702–707.

Rapport, S. I. (1991). Positron emission tomography in Alzheimer's disease in relation to disease pathogenesis: A critical review. *Cerebrovasc. Brain Metab. Rev.* **3**, 297–335.

Ratcliffe, P. J., and Wilcock, C. K. (1985). Cerebrovascular disease in dementia: The importance of atrial fibrillation. *Postgrad. Med. J.* **61**, 201–204.

Rocca, W. A., Hofman, A., Brayne, C., Breteler, M. M., Clarke, M., Copeland, J. R., Dartigues, J. F., Engedal, K., Hagnell, O., Heeren, T. J., Jonker, C., Lindesay, J., Lobo, A., Maun, A. H., Mölsä, P. K., Morgan, K., O'Canncr, D. W., du Silva Droux, A., Sulkava, R., Kay, D. W. K., and Amaducci L. (1991). The prevalence of vascular dementia in Europe: Facts and fragments from 1980–1990 studies. EURODEM-Prevalence Research Group. *Ann. Neurol.* **30**, 817–824.

Rockwood, K., Ebly, E., Hachinski, V., and Hogan, D. (1997). Presence and treatment of vascular risk factors in patients with vascular cognitive impairment. *Arch. Neurol. (Chicago)* **54**, 33–39.

Roman, G. C. (1999). A historical review of the concept of vascular dementia: Lessons from the past for the future. *Alzheimer Dis. Assoc. Disord.* **13**(Suppl. 3), S4–S8.

Román, G. C., Tatemichi, T. K., Erkinjuntti, T., Cummings, J. L., Masdeu, J. C., Garcia, J. H., Amaducci, L., Orgogozo, J. M., Brun, A., Hofman, A., Moody, D. M., O'Brien, M. D., Yamaguchi, T., Grafman, J., Drayer, B. P., Benett, D. A., Fisher, M., Ogata, J., Kokmen, E., Bermejo, F., Wolf, P. A., Gorelick, P. B., Bick, K. L., Pajecau, A. K., Bell, M. A., DeCorli, C., Gulebras, A., Korczyn, A. D., Boyousslausky, J., Hartman, A., and Scheinberg, P. (1993). Vascular dementia: Diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. *Neurology* **43**, 250–260.

Rosen, W. G., Terry, R. D., Fuld, P. A., Katyman, R., and Peck, A. (1980). Pathological verification of the ischemic score in diferentiation of dementias. *Ann. Neurol.* **7**, 486–488.

Ross, G. W., Petrovitch, H., White, L. R., Masaki, K. H., Li, C. Y., Curb, J. D., Yano, K., Rodriguez, B. L., Foley, D. J., Blanchette, P. L., and Havlik, R. (1999). Characterization of risk factors for vascular dementia: The Honolulu- Asia Aging Study. *Neurology* **53**, 337–343.

Rossi, R., Inzitari, D., Pantoni, L., del Ser, T., Erkinjuntti, T., Wallin, A., Bianchi, C., Badenas, J. M., and Beneke, M. (1999). Nimodipine in subcortical vascular dementia trial. *Alzheimer Dis. Assoc. Disord.* **13**(Suppl. 5), S159–S165.

Saletu, B., Paulus, E., Linzmayer, L., Anderer, P., Semlitsch, H. V., Grunberger, J., Wicke, L., Neuhold, A., and Podreka, I. (1995). Nicergoline in senile dementia of Alzheimer type and multi-infarct dementia: A double-blind, placebo-controlled, clinical and EEG/ERP mapping study. *Psychopharmacology (Berlin)* **117**, 385–395.

Schmidt, R. (1992). Comparison of magnetic resonance imaging in Alzheimer's disease, vascular dementia and normal aging. *Eur. Neurol.* **32**, 164–169.

Schmidt, T., Mechtler, L., Kinkel, P. R., Fazekas, F., and Kinkel, W. R. (1992). Cognitive impairment after hemispheric stroke: A clinical and computed tomogrpahic study. *Neurology* **422**, 176.

SHEP Cooperative Research Group. (1991). Prevention of stroke by antihypertensive drug treatment in older persons with isolated systolic hypertension. *JAMA, J. Am. Med. Assoc.* **265**, 3225–3264.

Skoog, I. (1994). Risk factors for vascular dementia: A review. *Dementia* **5**, 137–144.

Skoog, I. (1998). Status of risk factors for vascular dementia. *Neuroepidemiology* **17**, 2–9.

Skoog, I., Nilsson, L., Palmertz, B., Andreasson, L. A., and Svanborg, A. (1993). A population-based study of dementia in 85-year-olds. *N. Engl. J. Med.* **328**, 153–158.

Skoog, I., Palmertz, B., and Andreasson, L. A. (1994). The prevalence of white matter lesions on computed tomography of the brain in demented and non demented 85-year olds. *J. Geriatr. Psychiatry Neurol.* **7**, 169–175.

Skoog, I., Lernfelt, B., Landahl, S., Palmertz, B., Andreasson, L. A., Nilsson, L., Persson, G., Oden, A., and Svanborg, A. (1996). 15-year longitudinal study of blood pressure and dementia. *Lancet* **347**, 1141–1145.

Skoog, I., Hesse, C., Aevarsson, O., Landahl, S., Wahlström, J., Fredman, P., and Blennow, K. (1998). A population study of apoE genotype at the age of 5: Relation to dementia, cerebrovascular disease, and mortality. *J. Neurol. Neurosurg. Psychiatry* **64**, 37–43.

Slooter, A. J., Tang, M. X., van Duijn, C. M., Stern, Y., Ott, A., Bell, K., Breteler, M., Van Broeckhoven, C., Tatemichi, T. K., Tycko, B., Hofman, A., and Mayeux, R. (1997). Apolipoprotein E $\epsilon 4$ and the risk of dementia with stroke. A population-based investigation. *JAMA, J. Am. Med. Assoc.* **277**, 818–821.

Staessen, J. A., Fagard, R., Thijs, L. Celis, H., Arabidze, G. G., Birkenhager, W. H., Bulpitt, C. J., de Leeuw, P. W., Dollery, C. T., Fletcher, A. E., Fosette, F., Leenetti, G., Nachev, C., O'Brien, E. T., Rosefeld, J., Roolicio, J. L., Tuomilehto, J., and Zancletti, A. (1997). Randomized double-blind comparison of placebo and active treatment of older patients with isolated systolic hypertension. *Lancet* **350**, 757–764.

Starkstein, S. E., Sabe, L., Vazquez, S., Teson, A., Petracca, G., Chemerinski, E., Di Lorenzo, G., and Leiguarda, R. (1996). Neuropsychological, psychiatric, and cerebral blood flow findings in vascular dementia and Alzheimer's disease. *Stroke* **27**, 408–414.

Stengard, J. H., Pekkanen, J., Sulkava, R., Ehnholm, C., Erkinjuntti, T., and Nissinen, A. (1995). Apolipoprotein E polymorphism, Alzheimer's disease and vascular dementia among elderly Finnish men. *Acta Neurol. Scand.* **92**, 297–298.

Sulkava, R., Kainulainen, K., Verkkoniemi, A., Niinisto, L., Sobel, E., Davanipour, Z., Polvikoski, T., Haltia, M., and Kontula, K. (1996). APOE alleles in Alzheimer's disease and vascular dementia in a population aged 85+. *Neurobiol. Aging* **17**, 373–376.

Tamaki, K., Nakai, M., Yokota, T., and Ogata, J. (1995). Effects of aging and chronic hypertension on cerebral blood flow and cerebrovascular CO2 reactivity in the rat. *Gerontology* **41**, 11–17.

Tatemichi, T. K., Foulkes, M. A., Mohr, J. P., Hewitt, J. R., Hier, D. B., Price, T. R., and Wolf, P. A. (1990). Dementia in stroke survivors in the Stroke Data Bank cohort. Prevalence, incidence, risk factors, and computed tomographic findings. *Stroke* **21**, 858–866.

Tatemichi, T. K., Paik, M., Bagiella, E., Desmond, D. W., Stern, Y., Sano, M., Hauser, W. A., and Mayeux, R. (1994). Risk of dementia after stroke in a hospitalized cohort: Results of a longitudinal study. *Neurology* **44**, 1885–1891.

Tilvis, R. S., Strandberg, T. E., and Juva, K. (1998). Apolipoprotein E phenotypes, dementia and mortality in a prospective population sample. *J. Am. Geriatr. Soc.* **46**, 712–715.

Tomlinson, B. E., Blessed, G., and Roth, M. (1968). Observations on the brains of demented old people. *J. Neurol. Sci.* **7**, 331–356.

Treves, T. A., and Korczyn, A. D. (1999). Denbufylline in dementia: A double-blind controlled study. *Dement. Geriatr. Cogn. Disord.* **10**, 505–510.

Treves, T. A., Bornstein, N. M., Chapman, J., Klimovitzki, S., Verchovsky, R., Asherov, A., Veshchev, I. O., and Korczyn, A. D. (1996). APOE-$\epsilon 4$ in patients with Alzheimer disease and vascular dementia. *Alzheimer Dis. Assoc. Disord.* **10**, 189–191.

Verhey, F. R., Lodder, J., Rozendaal, N., and Jollès, J. (1996). Comparison of seven sets of criteria used for the diagnosis of vascular dementia. *Neuroepidemiology* **15**, 166–172.

Wetterling, T., Kanitz, R. D., and Borgis, K. J. (1994). The ICD-10 criteria for vascular dementia. *Dementia* **5**, 5185–5188.

Wetterling, T., Kanitz, R. D., and Borgis, K. J. (1996). Comparison of different diagnostic criteria for vascular dementia (ADDTC, DSM-IV, ICD-10, NINDS-AIREN). *Stroke* **27**, 30–36.

Wieringa, G. E., Burlinson, S., Rafferty, J. A., Gowland, E., and Burns, A. (1997). Apolipoprotein E genotypes and serum lipid levels in Alzheimer's disease and multi-infarct dementia. *Int. J. Geriatr. Psychiatry* **12**, 359–362.

Woo, J. I., Lee, J. H., Yoo, K. Y., Kim, C. Y., Kim, Y. I., and Shin, Y. S. (1998). Prevalence estimation of dementia in a rural area of Korea. *J. Am. Geriatr. Soc*. **46**, 983–987.

Yoshitake, T., Kiyohara, Y., Kato, I., Ohmura, T., Iwamoto, H., Nakayama, K., Ohmori, S., Nomiyama, K., Kawano, H., Ueda, K., Sneishi, K., Tsuneyoshi, M., and Fujishima, M. (1995). Incidence and risk factors of vascular dementia and Alzheimer's disease in a defined elderly Japanese population: The Hisayama Study. *Neurology* **45**, 1161–1168.

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12

Frontotemporal Dementias: From Classification Problems to Pathogenetic Uncertainties

The term frontotemporal dementia has been used to classify several clinical syndromes previously described based on a relatively homogeneous symptomatology. Patients in this diagnostic group present with an insidious and gradual change in personality and social conduct, early deficits of mental manipulation, sequencing and hierarchical organization processes, frontal-type amnesia, contrasting with preserved orientation, visuoconstructive and visuospatial abilities. Frontotemporal dementia may represent more than 20% of degenerative dementias and is currently considered as the third most common cause of dementia after Alzheimer's disease and Lewy body dementia. Biochemical and molecular genetic studies have made it possible to identify at least three biologically homogeneous subgroups of frontotemporal dementias: typical frontotemporal dementia cases with no tau or ubiquitin-positive neuronal and glial inclusions, two tauopathies, namely Pick's disease and frontotemporal dementia with parkinsonism linked to chromosome 17, and one ubiquitin-related disorder, the frontotemporal dementia with motor neuron disease. We provide here a detailed overview of current concepts regarding the clinical characteristics and etiopathogenesis of these conditions.

The evolution of the nomenclature of frontotemporal dementias (FTD) is complex and confusing. Arnold Pick (1892) provided the first detailed clinical description of FTD. Alois Alzheimer (1911) described the neuropathological lesions characteristic of Pick's disease and since then this term has been used to classify most patients with progressive early-onset dementia with massive deterioration of executive functions. Since the 1980s, several names have been proposed pointing to the predominant involvement of the frontal cortex, in contrast to the more frequent biparietal damage in Alzheimer's disease (AD), or the absence of specific histopathological lesions. In 1994, a consensus statement adopted the term FTD to cover a wide and highly heterogeneous nosographic spectrum, which includes demented patients with symptoms suggestive of either frontal lobe dysfunction, such as personality change or disinhibited behavior, or anterior temporal lobe dysfunction, such as hyperorality, associated or not with parkinsonism, motor neuron disease, and focal cortical degeneration, including progressive nonfluent aphasia and semantic dementia (Brun *et al*., 1994). Despite its evident usefulness in increasing clinical interrater reliability, the concept of FTD masks fundamental etiopathogenetic differences mainly in respect to the presence of tau pathology in neurons and glial cells. This chapter defines the clinical and neuropsychological features of the group of FTDs and provides a critical overview of recent progress in morphological, biochemical, and molecular genetic characterization of FTD subtypes.

I. Diagnosis of FTD: Epidemiological and Clinical Considerations

Frontotemporal dementia is the third most common cause of cortical dementia following AD and Lewy body disease (Neary *et al*., 1998). Estimates of its incidence vary depending on theoretical positions and nomenclature used. However, FTD is thought to represent more than 20% of degenerative dementias, raising the ratio of FTD:AD to 1:4 (Brun, 1993; Knopman, 1993; Kertesz and Munoz, 1998; Neary *et al*., 1998). The onset of disease is most commonly before the age of 65 years, yet sporadic cases with late-onset have been reported (Giannakopoulos *et al*., 1995). A positive family history of a similar disorder in a first-degree relative has been reported in as many as 50% of patients (Gustafson, 1987; Neary *et al*., 1998). Mutations in the tau gene on chromosome 17 and linkage to

chromosome 3 have been identified in a few families (Brown *et al*., 1995; Hutton *et al*., 1998; Poorkaj *et al*., 1998; Spillantini *et al*., 1998a). In particular, the identification of several intronic and exonic mutations in the tau gene in cases with FTD and parkinsonism has led to the definition of a new clinical subgroup of FTD referred to as frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) (Hutton *et al*., 1998; Poorkaj *et al*., 1998; Spillantini *et al*., 1998a).

Even though FTD can be often difficult to differentiate clinically from AD, the diagnosis is based on the presence of early personality and behavioral changes, which remain prominent in the course of the disease, symmetric or asymmetric cerebral atrophy confined to the frontal and anterior temporal cortex, cortical hypometabolism in the same areas, and normal electroencephalography (Elfgren *et al*., 1993; Mendez *et al*., 1993; Brun *et al*., 1994; Neary *et al*., 1998). The most common neurobehavioral syndrome in FTD is an insidious and gradual change in personality and social conduct characterized by disinhibition, mood disorders, irritability and aggressiveness contrasting with incoherent joviality, impulsivity, early loss of social awareness, aberrant behaviors, bulimia, and hyperorality (Brun *et al*., 1994; Neary *et al*., 1998). Global intelligence, as measured by the Wechsler Adult Intelligence Scale, is relatively preserved at the beginning of the disease without the systematic dissociation between verbal and performance IQ observed in AD (Miller *et al*., 1991). Patients are usually oriented in time and place and provide correct autobiographical information. Short-term memory is equally affected in both FTD and AD at the same degree of severity, except in its visuospatial component, which appears to be better preserved in FTD. Free recall is poor as in AD, but the performance is improved by the use of specific, directed questions rather than open-ended questions and by cues and multiple-choice alternative responses. This pattern of memory impairment is consistent with a ''frontal-type'' amnesia and reflects difficulties in retrieval and organization of the information rather than a pure storage deficit (for review, see Pasquier, 1999). Language deficits, including verbal stereotypia, echolalia, and progressive language impoverishment leading to mutism or press of speech, are frequent in FTD. Conversely, comprehension, reading, and naming skills are preserved (Mendez *et al*., 1993; Neary and Snowden, 1996; Pasquier, 1999). The most marked neuropsychological feature of FTD is the failure of mental manipulation, sequencing, and hierarchical organization processes. Performances in the Trail Making Test, a classical test of executive functions, is significantly worst in FTD than in AD cases. Verbal fluency is also impaired (Miller *et al*., 1991; Pasquier *et al*., 1995). In contrast, visuospatial and visuoconstructive abilities, which are affected early in AD, remain relatively intact in FTD (Neary *et al*., 1998). It should be kept in mind, however, that FTD patients may perform poorly on formal memory, perceptual, and spatial tests as a consequence of increased distractibility, poor self-control, and lack of concern for accuracy. In these cases, correct responses by cueing and fluctuations in test performance support the diagnosis of FTD (Neary *et al*., 1998; Pasquier, 1999). In most typical FTD cases, parkinsonian signs emerge only during late stages, but in FTDP-17, there are early key features of parkinsonism, such as muscular rigidity, slowness of movement, postural instability, and rarely tremor. These symptoms do not respond to pharmacologic treatment, which is usually successful in ameliorating the symptoms of idiopathic Parkinson's disease. Eye movement abnormalities and early-onset incontinence are also seen in FTDP-17 cases (Knopman *et al*., 1990; Foster *et al*., 1997).

Two other prototypic clinical sydromes are part of the FTD spectrum: primary progressive aphasia and semantic dementia (Mesulam, 1982; Weintraub *et al*., 1990; Hodges *et al*., 1992; Snowden *et al*., 1992). Primary progressive aphasia is a disorder of expressive language without marked behavioral abnormalities, which do not necessarily evolve to dementia. It is characterized by nonfluent spontaneous speech with numerous agrammatisms and phonemic paraphasias, increased word retrieval latency, impaired repetition, alexia, and agraphia. Verbal comprehension, visuoconstructive and visuospatial abilities, and memory are preserved, contrasting with the classical description of AD (Mesulam, 1982; Weintraub *et al*., 1990; Snowden *et al*., 1992). In semantic dementia, there is a severe loss of both verbal and visual semantic concepts with a preservation of other linguistic abilities. Speech is fluent and gramatically correct but the content is limited to a repetitive conversational repertoire. The loss of word meaning is compensated by the use of broad generic terms and semantic paraphasias. Prosopagnosia and associative visual agnosia are frequent, resulting from impaired manipulation of visual semantics. Conversely, perceptual matching, praxis, and memory tasks are performed normally (Hodges *et al*., 1992).

The association of Pick's disease and motor neuron disease was described many years ago (Mitsuyama and Takamiya, 1979). The rare association of typical FTD and progressive aphasia with motor neuron disease was recognized later, identifying the dementia as FTD with motor neuron disease (Neary *et al*., 1990; Ferrer *et al*., 1991; Sam *et al*., 1991; Caselli *et al*., 1993). The clinical evolution is usually rapid with early death from dysphagia. In these cases, the development of motor neuron disease symptomatology in patients with behavioral or language disorder would support the diagnosis of FTD or progressive aphasia, respectively. Although the coexistence of motor neuron disease and FTD is an epidemilogically marginal phenomenon, studies demonstrated that its biochemical background differs from that of typical FTD cases (for review, see Ince *et al*., 1998), suggesting that FTD with motor neuron disease represents a biologically separate entity.

II. Morphological Basis of FTD

Until the mid-1990s, the most widely accepted neuropathological definition of FTD was based on the presence of circumscribed frontal and/or anterior temporal atrophy and nonspecific histopathological changes, such as neuronal loss, spongiosis, and gliosis. Pick's disease had an equivocal place, as it was considered by some authors as a simple variant of FTD and by others as a true clinical entity (Knopman *et al*., 1990; Brun *et al*., 1994; Giannakopoulos *et al*., 1995). Progress in the biochemical characterization of filamentous nerve cell inclusions, as well as the detailed analysis of tau gene expression in FTDP-17 cases, has contributed to change this

TABLE 12.1 Tau Pathology Patterns in FTD Subtypes and Selected Tauopathies[a]

Disorder	Neuronal inclusion	Glial inclusion	Tau protein electrophoretic profile
Typical FTD	–	–	Six tau isoforms 55, 64, 69 kDa
FTDP-17	Globose NFT	+/–	55, 64, 69 kDa 64, 69 kDa
Pick's disease	PB	+	55, 64 kDa
FTD with MND	–	–	Six tau isoforms 55, 64, 69 kDa
CBD	Coiled/thread like	AP	64, 69 kDa
PSP	Globose NFT	TA	64, 69 kDa
AD	NFT	Rare	55, 64, 69 kDa

[a]PB, Pick bodies; CBD, corticobasal degeneration; MND, motor neuron disease, PSP, progressive supranuclear palsy; AP, astrocytic plaques; TA, tufted astrocytes. See text for details.

simplistic point of view radically. Although a global understanding of the molecular background of FTD has yet to be achieved, one can schematically consider that this group includes a large but poorly defined subgroup of typical FTD cases, two tauopathies, namely Pick's disease and FTDP-17, and one ubiquitin-related disorder, FTD with motor neuron disease (Table 12.1). The morphological and biochemical characteristics of each of these categories are summarized next.

A. Typical FTD

The neuropathological features of FTD consist of moderate to severe atrophy confined to the frontal and temporopolar regions and ventricular dilation (Brun, 1993; Mann *et al*., 1993; Neary *et al*., 1993; Fig. 12.1 top). The atrophy is symmetrical, but cases with asymmetrical gyral atrophy have also been reported (Knopman *et al*., 1990; Neary *et al*., 1993). According to the new neuropathological criteria for FTD, frontal and temporopolar cortices show a differential degree of loss of pyramidal cells, mainly in layers II and III, whereas those of layer V are affected mildly. Spongiform degeneration of layers II and III is often seen along with moderate to severe astrocytic gliosis in the gray and white matter (Brun, 1993; Mann *et al*., 1993; Brun *et al*., 1994). These lesions spread frequently to the insula and cingulate gyrus, whereas the posterior temporal, parietal, and occipital cortex are devoid of lesions in most cases. Although the involvement of the hippocampus, amygdala, and subcortical regions is mild in most patients with FTD, several authors reported cases with prominent cell loss and astrogliosis in the striatum, thalamus, and limbic structures and proposed the existence of possible neuropathological subgroups of this disorder (Knopman *et al*., 1990; Knopman, 1993; Neary *et al*., 1993). Such a possibility is supported by a neuropathological study of 32 FTD cases, which defined four subgroups (Giannakopoulos *et al*., 1995). The first group showed moderate to severe neuron loss and gliosis in the frontal and/or temporopolar cortex without

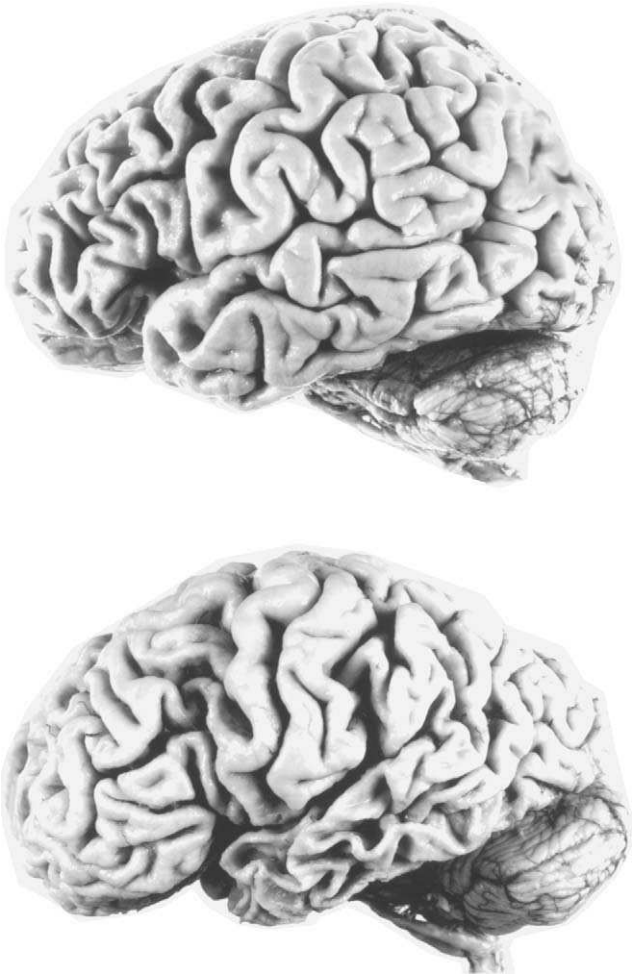

FIG. 12.1. Lateral surfaces of the cerebral cortex in a typical FTD case (top) and a Pick's disease case (bottom). Note the marked frontal atrophy in the FTD case as compared to the ''knife-edge'' atrophy of the temporopolar cortex in the Pick's disease case.

subcortical involvement. In the second group, neocortical cell loss was widespread and the striatum and substantia nigra displayed differential degrees of gliosis but no neuron loss. Another group of patients exhibited lesion distribution comparable to that observed in the second group, but with severe neuron loss in at least one subcortical region. Finally, a few cases were characterized by the preservation of the pyramidal neurons in the neocortex and variable subcortical changes (Fig. 12.2). Despite these differences in the topography of pathological changes, the crucial criterion for the diagnosis of FTD is the absence of tau- or ubiquitin-positive distinctive subcellular dysmorphic features, such as Alzheimer changes (except mild ones compatible with age), Pick bodies, Lewy bodies, and swollen neurons (Table 12.1; for review see Mann, 1998). It is worth noting that there is no clear relationship between the regional distribution of cell loss and clinical syndromes within the FTD spectrum. For instance, a positron emission tomography study has revealed a marked hypometabolism in the left posterior inferior temporal gyrus in cases with semantic dementia and significant anterolateral temporal atrophy. In these cases, clinical symptomatology

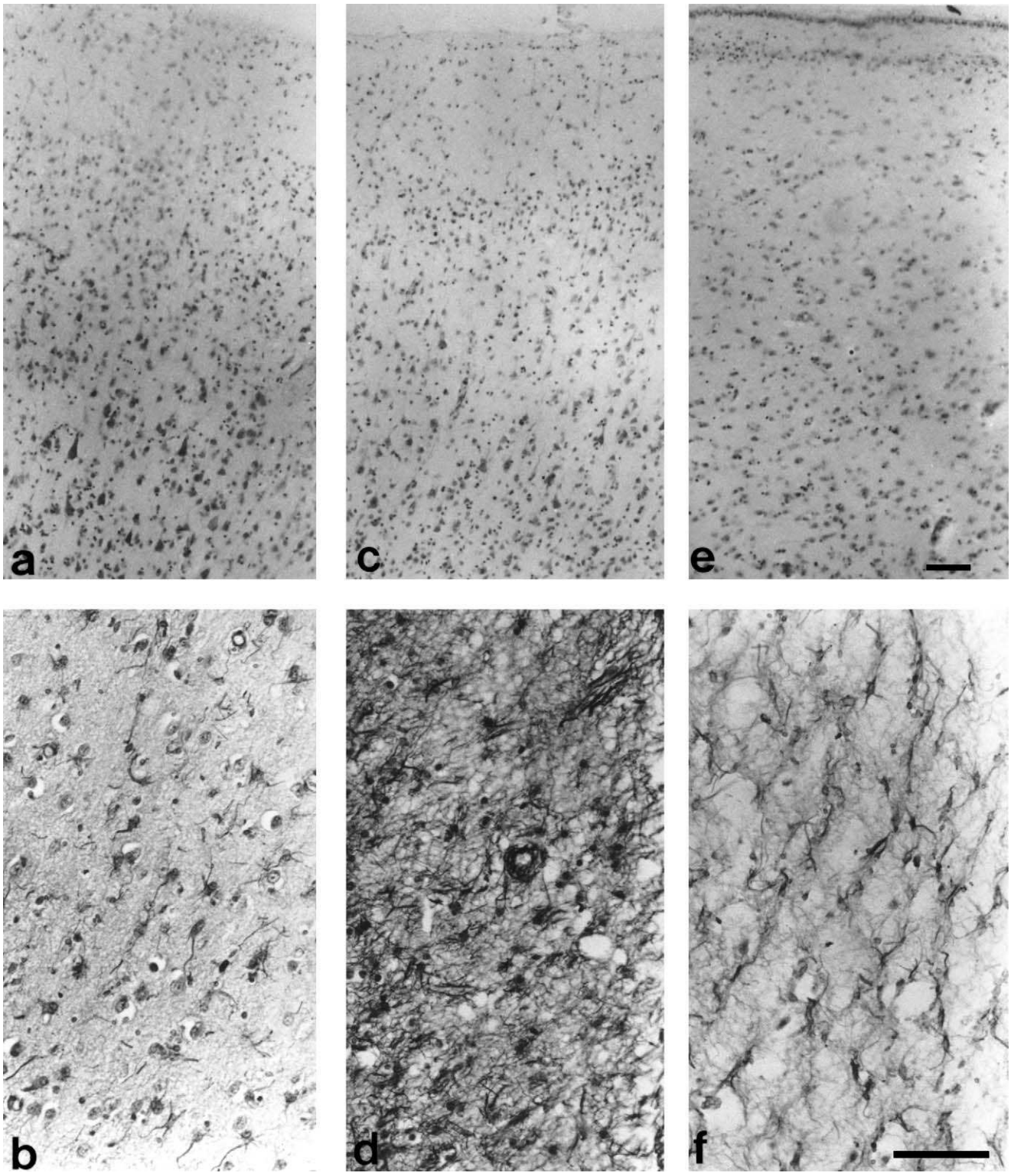

FIG. 12.2. Representative examples of pathologic changes in the frontal cortex (a–d) and striatum (e and f) in three typical FTD cases (a and b, c and d, e and f). Note the mild neuron loss in layers II and III (a) and the mild reactive astrocytosis (b) in the first case and the moderate loss of nerve cells (c) and the presence of both moderate gliosis and spongiform changes (d) in the second case. There is a marked depletion of striatal neurons (e) and mild reactive gliosis (f) in the third case. Materials were stained with cresyl violet (a, c, e) and Holzer (b, d, f) stains. Scale bar (on f): 100 μm. Reproduced with permission from Giannakopoulos *et al*., (1995).

depends on the disruption of temporal lobe circuits in regions distant from the structural damage (Mummery *et al*., 1999).

B. FTD-Related Tauopathies

The term ''tauopathies'' is applied to a highly heterogeneous group of disorders sharing filamentous tau protein deposits as their main pathological feature. Except for Pick's disease and FTDP-17 (Fig. 12.3), this group includes AD, Down syndrome, ''tangle-only'' dementia, argyrophilic grain disease, corticobasal degeneration, progressive supranuclear palsy, and several rare conditions, such as amyotrophic lateral sclerosis/parkinsonism dementia complex of Guam, Niemann–Pick disease type C, myotonic dystrophy, subacute sclerosing panencephalitis, and postencephalitic parkinsonism (for review, see Tolnay and Probst, 1999). Early language impairment, frontal symptoms, and personality changes support a diagnosis of Pick's disease and FTDP-17, whereas prominent akinetic-rigid syndrome, extrapyramidal motor dysfunction, apraxia, dystonia, and postural abnormalities suggest the presence of an atypical parkinsonian tauopathy such as corticobasal degeneration, progressive supranuclear palsy, and postencephalitic parkinsonism. However, the differential diagnosis is often very difficult. Extrapyramidal and subcortical involvement is well documented in Pick's disease, and clinical cases of corticobasal degeneration have been described with pathological features of Pick's disease (Riley *et al*., 1990; Rinne *et al*., 1994; Feany *et al*., 1996). Neuropathologically, progressive supranuclear palsy and postencephalitic parkinsonism are characterized by substantial neurofibrillary tangle (NFT) formation in the pallidum, subthalamic nucleus, substantia nigra or pons, and, to a lesser extent, in the striatum, oculomotor nuclei, medulla, and cerebellum (Hauw *et al*., 1994). In contrast to any other disease showing NFT, electron microscopy studies in progressive supranuclear palsy have revealed the coexistence of paired helical filaments (PHF) of the Alzheimer type and 15 nm straight filaments (Cervós-Navarro and Schumacher, 1994). Corticobasal degeneration is defined by the presence of achromatic neurons and tau-immunoreactive inclusions in both substantia nigra and neocortical layer II and by severe neuronal loss in the substantia nigra and basal ganglia (Hauw *et al*., 1994; Litvan *et al*., 1996). Although the introduction of NINCDS neuropathological criteria for progressive supranuclear palsy and related disorders has improved the accuracy of their neuropathological diagnosis, the differential diagnosis of these disorders remains challenging (Hauw *et al*., 1994; Litvan *et al*., 1996).

1. FTDP-17

The only neuropathological criterion that makes it possible to distinguish this condition from typical FTD is the abundant filamentous tau deposits in neurons and, in some cases, glial cells (Figs. 12.3a–12.3c; for review, see Foster *et al*., 1997; Spillantini *et al*., 1998b). The type of pathology in FTDP-17 depends largely on the location of mutations in the tau gene

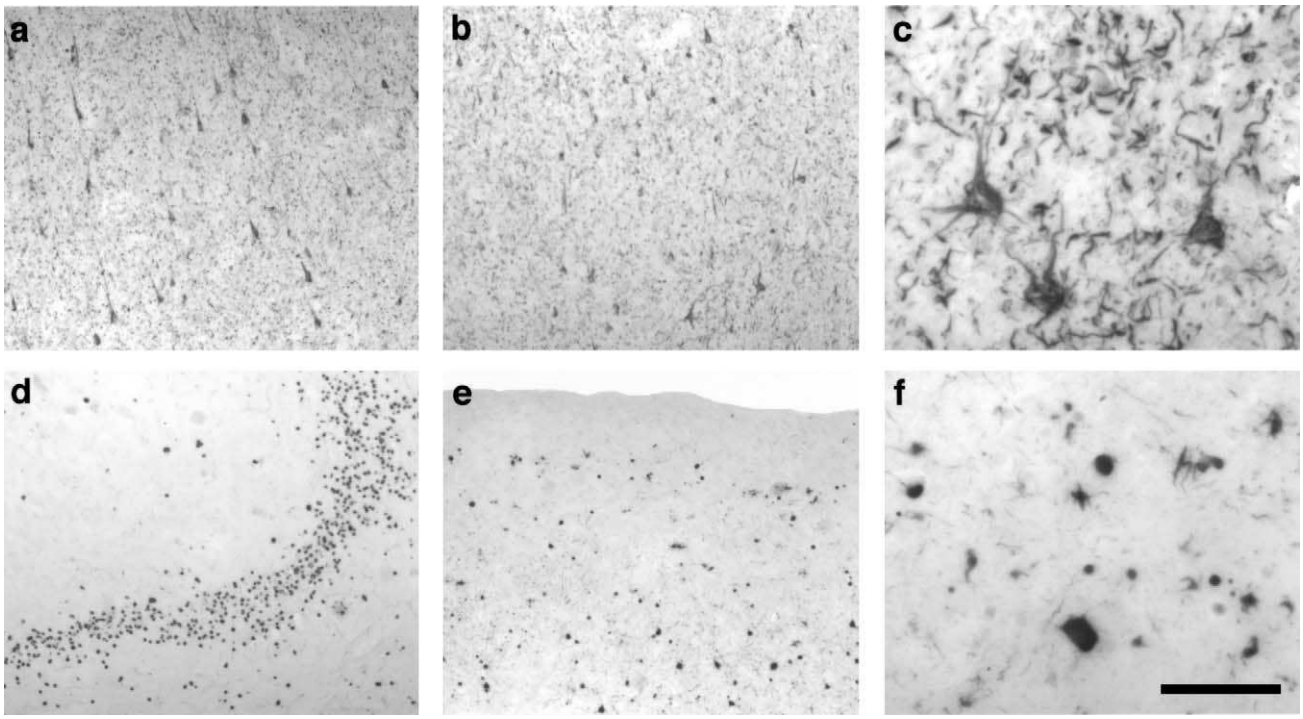

FIG. 12.3. Examples of characteristic lesion types and distribution in FTDP-17 (a–c) and Pick's disease (d–f). FTDP-17 displays moderate to high densities of NFT in the hippocampal formation (a, CA1 field) and neocortex (b, inferior temporal cortex). (c), A higher magnification of typical NFT and neurites stained with an antibody to hyperphosphorylated tau proteins in the temporal neocortex of an FTDP-17 case. Pick's disease is characterized by very high numbers of Pick bodies in the granule cell layer of the dentate gyrus (d) and in layers II and VI of the neocortex (e, frontopolar region). (f) Characteristic features of Pick bodies in the frontal neocortex as well as glial tangles, stained with an anti-tau antibody. Scale bar (on f): 150 μm (a, b, d, e) and 50 μm (c, f).

TABLE 12.2 Tau Inclusions in FTDP-17[a]

Mutation site	Biochemistry	EM	Pathogenesis
Introns (5′ splice site)	4 > 3 repeat tau	Twisted ribbons	Gain of function
Exons 9, 12, 13	Normal 4/3 repeat tau ratio	AD-PHF and SF	Loss of function
Exon 10	Mutant 4 repeat tau	Twisted ribbons	Loss of function

[a]EM, electron microscopy; PHF, paired helical filaments; SF, straight filaments. See text for details.

(Table 12.2). This gene contains 15 exons, and the six tau isoforms are encoded by 11 of them by alternative splicing of exons 2, 3, and 10 (Andreadis *et al*., 1992). Exons 9–13 encode four microtubule-binding repeats of 31 or 32 amino acids in the carboxy-terminal half of the tau molecule. The alternative splicing of exon 10 generates four or three repeat tau isoforms (Goedert *et al*., 1989). The most common FTDP-17 intronic mutations are located close to the splice-donor site of the intron following exon 10 and act by increasing splicing in exon 10 and production of the four repeat isoforms (Hutton *et al*., 1998; Spillantini and Goedert, 1998; Grover *et al*., 1999). This may result in an excess of tau over available binding sites on microtubules, thus inducing a gain of toxic function with unbound excess tau available for assembly into filaments (Goedert *et al*., 1998). Tau inclusions are found in neurons and glial cells as 6 to 22 nm-wide ribbons twisted every 90–300 nm quite similar to those reported in corticobasal degeneration (Spillantini *et al*., 1998c). Exonic mutations in FTDP-17 are missense mutations that induce microtubule destabilization by reducing the ability of mutated tau to interact with microtubules (partial loss of function). For instance, some of the exonic mutations in exons 10, 12, and 13 have been shown to decrease the microtubule-binding affinity of tau and to promote microtubule assembly (Hasegawa *et al*., 1998). The ratio of four to three repeat tau isoforms is preserved in FTDP families with missense mutations in exons 9, 12, and 13. Tau inclusions contain all six tau isoforms, and filaments are ultrastructurally comparable to those seen in AD (for review, see Hardy *et al*., 1998; Spillantini and Goedert, 1998; Spillantini *et al*., 1998c). Exon 10 mutations result in tau inclusions, which contain the mutant four repeat form and by electron microscopy filaments are similar to those obtained in intronic mutations.

2. Pick's Disease

Pick's disease is characterized by a progressive frontotemporal lobar atrophy, gliosis, severe neuronal loss, αB-crystallin-immunoreactive ballooned neurons, and the presence of argyrophilic (but Gallyas-negative) neuronal inclusions, the Pick bodies, in the cerebral cortex and some subcortical structures (Figs. 12.3d–12.3f). The ''knife-edge'' cortical atrophy is frequently asymmetric and predominates in the frontal and temporopolar regions, with the posterior part of frontal and temporal lobes being less affected (Yoshimura, 1989; Brion *et al*., 1991; Kosaka *et al*., 1991; Fig. 12.1 bottom). In these regions, cortical atrophy mainly involves the supragranular layers. The parietal and occipital cortices are usually spared, but panencephalitic and parietal variants of Pick's disease have been reported (Cambier *et al*., 1981; Shibayama *et al*., 1983). The hippocampal formation displays severe atrophy accompanied by high densities of Pick bodies, especially in the dentate gyrus, where very high densities were reported (Hof *et al*., 1994). In subcortical structures, pathologic changes are observed frequently in the basal ganglia, amygdala, nucleus basalis of Meynert, substantia nigra, locus coeruleus, and central gray matter (Forno *et al*., 1989; Arima and Akashi, 1990; Brion *et al*., 1991; Kosaka *et al*., 1991). The distribution of Pick bodies in neocortical layers differs from that of NFT in AD in that there is a preferential involvement of small pyramidal neurons in layer II and the superficial portion of layer III. Moreover, layer VI is affected severely in Pick's disease, suggesting that certain corticosubcortically projecting neurons are involved in PD that may be resistant in AD (Hof *et al*., 1994).

Ultrastructurally, Pick bodies consist of bundles of disorganized 10 to 15 nm straight filaments, which may be mixed with PHF-like of 130 to 160 nm periodicity, and share antigenic determinants with NFT (Hof *et al*., 1994; for review, see Delacourte *et al*., 1996). In particular, Pick bodies are associated with phosphorylated neurofilament epitopes identical to those found in NFT, as well as with other markers, such as the microtubule-associated protein tau and ubiquitin, indicating that, like NFT, Pick bodies may derive from altered components of the neuronal cytoskeleton. Moreover, there is a coexistence of Pick bodies and NFT in the brains of most patients with Pick's disease, whereas diffuse Aβ deposits are also found in 30% of cases (Hof *et al*., 1994). Observations in aged transgenic mices expressing the human medium molecular weight neurofilament protein subunit revealed the formation of lesions morphologically similar to Pick bodies and NFT in the neocortex. These findings suggest that although the laminar distribution of neuropathological lesions differs between AD and Pick's disease, common biochemical mechanisms leading to alterations of comparable cellular constituents exist in these disorders (Katzman and Kawas, 1994).

In addition to neuronal pathology, there is a marked neuritic and glial tau pathology in Pick's disease (Table 12.1; Buée-Scherrer *et al*., 1996; Feany *et al*., 1996; Probst *et al*., 1996). Immunostaining with phosphorylation-dependent anti-tau antibodies showed a dense network of immunoreactive axons in the vicinity of Pick body-containing neurons that could be differentiated easily from AD dendritic threads. In contrast to AD, several types of glial cytoskeletal alterations have been described in Pick's disease and appear to be a consistent finding in progressive supranuclear palsy, post-encephalitic parkinsonism, and corticobasal degeneration,

indicating that in these diseases, glial elements may participate significantly in the pathologic tau profile (Feany and Dickson, 1995; Buée-Scherrer *et al*., 1996; Feany *et al*., 1996). Tau- and ubiquitin-immunoreactive cortical and white matter astrocytic inclusions are mostly observed in the middle and temporal gyri, which are the most severely affected cerebral regions. In progressive supranuclear palsy, widespread glial tangle pathology referred to as tufted and thorn-shaped astrocytes and coiled bodies has been reported in the striatum, thalamus, and cerebral cortex, whereas consistent amyloid-negative cortical astrocytic plaque formation has been observed in corticobasal degeneration (for review, see Chin and Goldman, 1996). It is also worth noting that tau filaments in Pick's disease contain only three repeat isoforms (Delacourte *et al*., 1998), whereas only four repeat isoforms are found in progressive supranuclear palsy and corticobasal degeneration (Mailliot *et al*., 1998). These differences in the molecular composition of tau protein, as well as the electrophoretic patterns described later, permit a reliable identification of Pick's disease cases among tauopathies (Table 12.1).

3. Biochemical Characterization of FTD-Related Tauopathies

Electrophoresis of tau proteins is a powerful tool for discriminating FTD-related tauopathies. The electrophoretic mobility of tau protein, is slowed by excessive phosphorylation. Hyperphosphorylated tau proteins in brains from AD, Down syndrome, Pick's disease disease with NFT, and nondemented elderly individuals appear as the tau 55, 64, and 69 triplet (Delacourte *et al*., 1990; Lee *et al*., 1991). A similar immunoblotting pattern has been found in postencephalitic parkinsonism and FTDP-17 family Seattle A (Spillantini *et al*., 1996; Buée-Scherrer *et al*., 1997). As in progressive supranuclear palsy and corticobasal degeneration, some FTDP-17 families displayed the tau 64 and 68 doublet (Ksiezak-Reding *et al*., 1994; Buée-Scherrer *et al*., 1996; Spillantini *et al*., 1998b). Among tauopathies, typical Pick's disease cases with Pick bodies, but without NFT, are the only one to show the tau 60 and 64 doublet (Table 12.1; Delacourte *et al*., 1996).

If these observations in brain tissue are highly promising, this is not the case for the biochemical analysis of cerebrospinal fluid. In fact, such data in FTD cases are still very scarce and contradictory. An elevation of cerebrospinal fluid tau concentrations has been reported in FTD, but not in progressive supranuclear palsy and corticobasal degeneration (Arai *et al*., 1997; Green *et al*., 1999). A specific increase in the concentrations of the monoamine metabolite 3-methoxy-4-hydroxyphenylglycol, as well as a decrease in corticotropin-releasing factor, and somatostatin levels have been reported in the cerebrospinal fluid of FTD cases compared to controls (Minthon *et al*., 1997; Sjögren *et al*., 1998), although the significance of these observations has not yet been demonstrated.

C. FTD with Motor Neuron Disease: A Separate Case?

The lesion distribution in FTD cases with motor neuron disease differs from that observed in typical FTD cases in that there is a preferential involvement of hippocampus and subcortical structures and, in typical cases, marked hypoglossal and anterior horn degeneration (Deymeer *et al*., 1989; Ferrer *et al*., 1991). Immunocytochemically, this disorder is characterized by the presence of ubiquitin-positive and tau-negative intraneuronal inclusions. The term ''ubiquitin-specific inclusions'' has been introduced to designate these inclusions, invisible on classical histological stains, which are principally found in the dentate gyrus and frontal cortex of FTD cases with motor neuron disease. The main constituent of these inclusions has not yet been identified (for review, see Ince *et al*., 1998). The ubiquitin-specific inclusions are also observed in amyotrophic lateral sclerosis and neuroaxonal dystrophy, and it has been postulated that these diseases may have a common origin (Arima *et al*., 1998; Tolnay and Probst, 1999). However, several FTD cases without clinical symptoms of motor neuron disease exhibit these inclusions, pointing to the necessity for further studies to better delineate these entities.

III. Conclusions

As is frequently the case in dementia research, the existence of complex relationships between clinical and biological descriptions is a source of confusion. Despite this, progress in the morphological and biochemical characterization of FTD has contributed to define the spectrum of these syndromes better. Clearly, the clinical umbrella of FTD, although conceptually weak, had the merit to increase the diagnostic reliability among clinicians. Until neuropsychological, neuroimaging, and biochemical markers become available to allow for a valid premortem distinction among the different subgroups of FTD, the broad use of the term FTD (including its prototypical clinical syndromes) is recommended. However, detailed postmortem investigations of FTD cases using modern morphological and biochemical techniques is warranted to classify them in biologically homogeneous diagnostic groups. Recent advances in the understanding of FTD-related tauopathies have demonstrated that the events leading to a filamentous tau pathology are sufficient for neurodegeneration and onset of dementia in the absence of significant amyloid pathology. In this respect, one kindred with FTDP-17 (Seattle family A) displayed tau ultrastructural and biochemical characteristics identical to those found in AD, raising the question of the relationship between these two disorders (Goedert *et al*., 1998). Moreover, the ubiquitin-related immunocytochemical patterns in FTD with motor neuron disease and amyotrophic lateral sclerosis suggest intriguing biological similarities between these disorders. Although these observations imply that the biological background of some forms of FTD could be defined better in the near future, the pathogenesis of most typical FTD cases remains obscure. An immunocytochemical study of presenilin-1 and apoptosis-related proteins in these FTD cases revealed that neurons containing presenilin-1 are preserved in affected cortical areas, but Bax and Bcl-x_L were not activated (Giannakopoulos *et al*., 1999). This is consistent with a report showing that the glutamate-induced death of cerebellar granular cells in culture does not involve Bax expression and suggests that alternative mechanisms of cell death that do not require the activation of this protein occur in typical FTD (Dessi *et al*., 1993;

Giannakopoulos *et al*., 1999). Conceptually, the biological research on FTD should aim at resolving two enigmas to permit the development of an etiological classification of these disorders: (1) how the same symptoms correspond to several, biologically distinct groups, which in turn converge into clinically unrelated dementing conditions, and (2) what mechanisms are involved in cell loss in FTD.

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References

Alzheimer, A. (1911). Über eigenartige Krankheitsfälle des späteres Alters. *Z. Neurol*. **4**, 356–385.

Andreadis, A., Brown, W. M., and Kosik, K. S. (1992). Structure and novel exons of the human tau gene. *Biochemistry* **31**, 10626–10633.

Arai, H., Morikawa, Y., Higuchi, M., Matsui, T., Clark, C. M., Miura, M., Machida, N., Lee, V. M., Trojanowski, J. Q., and Sasaki, H. (1997). Cerebrospinal fluid tau levels in neurodegenerative diseases with distinct tau-related pathology. *Biochem. Biophys. Res. Commun*. **236**, 262–264.

Arima, K., and Akashi, T. (1990). Involvement of the locus coeruleus in Pick's disease with or without Pick body formation. *Acta Neuropathol*. **79**, 629–633.

Arima, K., Ogawa, M., Sunohara, N., Nishio, T., Shimomura, Y., Hirai, S., and Eto, K. (1998). Immunohistochemical and ultrastructural characterization of ubiquitinated eosinophilic fibrillary neuronal inclusions in sporadic amyotrophic lateral sclerosis. *Acta Neuropathol*. **96**, 75–85.

Brion, S., Plas, J., and Jeanneau, A. (1991). Maladie de Pick. Point de vue anatomo-clinique. *Rev. Neurol*. **147**, 693–704.

Brown, J., Ashworth, A., Gydesen, S., Sorensen, A., Rossor, M., and Hardy, J. (1995). Familial non-specific dementia maps to chromosome 3. *Hum. Mol. Genet*. **4**, 1625–1628.

Brun, A. (1993). Frontal lobe degeneration of non-Alzheimer type revisited. *Dementia* **4**, 126–131.

Brun, A., Englund, B., Gustafson, L., Passant, U., Mann, D. M. A., Neary, D., and Snowden, J. S. (1994). Clinical and neuropathological criteria for frontotemporal dementia. *J. Neurol. Neurosurg. Psychiatry* **57**, 416–418.

Buée-Scherrer, V., Hof, P. R., Buée, L., Leveugle, B., Vermersch, P., Perl, D. P., Olanow, C. W., and Delacourte, A. (1996). Hyperphosphorylated tau proteins differentiate corticobasal degeneration and Pick's disease. *Acta Neuropathol*. **91**, 351–359.

Buée-Scherrer, V., Buée, L., Leveugle, B., Perl, D. P., Vermersch, P., Hof, P. R., and Delacourte, A. (1997). Pathological tau proteins in postencephalitic parkinsonism: Comparison with Alzheimer's disease and other neurodegenerative disorders. *Ann. Neurol*. **41**, 277–281.

Cambier, J., Masson, M., Dairou, R., and Henin, D. (1981). Etude anatomo-clinique d'une forme pariétale de la maladie de Pick. *Rev. Neurol*. **137**, 33–38.

Caselli, R. J., Windenbank, A. J., and Petersen, R. C. (1993). Rapidly progressive aphasic dementia and motor neuron disease. *Ann. Neurol*. **33**, 200–207.

Cervós-Navarro, J., and Schumacher, K. (1994). Neurofibrillary pathology in progressive supranuclear palsy (PSP). *J. Neural Transm. Suppl*. **42**, 153–164.

Chin, S. S. M., and Goldman, J. E. (1996). Glial inclusions in CNS degenerative diseases. *J. Neuropathol. Exp. Neurol*. **55**, 499–508.

Delacourte, A., Flament, S., Dibe, E. M., Hublau, P., Sablonnière, B., Hémon, B., Scherrer, V. and Défossez, A. (1990). Pathological proteins Tau 64 and 69 are specifically expressed in the somatodendritic domain of the degenerating cortical neurons during Alzheimer's disease. Demonstration with a panel of antibodies against Tau proteins. *Acta Neuropathol*. **80**, 111–117.

Delacourte, A., Robitaille, Y., Sergeant, N., Buée, L., Hof, P. R., Wattez, A., Laroche-Cholette, A., Mathieu, J., Chagnon, P., and Gauvreau, D. (1996). Specific pathological tau protein variants characterize Pick's disease. *J. Neuropathol. Exp. Neurol*. **55**, 159–168.

Delacourte, A., Sergeant, N., Wattez, A., Gauvreau, D., and Robitaille, Y. (1998). Vulnerable neuronal subset in Alzheimer's and Pick's disease are distinguished by their tau isoform distribution and phosphorylation. *Ann. Neurol*. **43**, 193–204.

Dessi, F., Charriaut-Marlangue, C., Khrestchatisky, M., and Ben-Ari, Y. (1993). Glutamate-induced neuronal death is not a programmed cell death in cerebellar culture. *J. Neurochem*. **60**, 1953–1955.

Deymeer, F., Smith, T. W., DeGirolami, U., and Drachman, D. A. (1989). Thalamic dementia and motor neuron disease. *Neurology* **39**, 58–61.

Elfgren, C., Passant, U., and Risberg, J. (1993). Neuropsychological findings in frontal lobe dementia. *Dementia* **4**, 214–219.

Feany, M. B., and Dickson, D. W. (1995). Widespread cytoskeletal pathology characterizes corticobasal degeneration. *Am. J. Pathol*. **146**, 1388–1393.

Feany, M. B., Mattiace, L. A., and Dickson, D. W. (1996). Neuropathologic overlap of progressive supranuclear palsy, Pick's disease and corticobasal degeneration. *J. Neuropathol. Exp. Neurol*. **55**, 53–67.

Ferrer, I., Roig, C., Espino, A., Peiro, G., and Matias Guiu, X. (1991). Dementia of frontal lobe type and motor neuron disease. A Golgi study of the frontal cortex. *J. Neurol. Neurosurg. Psychiatry* **54**, 932–934.

Forno, L. S., Eng, L. F., and Selkoe, D. J. (1989). Pick bodies in the locus ceruleus. *Acta Neuropathol*. **79**, 10–17.

Foster, N. L., Wilhelmsen, K., Sima, A. A. F., Jones, M. Z., D'Amato, C. J., and Gilman, S. (1997). Frontotemporal dementia and parkinsonism linked to chromosome 17: A consensus statement. *Ann. Neurol*. **41**, 706–715.

Giannakopoulos, P., Hof, P. R., and Bouras, C. (1995). Dementia lacking distinctive histopathology: Clinicopathological evaluation of 32 cases. *Acta Neuropathol*. **89**, 346–355.

Giannakopoulos, P., Kövari, E., Savioz, A., De Bilbao, F., Dubois-Dauphin, M., Hof, P. R., and Bouras, C. (1999). Differential distribution of presenilin-1, Bax, and Bcl-X_L in Alzheimer's disease and frontotemporal dementia. *Acta Neuropathol*. 98, 141–149.

Goedert, M., Spillantini, M. G., Jakes, R., Rutherford, D., and Crowther, R. A. (1989). Multiple isoforms of human microtubule-associated protein tau: Sequence and localization in neurofibrillary tangles of Alzheimer's disease. *Neuron* **3**, 519–526.

Goedert, M., Spillantini, M. G., and Davis, S. W. (1998). Filamentous nerve cell inclusions in neurodegenerative diseases. *Curr. Opin. Neurobiol*. **8**, 619–632.

Green, A. J., Harvey, R. J., Thompson, E. J., and Rossor, M. N. (1999). Increased tau in the cerebrospinal fluid of patients with frontotemporal dementia and Alzheimer's disease. *Neurosci. Lett*. **259**, 133–135.

Grover, A., Houlden, H., Baker, M., Adamson, J., Lewis, J., Prihar, G., Pickering-Brown, S., Duff, K., and Hutton, M. (1999). 5′ splice site mutations in tau associated with the inherited dementia FTDP-17 affect a stem-loop structure that regulates alternative splicing of exon 10. *J. Biol. Chem*. **274**, 15134–15143.

Gustafson, L. (1987). Frontal lobe degeneration of non-Alzheimer type. II. Clinical picture and differential diagnosis. *Arch. Gerontol. Geriatr*. **6**, 209–223.

Hardy, J., Duff, K., Gwinn-Hardy, K., Perez-Tur, J., and Hutton, M. (1998). Genetic dissection of Alzheimer's disease and related dementias: Amyloid and its relationship to tau. *Nat. Neurosci*. **1**, 355–358.

Hasegawa, M., Smith, M. J., and Goedert, M. (1998). Tau proteins with FTDP-17 mutations have a reduced ability to promote microtubule assembly. *FEBS Lett*. **437**, 207–210.

Hauw, J.-J., Daniel, S. E., Dickson, D., Horoupian, D. S., Jellinger, K., Lantos, P. L., McKee, A., Tabaton, M., and Litvan, I. (1994). Preliminary NINDS neuropathologic criteria for Steele-Richardson-Olszewski syndrome (progressive supranuclear palsy). *Neurology* **44**, 2015–2019.

Hodges, J. R., Patterson, K., Oxburry, S., and Funnell, E. (1992). Semantic dementia. Progressive fluent aphasia with temporal lobe atrophy. *Brain* **115**, 1783–1806.

Hof, P. R., Bouras, C., Perl, D. P., and Morrison, J. H. (1994). Quantitative neuropathologic analysis of Pick's disease cases: Cortical distribution of Pick bodies and coexistence with Alzheimer's disease. *Acta Neuropathol*. **87**, 115–124.

Hutton, M., Lendon, C. L., Rizzu, P., Baker, M., Froelich, S., Houlden, H., Pickering-Brown, S., Chakraverty, S., Isaacs, A., Grover, A., Hackett, J., Adamson, J., Lincoln, S., Dickson, D., Davies, P., Petersen, R. C., Stevens, M., de Graaff, E., Wauters, E., van Baren, J., Hillebrand, M., Joosse, M., Kwon, J. M., Nowotny, P., and Heutink, P. (1998). Association of missense and 5'-splice site mutations in tau with the inherited dementia FTDP-17. *Nature (London)* **393**, 702–705.

Ince, P. G., Lowe, J., and Shaw, P. J. (1998). Amyotrophic lateral sclerosis: Current issues in classification, pathogenesis and molecular pathology. *Neuropathol. Appl. Neurobiol*. **24**, 104–117.

Katzman, R., and Kawas, C. (1994). The epidemiology of dementia and Alzheimer's disease. *In* "Alzheimer Disease" (R. Katzman and K. L. Bick, eds.), pp. 105–122. Raven Press, New York.

Kertesz, A., and Munoz, D. (1998). Pick's disease, frontotemporal dementia, and Pick complex. *Arch. Neurol. (Chicago)* **55**, 302–304.

Knopman, D. S. (1993). Overview of dementia lacking distinctive histopathology: Pathological designation of a progressive dementia. *Dementia* **4**, 132–136.

Knopman, D. S., Mastri, A. R., Frey, W. H., Sung, J. H., and Rustan, T. (1990). Dementia lacking distinctive histologic features: A common non-Alzheimer degenerative dementia. *Neurology* **40**, 251–266.

Kosaka, K., Ikeda, K., Kobayashi, K., and Mehraein, P. (1991). Striatopallidonigral degeneration in Pick's disease: A clinicopathological study of 41 cases. *J. Neurol*. **238**, 151–160.

Ksiezak-Reding, H., Morgan, K., Mattiace, L. A., Davies, P., Liu, W. K., Yen, S. H., Weidenheim, K., and Dickson, D. W. (1994). Ultrastructure and biochemical composition of paired helical filaments in corticobasal degeneration. *Am. J. Pathol*. **145**, 1496–1508.

Lee, V. M.-Y., Balin, B. J., Otvos, L., Jr., and Trojanowski, J. Q. (1991). A68: A major subunit of paired helical filaments and derivatized forms of normal tau. *Science* **251**, 675–678.

Litvan, I., Hauw, J.-J., Bartko, J. J., Lantos, P. L., Daniel, S. E., Horoupian, D. S., McKee, A., Dickson, D., Bancher, C., Tabaton, M., Jellinger, K., and Anderson, D. W. (1996). Validity and reliability of the preliminary NINDS neuropathologic criteria for progressive supranuclear palsy and related disorders. *J. Neuropathol. Exp. Neurol*. **55**, 97–105.

Mailliot, C., Sergeant, N., Bussière, T., Caillet-Boudin, M. L., Delacourte, A., and Buée, L. (1998). Phosphorylation of specific sets of tau isoforms reflect different neurofibrillary degeneration processes. *FEBS Lett*. **433**, 201–204.

Mann, D. M. (1998). Dementia of frontal type and dementias with subcortical gliosis. *Brain Pathol*. **8**, 325–338.

Mann, D. M. A., South, P. W., Snowden, J. S., and Neary, D. (1993). Dementia of frontal lobe type: Neuropathology and immunohistochemistry. *J. Neurol. Neurosurg. Psychiatry* **56**, 605–614.

Mendez, M. F., Selwood, A., Mastri, A. R., and Frey, W. H., II (1993). Pick's disease versus Alzheimer's disease: A comparison of clinical characteristics. *Neurology* **43**, 289–292.

Mesulam, M. M. (1982). Slowly progressive aphasia without generalized dementia. *Ann. Neurol*. **11**, 592–598.

Miller, B. L., Cummings, J. L., Villanueva-Meyer, J., Boone, K., Mehringer, C. M., Lesser, I. M., and Mena, I. (1991). Frontal lobe degeneration: Clinical, neuropsychological and SPECT characteristics. *Neurology* **41**, 1374–1382.

Minthon, L., Edvinsson, L., and Gustafson, L. (1997). Somatostatin and neuropeptide Y in cerebrospinal fluid: Correlations with severity of disease and clinical signs in Alzheimer's disease and frontotemporal dementia. *Dementia Geriatr. Cogn. Disord*. **8**, 232–239.

Mitsuyama, Y., and Takamiya, S. (1979). Presenile dementia with motor neuron disease in Japan: A new entity? *Arch. Neurol. (Chicago)* **36**, 592–593.

Mummery, C. J., Patterson, K., Wise, R. J., Vandenbergh, R., Price, C. J., and Hodges, J. R. (1999). Disrupted temporal lobe connections in semantic dementia. *Brain* **122**, 61–73.

Neary, D., and Snowden, J. S. (1996). Clinical features of frontotemporal dementia. *In* "Frontotemporal Dementia" (F. Pasquier, F. Lebert, and P. Scheltens, eds.), pp. 31–47. ICG, Dordrecht, The Netherlands.

Neary, D., Snowden, J. S., Mann, D. M. A., Northen, B., Goulding, P. J., and MacDermott, N. (1990). Frontal lobe dementia and motor neuron disease. *J. Neurol. Neurosurg. Psychiatry* **53**, 23–32.

Neary, D., Snowden, J. S., and Mann, D. M. A. (1993). The clinical pathological correlates of lobar atrophy. *Dementia* **4**, 154–159.

Neary, D., Snowden, J. S., Gustafson, L., Passant, U., Stuss, D., Black, S., Freedman, M., Kertesz, A., Robert, P. H., Albert, M., Boone, K., Miller, B. L., Cummings, J., and Benson, D. F. (1998). Frontotemporal lobe degeneration: A consensus on clinical diagnostic criteria. *Neurology* **51**, 1546–1554.

Pasquier, F. (1999). Early diagnosis of dementia: Neuropsychology. *J. Neurol*. **246**, 6–15.

Pasquier, F., Lebert, F., Grymonprez, L., and Petit, H. (1995). Verbal fluency in dementia of frontal lobe type and dementia of Alzheimer type. *J. Neurol. Neurosurg. Psychiatry* **58**, 81–84.

Pick, A. (1892). Über die Beziehungen der senilen Hirnatrophie zur Aphasie. *Prager Med. Wochenschr.* **17**, 165–167.

Poorkaj, P., Bird, T. D., Wijsman, E., Nemens, E., Garruto, R. M., Anderson, L., Andreadis, A., Wiederholt, W. C., Raskind, M., and Schellenberg, G. D. (1998). Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Ann. Neurol*. **43**, 815–825.

Probst, A., Tolnay, M., Langui, D., Goedert, M., and Spillantini, M. G. (1996). Pick's disease: Hyperphosphorylated tau protein segregates to the somatoaxonal compartment. *Acta Neuropathol*. **92**, 588–596.

Riley, D. E., Lang, A. E., Lewis, A., Resch, L., Ashby, P., Hornykiewicz, O., and Black, S. (1990). Cortico-basal ganglionic degeneration. *Neurology* **40**, 1203–1212.

Rinne, J. O., Lee, M. S., Thompson, P. D., and Marsden, C. D. (1994). Corticobasal degeneration: A clinical study of 36 cases. *Brain* **117**, 1183–1196.

Sam, M., Gutmann, L., Schochet, S. S., and Doshi, H. (1991). Pick's disease: A case clinically resembling amyotrophic lateral sclerosis. *Neurology* **41**, 1831–1833.

Shibayama, H., Kitoh, J., Marui, Y., Kobayashi, H., Iwase, S., and Kayukawa, Y. (1983). An unusual case of Pick's disease. *Acta Neuropathol*. **59**, 79–87.

Sjögren, M., Minthon, L., Passant, U., Blennow, K., and Wallin, A. (1998). Decreased monoamine metabolites in frontotemporal dementia and Alzheimer's disease. *Neurobiol. Aging* **19**, 379–384.

Snowden, J. S., Neary, D., Mann, M. A., Goulding, P. J., and Testa, H. J. (1992). Progressive language disorder due to lobar atrophy. *Ann. Neurol*. 31, 174–183.

Spillantini, M. G., and Goedert, M. (1998). Tau protein pathology in neurodegenerative diseases. *Trends Neurosci*. **21**, 428–433.

Spillantini, M. G., Crowther, R. A., and Goedert, M. (1996). Comparison of the neurofibrillary pathology in Alzheimer's disease and familial presenile dementia with tangles. *Acta Neuropathol*. **92**, 42–48.

Spillantini, M. G., Murell, J. R., Goedert, M., Farlow, M. R., Klug, A., and Ghetti, B. (1998a). Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 4113–4118.

Spillantini, M. G., Crowther, R. A., Kamphorst, W., Heutink, P., and Van Swieten, J. C. (1998b). Tau pathology in two Dutch families with mutations in the microtubule-binding region of tau. *Am. J. Pathol*. **153**, 1359–1363.

Spillantini, M. G., Bird, T. D., and Ghetti, B. (1998c). Frototemporal dementia and parkinsonism linked to chromosome 17: A new group of tauopathies. *Brain Pathol*. **8**, 387–402.

Tolnay, M., and Probst, A. (1999). Tau protein pathology in Alzheimer's disease and related disorders. *Neuropathol. Appl. Neurobiol*. **25**, 171–187.

Weintraub, S., Rubin, N. P., and Mesulam, M. M. (1990). Primary progressive aphasia: Longitudinal course, neuropsychological profile and language features. *Arch. Neurol*. (*Chicago*) **47**, 1329–1335.

Yoshimura, N. (1989). Topography of Pick body distribution in Pick's disease: A contribution to understanding the relationship between Pick's and Alzheimer's diseases. *Clin. Neuropathol*. **8**, 1–6.

Dennis W. Dickson

13

Progressive Supranuclear Palsy and Corticobasal Degeneration

Progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) are sporadic neurodegenerative disorders of mid to late adult life that have shared clinical and pathologic features, but also notable differences that warrant their separation as clinicopathologic entities. Among shared clinical features are extrapyramidal signs similar to Parkinson's disease, but neither PSP nor CBD is responsive to levodopa therapy. Motor abnormalities in PSP are usually symmetrical, whereas asymmetry is the hallmark of CBD. Focal cortical signs, such as apraxia and aphasia, are common in CBD, but rare in PSP. Dementia is more common in CBD than PSP. Severe vertical gaze palsy early in the disease course is common in PSP, but is uncommon or a late manifestation of CBD. Pathologically, both PSP and CBD are associated with neuronal and glial filamentous inclusions that are composed of tau protein. The morphology of neuronal and glial lesions differs in PSP and CBD, but there are a number of lesions with transitional or overlapping features. Distribution of the lesions shows considerable overlap, but the overall distribution of the lesions differs in PSP and CBD. Cortical gray and white matter lesions are prominent in CBD, whereas deep gray matter lesions are more common in PSP. Biochemical studies of brain tissue from PSP and CBD show similar alterations in tau protein. Abnormal tau proteins in PSP and CBD are relatively insoluble and hyperphosphorylated. Furthermore, they appear to be composed of tau enriched in specific tau mRNA splice forms, specifically tau derived from alternative spicing of exon 10, which generates tau with four repeat regions in the microtubule-binding domain. It is unclear if this is due to disease-related differential expression of these tau isoforms, involvement of cell types that express these isoforms preferentially, or selective assembly of these specific tau isoforms in the lesions. Both PSP and CBD are considered to be nonfamilial or sporadic ''tauopathies,'' but genetic studies suggest that polymorphisms in the tau gene may confer some degree of genetic risk for these disorders. Given the relative rarity of these conditions, further clinical and pathologic studies are needed to define the diagnostic boundaries and to develop biologic markers for their clinical and pathologic differentiation.

I. Introduction

Progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) are sporadic neurodegenerative disorders of mid to late adult life that have only recently been recognized as clinicopathologic entities. They were first reported in the medical literature in the 1960s (Steele *et al*., 1964; Rebeiz *et al*., 1967), and the term CBD was coined in the 1980s (Gibb *et al*., 1989). Molecular methods have been applied to these disorders, and these studies raise questions about how PSP and CBD might be related, as the biochemical changes in the brain are very similar (reviewed in Buée and Delacourte, 1999). Although some clinical and pathological features are shared by PSP and CBD, there are notable differences that warrant their separation as clinicopathologic entities (reviewed in Litvan and Agid, 1992; Litvan *et al*., 2000).

Among the shared clinical features are extrapyramidal signs. Individuals with CBD and PSP have bradykinesia, rigidity, and gait abnormalities that bring to mind Parkinson's disease. However, neither PSP nor CBD typically has a sustained clinical response to levodopa like Parkinson's disease. Furthermore, rest tremor, one of the cardinal clinical features of Parkinson's disease, is uncommon in PSP and CBD. Motor abnormalities in PSP are usually symmetrical, whereas asymmetry is the hallmark of CBD. Focal cortical signs help differentiate CBD and PSP. Apraxia and aphasia are common in CBD, but are decidedly atypical in PSP. Dementia is common in CBD, and retrospective studies of pathologically confirmed cases of CBD indicate that this may be one of the most common presentations of CBD. Dementia is not prominent in PSP, but many individuals develop a frontal lobe syndrome in advanced stages of the disease. Supranuclear gaze palsy early in the disease course, which is common in PSP, is another clinical feature that distinguishes PSP from CBD.

Pathologically, both PSP and CBD are associated with neuronal and glial filamentous inclusions that are composed primarily of the microtubule-associated protein tau. The distribution of neuronal and glial lesions shows a considerable

degree of overlap, but the overall pattern differs (Dickson, 1999b). For example, cortical and white matter pathology is more prominent in CBD, whereas deep gray matter areas are affected more in PSP. The morphology of neuronal and glial lesions is different in PSP and CBD (Komori *et al*., 1998), but there are no clear boundaries that make individual lesions pathognomonic.

Biochemical studies of brain tissue from PSP and CBD show alterations in tau protein (reviewed in Buée and Delacourte, 1999). These abnormal tau proteins, which are probably derived from the filamentous lesions, are relatively insoluble and hyperphosphorylated. In PSP and CBD there is also evidence of enrichment of specific tau mRNA splice tau in the abnormal tau isolated by detergent extraction. Whether there is differential overexpression of these tau isoforms or differential assembly of specific tau isoforms in the lesions remains to be determined. Given the pivotal role that tau abnormalities are increasingly felt to play in neurodegenerative diseases, PSP and CBD are considered members of the class of neurodegenerative disorder referred to as ''tauopathies'' (Hardy and Gwinn-Hardy, 1998). Other disorders in this class include Pick's disease and frontotemporal dementia with parkinsonism linked to mutations on chromosome 17 (FTDP-17) (Foster *et al*., 1997; Poorkaj *et al*., 1998; Spillantini *et al*., 1998a). All tauopathies have selective neurodegeneration with filamentous inclusions containing tau protein. Some are sporadic (Pick's disease, PSP, and CBD), whereas others are hereditary (FTDP-17). Given the relative rarity of these conditions, it is not surprising that the range of clinical and pathologic phenotypes of these disorders is in a state of flux. Of particular note are transitional cases with overlapping clinical and pathologic features, even among those tauopathies with a defined genetic basis. Further studies will shed light on the relationship of genotype to phenotype.

II. Clinical Features

A. Progressive Supranuclear Palsy

PSP is one of the major causes of levodopa-nonresponsive parkinsonism (Rajput *et al*., 1984). Given the fact that it is associated with additional clinical features not typical of Parkinson's disease, it is considered one of the ''parkinsonism-plus'' syndromes. One of the earliest clinical features of PSP is unexplained falls. Later in the course the characteristic clinical picture emerges, which features postural instability, vertical gaze paresis, nuchal rigidity, and dysarthria (Rajput *et al*., 1991; Golbe, 1993). PSP is an uncommon disorder, with prevalence rate estimates of about 1.5 per 100,000 (Golbe *et al*., 1988; Bower *et al*., 1999a), compared to 100 to 150 per 100,000 for Parkinson's disease. New cases occur at a rate of about 3–4 per million population per year (Rajput *et al*., 1984). Along with falls, gait disturbance is common in early stages of PSP (Golbe, 1993). Gait problems are progressive and disabling, leading eventually to confinement to chair or bed within about 5 years.

Eye movement abnormalities are the hallmark of the clinical presentation of PSP (Steele *et al*., 1964) and are characterized initially by impairment of downward gaze, followed later by difficulties with upward gaze and even horizontal gaze. There is usually preservation of the oculocephalic reflexes (''dolls eye'' test). Blepharospasm and low blink rate are also common (Golbe, 1993). A supranuclear downward gaze palsy may develop in the course of CBD, but it is not usually as prominent as in PSP.

Other clinical features seen to varying degrees in PSP include dysarthria and dysphagia. As noted previously, focal cortical signs, such as aphasia or apraxia, are absent in PSP. Some degree of mental dysfunction, but not severe dementia, is common in PSP (Maher *et al*., 1985). Cognitive dysfunction in PSP is consistent with a subcortical and frontal lobe disorder. Attention is impaired and cognitive processing is slowed (Dubois *et al*., 1988). Frontal lobe signs may be prominent (Agid *et al*., 1987; Litvan *et al*., 1989; Grafman *et al*., 1990).

Structural imaging is of limited value in differentiating PSP from other parkinsonism-plus disorders. Narrowing of the anteroposterior midbrain diameter (usually < 15 mm, normal being > 18 mm) and dilation of the third ventricle are characteristic, but not completely diagnostic (Golbe, 1993; Gimenez-Roldan *et al*., 1994). SPECT and PET scanning show bilateral frontal hypoactivity in PSP (D'Antona *et al*., 1985; Foster *et al*., 1988; Leenders *et al*., 1988). Striatal dopaminergic deficits are also common in PSP (Baron *et al*., 1986; Brooks *et al*., 1990). Studies show loss of both dopamine and dopamine receptors. In contrast, Parkinson's disease is associated with decreases in striatal dopamine with preservation of dopamine receptors (Brooks *et al*., 1992). This reflects the fact that in PSP there is not only damage to dopaminergic neurons projecting from the substantia nigra to the striatum, but also to intrinsic neurons of the striatum.

There are no specific diagnostic tests for PSP and it is not uncommon for an individual to carry a diagnosis of Parkinson's disease for years before a correct diagnosis is made (Rajput *et al*., 1991). Indeed, in retrospective autopsy series, cases with pathologically confirmed PSP have been found to carry a clinical diagnosis of Parkinson's disease (Bower *et al*., 1999b). It has been suggested that only with longitudinal evaluation is it possible to accurately diagnose parkinsonian disorders (Rajput *et al*., 1991).

The disorders most often mistaken for PSP are multiple system atrophy (MSA), CBD, and diffuse Lewy body disease (DLBD) based on evaluation of brains submitted to the Society for Progressive Supranuclear Palsy brain bank (unpublished data). Cases submitted to the brain bank with a diagnosis of PSP were found to have PSP 75% of the time with MSA, CBD, and DLBD accounting for most of the misdiagnoses. Individual cases with basal ganglia infarcts or Alzheimer's disease (AD) were also misdiagnosed as PSP.

Of the disorders that can be mistaken for PSP, two of them are not associated with tau pathology, but rather by inclusion bodies composed of synuclein (Dickson, 1999a). These are MSA, with its synuclein-positive, glial cytoplasmic inclusions (Lantos, 1998), and DLBD, which is characterized by Lewy bodies composed of synuclein (Dickson, 1999a).

B. Corticobasal Degeneration

CBD was first described in 1967 as ''corticodentatonigral degeneration with neuronal achromasia'' in a patient with an asymmetrical cortical syndrome (Rebeiz *et al*., 1967, 1968).

While prevalence rates have not been estimated, it is generally accepted that CBD is less common that PSP. True prevalence rates of CBD are difficult to estimate as clinical and pathologic criteria for diagnosis of CBD are undergoing reappraisal. The range of clinical presentations for CBD is expanding based on recent clinicopathologic studies (Lang *et al*., 1994). Original descriptions of CBD emphasized a progressive asymmetrical apraxia and rigidity syndrome (Gibb *et al*., 1989; Riley *et al*., 1990), but more recent reports have indicated that individuals with postmortem findings of CBD may also present with focal cortical syndromes, such as frontal dementia and aphasia (Gibb *et al*., 1989; Paulus and Selim, 1990; Arima *et al*., 1994; Lang *et al*., 1994; Bergeron *et al*., 1996, 1998; Ikeda *et al*., 1996). Furthermore, the clinical syndrome of progressive asymmetrical apraxia and rigidity can be due to a variety of pathologic disorders (Boeve *et al*., 1999), suggesting that CBD is a focal cortical degenerative disorder and that the clinical phenotype is a reflection of the location of the dominant cortical pathology. The typical clinical phenotype corresponds to damage to the dorsal perirolandic, superior frontal, and superior parietal cortices, whereas cases with aphasia show pathology in the perisylvian region.

The initial signs of typical cases of CBD are unilateral or asymmetrical apraxia, rigidity, and dystonia. This may be associated with myoclonic jerks, grasp reflex, cortical sensory impairment, and alien limb sign. The affected hand may develop dystonic flexion contractures. Cognitive impairment is not universal in CBD, but is seemingly more frequent than in PSP. As mentioned previously, some cases come to autopsy with a primary diagnosis of degenerative dementia, without asymmetrical cortical signs ever noted in clinical records. Dementia in CBD most often has features of a frontal lobe dementia marked by personality change, disorder of conduct, impaired attention, and distractibility. Frontal lobe signs, including grasp reflex, forced groping, utilization behavior, and intermanual conflict, characteristically are unilateral at onset and markedly asymmetric in CBD.

The alien limb phenomenon, which describes involuntary movement, such as elevation of the arm or leg into the air (Doody and Jankovic, 1992), is often emphasized in CBD, but it is neither specific to CBD nor found in many cases. Cortical sensory deficits due to parietal lobe involvement and characterized by graphesthesia and astereognosis are frequent in CBD.

Structural imaging may be helpful in CBD, where magnetic resonance imaging may show asymmetrical atrophy of the superior parietal lobule variably extending into frontal regions, with less prominent atrophy elsewhere (Soliveri *et al*., 1999). Structural changes become more obvious as the disease progresses. Initial evaluations may show no asymmetrical cortical atrophy. These findings are not specific, as Pick's disease, nonspecific frontotemporal dementia, primary progressive aphasia, and some individuals with FTDP-17 may have asymmetrical cortical atrophy. Magnetic resonance imaging scans in CBD may show hyperintense signals in white matter in regions of brain atrophy and sometimes in the corpus callosum (Yamaguchi *et al*., 1998; Doi *et al*., 1999).

Functional imaging is often informative in CBD. The hallmark finding is asymmetrical hypometabolism in superior frontal and parietal lobes (Sawle *et al*., 1991; Blin *et al*., 1992). Hypometabolism is sometimes also detected in caudate, putamen, and thalamus. PET scans assessing dopamine metabolism [^{18}F]DOPA may show reduction of striatal and medial frontal uptake (Sawle *et al*., 1991). Striatal uptake is usually most severely impaired contralateral to the clinically most affected limbs.

III. Neuropathology

A. Progressive Supranuclear Palsy

Gross examination of the brain may be unrevealing in PSP, but the most common pathological findings are a mild degree of frontal and midbrain atrophy (Fig. 13.1). The third ventricle and aqueduct of Sylvius may be dilated (Fig. 13.1). The substantia nigra invariably shows some degree of depigmentation (Fig. 13.2). In most cases the substantia nigra shows marked pathology. In contrast, the locus ceruleus shows less pigment loss. The subthalamic nucleus may be noticeably smaller than expected (Fig. 13.3), and the superior cerebellar peduncle and the hilus of the cerebellar dentate nucleus may be attenuated and gray due to myelinated fiber loss (Fig. 13.2).

Microscopic findings include neuronal loss and fibrillary gliosis affecting multiple systems. Nuclei with the most marked and consistent pathology are the globus pallidus, subthalamic nucleus, and substantia nigra. Other parts of the basal ganglia, diencephalon, and brain stem are affected to a variable degree. PSP is a multisystem degeneration with involvement of corticopontine, olivopontocerebellar, dentatorubrothalamic, pallidoluysolnigral, and corticostriatal systems. The reticular activating system is also affected. The basis for selective vulnerability of these mostly extrapyramidal motor systems to degeneration is unknown.

In addition to the changes visible with routine histologic methods, silver stains or immunostaining for the tau protein reveal neurofibrillary tangles (NFT) as well as glial inclusions (Fig. 13.4). The distribution of pathology is highly characteristic of PSP. In fact, pathologic diagnosis (Hauw *et al*., 1994) is contingent on pathology in specific nuclei and tracts as there is no single pathognomonic lesion (Jellinger and Bancher, 1992; Lantos, 1994; Feany *et al*., 1996). The globus pallidus and pars reticularis of the substantia nigra may show, in addition to neuronal loss, gliosis, and NFT, extensive iron pigment deposition and granular neuroaxonal spheroids. The striatum and thalamus, especially ventral anterior and lateral nuclei, may have gliosis, but neuronal loss is usually too subtle to detect with routine methods. The basal nucleus of Meynert usually has mild cell loss, but more noticeable neurofibrillary pathology. The brain stem regions that are affected include the superior colliculus, periaqueductal gray matter, oculomotor nuclei, locus ceruleus, pontine nuclei, pontine tegmentum, vestibular nuclei, medullary tegmentum, and inferior olives. The cerebellar dentate nucleus is frequently affected and may show grumose degeneration (Arai, 1987; Mizusawa *et al*., 1989; Cruz-Sanchez *et al*., 1992b). The latter is a type of degeneration associated with clusters of degenerating presynaptic terminals around dentate neurons (Fig. 13.5). Grumose degeneration is not specific for PSP, but PSP may be the most common condition in which it is routinely detected. The

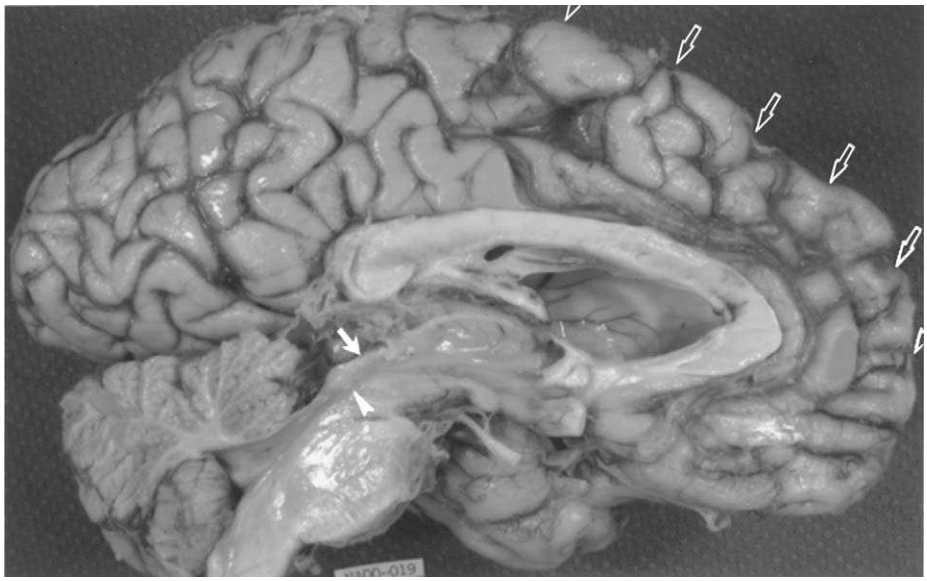

FIG. 13.1. The medial surface of the brain in PSP often shows mild frontal atrophy (open arrows), whereas midbrain atrophy is more consistent and often marked. The midbrain tectal plate (closed arrow) is small and the aqueduct of Sylvius (arrowhead) is dilated.

dentatorubrothalamic pathway consistently shows fiber loss (Fig. 13.5). The cerebellar cortex is generally preserved, but there may be mild Purkinje and granular neuronal loss with scattered Purkinje cell dystrophic axons (''torpedoes''). A more consistent finding in the cerebellum is the presence of glial cells with fibrillary inclusions in the cerebellar white matter. Spinal cord involvement is common, where neuronal inclusions can be found in anterior horn and intermediolateral cells (Jellinger and Bancher, 1992).

Neuronal loss is usually greatest in the ventrolateral tier of the substantia nigra, as in Parkinson's disease, but there is often more extensive neuronal loss throughout all regions of the substantia nigra (Jellinger, 1971). Dopaminergic neurons of the ventrolateral tier of the pars compacta project to the striatum, and loss of this population of neurons presumably accounts for extrapyramidal symptoms. Neuronal cell loss is accompanied by fibrillary gliosis and globose-shaped NFT (Fig. 13.6). Neuronal loss and NFT may be prominent in the globus pallidus. Large striatal neurons appear especially vulnerable, and loss of up to 30–40% of these cells has been reported (Oyanagi *et al*., 1988). Neuronal loss in the basal nucleus of Meynert ranges from 13 to 54% (Tagliavini *et al*., 1984), but neuronal lesions are common. The involvement of the basal nucleus accounts for cholinergic deficits that have

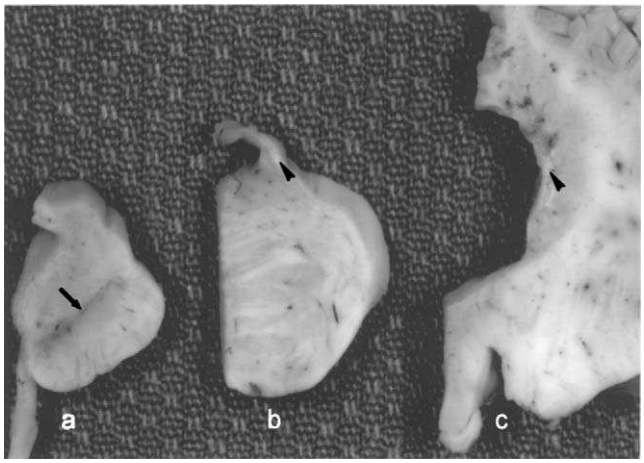

FIG. 13.2. Sections of brain stem and cerebellum in PSP show variable, often marked loss of pigment in the substantia nigra (a, arrow) and locus ceruleus. The superior cerebellar peduncle in the pons (b, arrow) is often attenuated and gray colored due to loss of myelinated fibers. The cerebellar dentate nucleus is typically atrophic and the hilus is soft and gray (c, arrow) due to myelinated fiber loss.

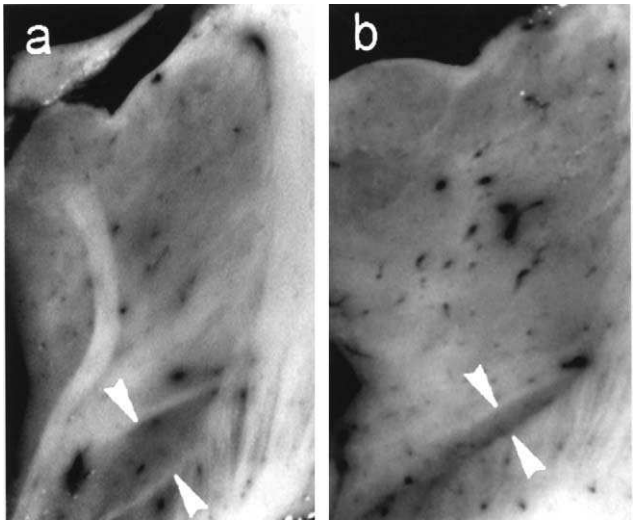

FIG. 13.3. The subthalamic nucleus in PSP is typically small (b, arrowheads). In contrast, the subthalamic nucleus is normal in most other neurodegenerative disorders (a, arrowheads), including Alzheimer's disease, Lewy body disease, and CBD.

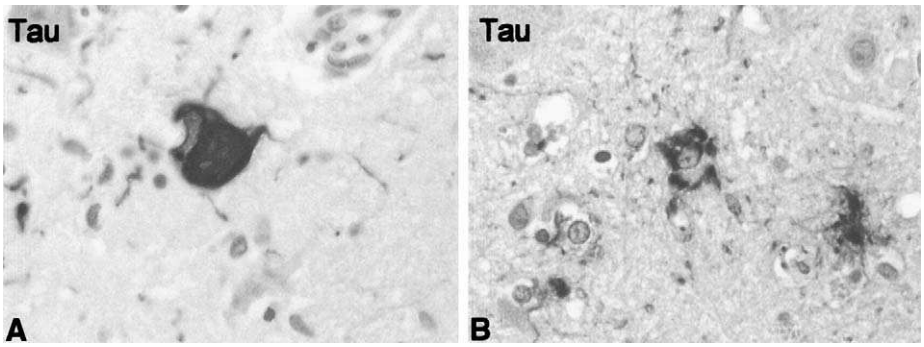

FIG. 13.4. Tau immunostaining reveals the two cardinal lesions in PSP: neurofibrillary tangles (A) and tufted astrocytes (B). The tufted astrocytes are most abundant in motor cortex and the corpus striatum, whereas NFT are prevalent in a number of basal ganglia and brain stem nuclei. Tau-immunoreactive inclusions in oligodendrocytes (''coiled bodies''; see Fig. 13.10) are found to a variable degree in white matter tracts, especially in the thalamic fasciculus.

been reported in PSP (Agid *et al*., 1987). Nuclei in the pontine base have NFT, whereas glial lesions are less abundant than in the basal ganglia (Fig. 13.6).

Cortical gray matter pathology is less pronounced than deep gray matter pathology, but lesions are common in motor and premotor cortices (Hauw *et al*., 1990; Hof *et al*. 1992; Vermersch *et al*., 1994) (Fig. 13.6). Early studies emphasized the lack of neocortical pathology in PSP (Steele *et al*., 1964; Ishino and Otsuki, 1976), but more recent studies using immunocytochemical and special silver impregnation methods

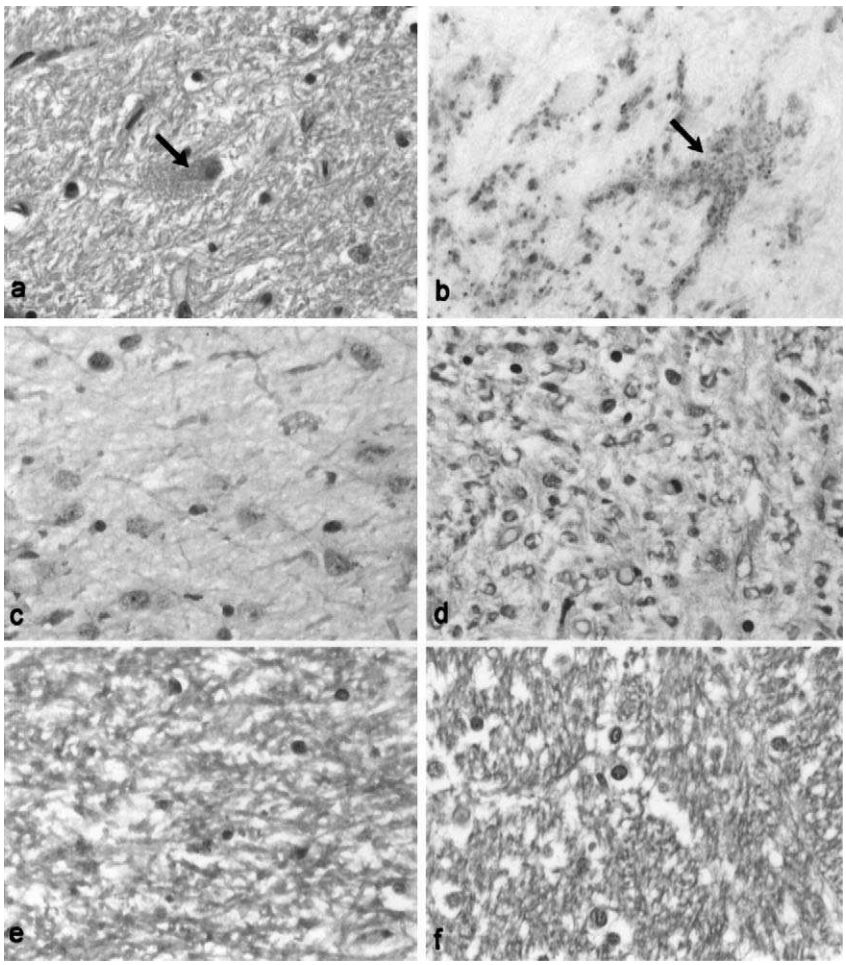

FIG. 13.5. The cerebellar dentate nucleus and its outflow pathway show pathology in typical cases of PSP. On hematoxylin–eosin stained materials the number of neurons is decreased and many of them have ill-defined indistinct borders (a) due to increased dystrophic synaptic terminals, which can be best illustrated with synaptophysin immunostaining (b). The dentate hilus (c) and the superior cerebellar peduncle (d) are depleted of myelinated fibers (Luxol fast blue stain for myelin), whereas nearby myelinated fibers are preserved (e, amiculum of dentate nucleus; f, middle cerebellar peduncle).

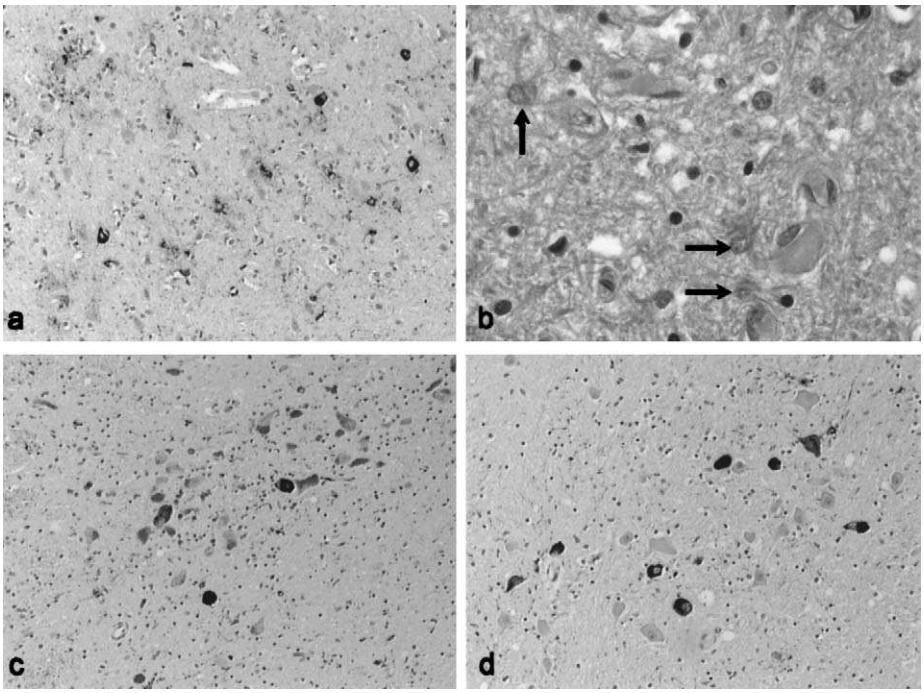

FIG. 13.6. The motor cortex has tau-immunoreactive lesions in the form of tufted astrocytes and scattered NFT (a). The subthalamic nucleus (b) and substantia nigra have marked neuronal loss accompanied by reactive fibrillary astrocytes (arrows) that can be detected readily with hematoxylin–eosin stains. The substantia nigra (c) and pontine nuclei (d) have neurons with NFT. When the neuronal loss is extensive, there may be only a small number of NFT in the substantia nigra (c).

indicate consistent involvement of the frontal cortex in PSP (Hauw *et al*., 1990; Hof *et al*., 1992; Braak *et al*., 1992; Vermersch *et al*., 1994; Bergeron *et al*., 1997; Bigio *et al*., 1999). Neocortical NFT and glial tangles are concentrated in the precentral gyrus (Hauw *et al*., 1990; Hof *et al*., 1992). Studies suggest that cortical neurofibrillary pathology may be greater in cases of PSP with dementia (Bigio, *et al*., 1999). The white matter beneath the motor cortex is also the site of glial pathology. More widespread cerebral white matter pathology is uncommon in PSP, although scattered glia may be encountered in cerebral white matter throughout the cerebrum.

The limbic lobe is relatively preserved in PSP. Neurofibrillary pathology in the hippocampus is variable and not intrinsic to PSP, but is rather more consistent with concurrent age-related pathology (Braak *et al*., 1992). When present, the distribution of NFT in the hippocampal formation is qualitatively similar to that seen in aging and AD. The exception to this rule is the frequent involvement of the dentate granule cells in PSP (Hof *et al*., 1992). The degree of neurofibrillary degeneration may correlate with the degree of cognitive impairment in PSP as in aging and AD (Braak and Braak, 1991).

While NFT in PSP often have a rounded or globose appearance, flame-shaped NFT are also detected. The Gallyas silver iodine method is sensitive to pathological structures in PSP and demonstrates, in addition to NFT, fibrillary inclusions in glial cells and cell processes. As in AD, NFT in PSP contain abnormally phosphorylated tau protein (Iqbal *et al*., 1989; Greenberg and Davies, 1990). Immunocytochemistry for tau protein is a sensitive method for detecting NFT in PSP, but it also reveals a wide variety of inclusions in both neurons and glia, including pretangles, neuropil threads, and tufts of abnormal fibers. Pretangles are neurons with nonfilamentous granular cytoplasmic tau immunoreactivity. The neurons that are most vulnerable to degeneration display this change, which is felt to be a precursor to the filamentous lesion. Antibodies that are most sensitive for detecting pretangles are those that recognize phosphorylated epitopes or conformational changes in tau proteins (Jicha *et al*., 1999).

The tufts of abnormal fibers are most common in motor cortex and striatum (Fig. 13.6). They have been shown to be fibrillary tau inclusions in astrocytes (Nishimura *et al*., 1992; Yamada *et al*., 1993; Iwatsubo *et al*., 1994). Tufted astrocytes account for much of the cortical pathology observed in PSP. Tau immunohistochemistry also reveals tau-positive fibers, so-called ''neuropil threads'' (Braak *et al*., 1986; Probst *et al*., 1988; Ikeda *et al*., 1994), and small round glial cells in the white matter of areas affected by PSP. The tau-positive glial cells in white matter, also referred to as ''coiled bodies,'' have been shown to be oligodendroglial inclusions (Yamada *et al*., 1992; Iwatsubo *et al*., 1994; Nishimura *et al*., 1995; Yamada and McGeer, 1995). The thread-like processes in white matter are not as numerous as in CBD, but in both disorders have been shown to be within both axons and the outer mesaxon of myelinated fibers (Arima *et al*., 1997). In gray matter, tau-positive threads are much less common in PSP than in AD (Davis *et al*., 1992) and CBD.

The NFT in PSP may be distinguished from NFT in AD by the paucity of ubiquitin immunoreactivity (Bancher *et al*., 1987; Cruz-Sanchez *et al*., 1992a). They are also far less fluorescent with thioflavin S stains. In fact, NFT may be completely missed if thioflavin S is the only method used to evaluate neuropathology in PSP. Certain neurofilament

epitopes may also distinguish NFT in PSP from those in AD (Schmidt *et al*., 1988). A more recent study with a panel of anti-tau antibodies recognizing epitopes along the length of the tau molecule suggested that full-length tau proteins are incorporated into the lesions in both AD and PSP (Schmidt *et al*., 1996). The observed differences must therefore reflect differences in packing, conformation, or posttranslational modification. While NFT in AD are composed mostly of 22 nm-diameter paired helical filaments (PHF) and a minor component of 15 to 18 nm diameter straight filaments (Terry, 1963), NFT in PSP are composed of straight 15 to 18 nm filaments (Tellez-Nagel and Wisniewski 1973; Powell *et al*., 1974; Roy *et al*., 1974; Bugiani *et al*., 1979; Yagashita *et al*., 1979). The abnormal filaments in glial cells in PSP also contain straight filaments (Nishimura *et al*., 1992).

B. Corticobasal Degeneration

The gross examination of the brain characteristically reveals asymmetrical narrowing of cortical gyri, most marked in pre- and postcentral regions. The atrophy is often best appreciated from a dorsal view rather than from a lateral or medial view (Figs. 13.7 and 13.8). This dorsal frontoparietal atrophy merges with less severe atrophy in ventral frontal and posterior parietal regions, whereas the temporal and occipital cortical regions are relatively preserved. The brain stem and cerebellum are not consistently reduced in size, but pigment loss is common in the substantia nigra (Fig. 13.8 and 13.9). There may also be mild atrophy of the midbrain tegmentum and enlargement of the aqueduct of Sylvius. In contrast to PSP, the superior cerebellar peduncle and the subthalamic nucleus are grossly normal.

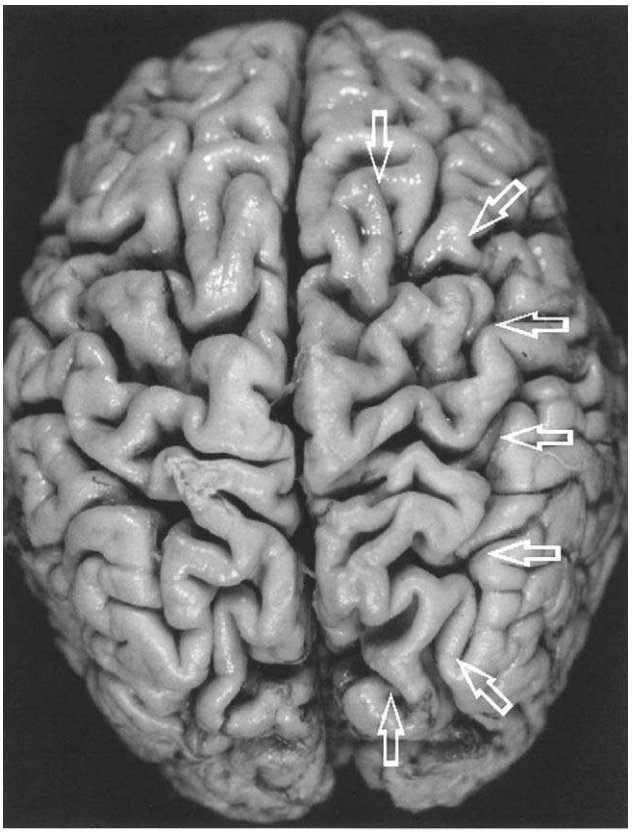

FIG. 13.7. The cortical atrophy in CBD is often best appreciated from a dorsal perspective. The superior frontal gyrus and superior parietal lobule are affected preferentially (arrows) in typical cases of PSP.

The cerebral white matter in affected areas is often attenuated and may have a gray discoloration or a gelatinous consistency in severe cases. The anterior corpus callosum is sometimes thinned and the frontal horn of the lateral ventricle is frequently dilated. Thinning of the corpus callosum has been used as a diagnostic feature of CBD in clinical imaging studies (Yamaguchi *et al*., 1998). The anterior limb of the internal capsule may show attenuation as well, but other white matter tracts, such as the optic tract, anterior commissure, and fornix, are preserved.

On microscopic examination, sections of atrophic frontoparietal cortex show moderately severe nerve cell loss and subcortical myelin pallor with gliosis. There is disruption of the normal pattern of cortical lamination, superficial spongiosis, and diffuse astrocytic and microglial proliferation. These findings are not dissimilar to those of nonspecific focal cortical degenerations, but several histologic features readily distinguish CBD from dementia lacking distinctive histopathology (Knopman *et al*., 1990).

In affected areas, especially the superior frontal gyrus, swollen and vacuolated cortical neurons, referred to as ballooned neurons (Fig. 13.10), are scattered in middle and lower cortical layers (Figs. 13.10 and 13.11) Ballooned neurons may be found in anterior cingulate, amygdala, and claustrum, where they are of less diagnostic significance, as they can be found in the limbic lobe in several different disorders. In contrast, ballooned neurons in the superior frontal and parietal lobes are decidedly uncommon in other disorders and nearly specific for CBD. The only exception is Pick's disease. In Pick's disease, ballooned neurons are most numerous in frontotemporal distribution, but a few may occasionally be detected in the parietal lobes (Constandinidis *et al*., 1974; Dickson, 1998). Ballooned neurons are eosinophilic and weakly argyrophilic. They may show irregular or tortuous swelling of proximal dendrites. Some ballooned neurons contain granulovacuolar bodies. They lack apparent Nissl substance, which was the basis for the term ''achromasia.'' Ballooned neurons are also referred to as ''Pick cells'' in Pick's disease (Dickson, 1998).

Ballooned neurons are strongly immunoreactive for phosphorylated neurofilaments (Dickson *et al*., 1986; Smith *et al*., 1992) and αB-crystallin (Lowe *et al*., 1992). They show variable immunoreactivity for tau and ubiquitin (Smith *et al*., 1992; Feany and Dickson, 1995; Halliday *et al*., 1995). They are negative for epitopes specific to neurofibrillary tangles (Dickson *et al*., 1986). Ultrastructurally, the cytoplasm of the ballooned neurons contains accumulations of intermediate-sized filaments 9–16 nm in diameter, interspersed with other cytoplasmic elements (Clark *et al*., 1986).

In addition to ballooned neurons scattered neurons in atrophic cortical areas have tau immunoreactivity. Tau-immunoreactive neuronal lesions are structurally pleomorphic. In some neurons the immunoreactivity is densely packed into a small inclusion body somewhat reminiscent of a Pick body or a small NFT. In other neurons the filamentous inclusions are

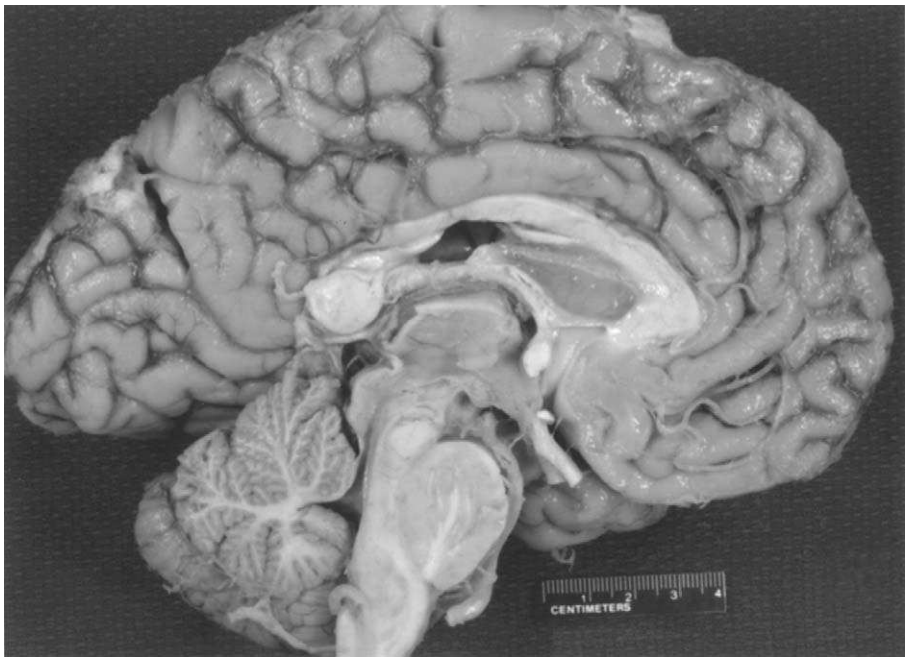

FIG. 13.8. The medial view of the brain in CBD may be unrevealing. Specifically absent is noticeable brain stem atrophy. The midbrain tectum is preserved. The aqueduct of Sylvius is not dilated.

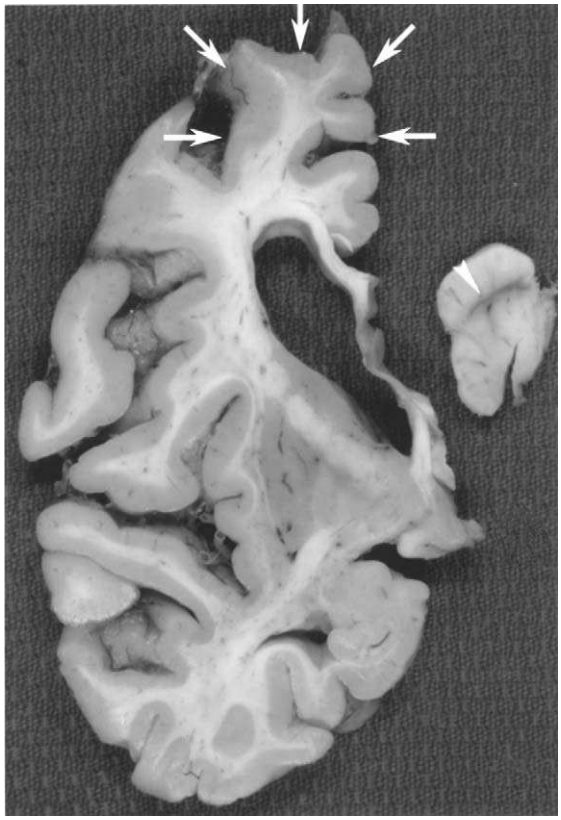

FIG. 13.9. On coronal sections of the brain, atrophy of the superior frontal gyrus (arrows) is disproportionate to that seen in any of the other gyri. Even the adjacent cingulate gyrus may be normal. The frontal horn of the lateral ventricle is typically enlarged. Midbrain sections show loss of black neuromelanin pigment in the substantia nigra (arrowhead), but there may also be an increase in rust-colored pigment.

more dispersed and disorderly. In contrast to NFT of AD, where lesions are readily detected with a host of diagnostic silver stains and even with thioflavin fluorescence microscopy, neuronal lesions in CBD are not easily seen and are often completely negative, especially with thioflavin S. This points to the fact that tau pathology in CBD is different from that in aging and AD. Other differences in neurofibrillary pathology in CBD compared to AD include less ubiquitination and a restricted tau isoform composition. These features are shared with the neurofibrillary pathology of PSP. Neurofibrillary lesions in brain stem monoaminergic nuclei, such as the locus coeruleus and substantia nigra, sometimes resemble the globose NFT of PSP, but more often are ill-defined amorphous inclusions (Fig. 13.12).

In addition to fibrillary lesions in perikarya of neurons, the neuropil of CBD invariably contains an assortment of tau-immunoreactive cell processes (Wakabayashi *et al*., 1994; Mori *et al*., 1994; Feany and Dickson, 1995; Takahashi *et al*., 1996) (Fig. 13.10). While these lesions are sometimes referred to as ''neuropil threads'' (Komori *et al*., 1997) after the lesions described in AD (Braak *et al*., 1986), it may be incorrect to use this term in CBD. In AD, virtually all neuropil threads are neuronal in origin as demonstrated by immunoelectron microscopy (Yamaguchi *et al*., 1990). In contrast, in CBD only a small fraction of thread-like structures are double labeled with neurofilament antibodies (Feany and Dickson, 1995), which indicates that many thread-like processes in CBD are probably glial rather than neuronal. In CBD, thread-like processes and shorter, stubbier tau-immunoreactive lesions with fuzzy rather than smooth profiles. They are usually profuse in affected areas of gray and white matter. The predominance of tau immunoreactivity in cell processes is an important attribute of CBD and a useful feature in differentiating it from other disorders. In other disorders with which CBD

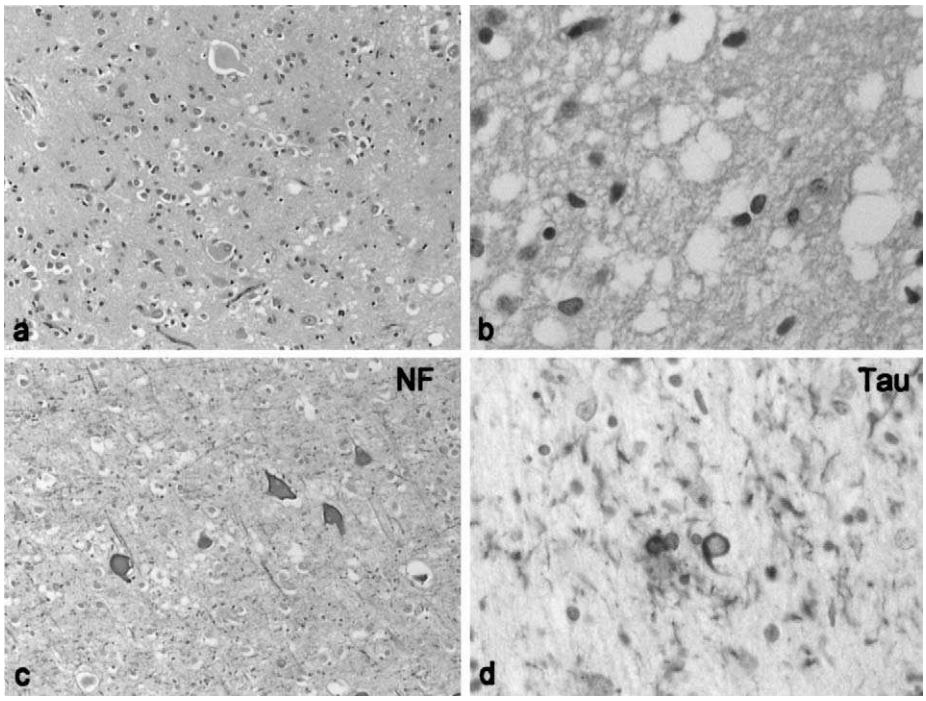

FIG. 13.10. The superior frontal and parietal cortices typically have microvacuolation and scattered swollen neurons (a). Microvacuolation often produces a spongiform appearance in the superficial cortical layers (b). The swollen or ballooned neurons are detected easily with neurofilament immunostaining (c). Tau immunostaining reveals many cell processes in both gray and white matter, as well as numerous coiled bodies that have been shown to be inclusions in oligodendrocytes (d).

can be confused, tau-related pathology is more often located in cell bodies (e.g., NFT and Pick bodies) and the proximal cell processes of neurons and glia.

A host of tau-immunoreactive astrocytic lesions have been described in various neurodegenerative diseases, including CBD. Astrocytic tau-immunoreactive lesions have been referred to as ''tufted astrocytes,'' ''star-like tufts,'' ''spiderlike radiating fibers,'' ''thorn-shaped astrocytes,'' and ''gliofibrillary tangles'' (reviewed in Chin and Goldman, 1996; Komori, 1999). The terminology is not standardized, and it is likely that the different morphologies and terms for the lesions reflect methodological as much as biologic differences. In particular, the morphology of the lesions varies dramatically, depending on the thickness of the tissue section. In routine 5- to 7-μm-thick sections, only a small cross-sectional sample of the lesion is included, whereas in thicker (40–100 μm) sections the lesions are visible in their entirety. The different morphologies of tau-immunoreactive astrocytic lesions may also be a function of which type of astrocytes (protoplasmic vs fibrous, subpial vs parenchymal) is affected.

The most characteristic tau-immunoreactive astrocytic lesion in CBD, particularly in the neocortex, is an annular cluster of short stubby processes that may be highly suggestive of an Alzheimer-type neuritic plaque (Uchihara *et al*., 1994;

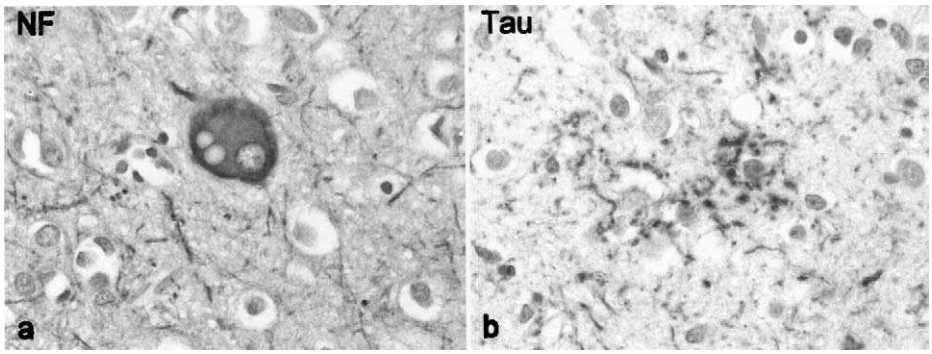

FIG. 13.11. Cardinal lesions in CBD—ballooned neurons (a) and astrocytic plaques (b)—are best demonstrated with immunostaining for neurofilament (or αB-crystallin) (a) and for tau (b). In the cortex, astrocytic plaques are characterized by radial arrays of short stubby and irregular cell processes around a central nucleus that can be shown to be an astrocyte with double immunostaining with astrocyte markers such as GFAP.

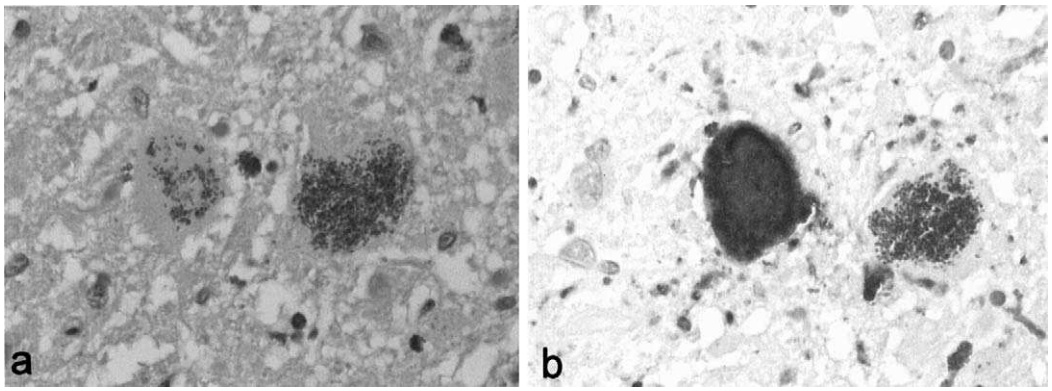

FIG. 13.12. In brain stem monoaminergic nuclei, neurons have amorphous ill-defined slightly basophilic inclusions (''corticobasal bodies'') with hematoxylin–eosin (a) that can be shown to be intensely positive for tau with immunostaining (b). These neuronal lesions are most common in neurons that also contain neuromelanin [small dark granules in neurons at the right of each corticobasal body in (a) and (b)].

Feany and Dickson, 1995). These lesions, in contrast to Alzheimer plaques, do not contain amyloid based on histochemical, fluorescent, and immunocytochemical methods for detecting amyloid. The only exception is in rare cases of coexistent CBD and AD. In further contradistinction with neuritic plaques in AD, most of the tau immunoreactivity is not within dystrophic neuronal processes, but rather processes of glial cells. Double immunostaining for tau and glial fibrillary acid protein, vimentin, or CD44, which are all markers with varying degrees of specificity for astrocytes, demonstrates that the tau-positive cell processes in these lesions are derived from astrocytes (Feany and Dickson, 1995). These lesions are called ''astrocytic plaques'' (Feany and Dickson, 1995) (Fig 13.11). Astrocytic plaques differ from the tufted astrocytes seen in PSP, and the two lesions do not appear to coexist in the same brain (Komori *et al*., 1998). When the astrocytic plaque, with its distinct annular array of tau-immunoreactive processes, is detected with Gallyas silver stains or immunocytochemistry in the neocortex or neostriatum, it should suggest a diagnosis of CBD until proven otherwise. The astrocytic plaque may be the most specific histopathologic lesion of CBD. Other less distinctive tau-immunoreactive astrocytic lesions are also seen in CBD, and the latter, in particular, may have overlapping morphological features with tau-positive astrocytes of other tauopathies.

In addition to cortical pathology, deep gray matter is consistently affected in CBD. In the basal ganglia, thread-like processes are often extensive in the pencil fibers of the striatum. There may also be a few astrocytic plaques in the striatum. Neuronal inclusions are also common in the striatum and globus pallidus. The internal capsule often has many thread-like processes, especially in the vicinity of the thalamic fasciculus. The subthalamic nucleus usually has a normal neuronal population, but a few neurons may have tau inclusions and there may be a number of thread-like lesions in the nucleus. Fibrillary gliosis typical of PSP is not found in the subthalamic nucleus in CBD, but it is not uncommon to find tau-positive processes, glial inclusions, and a few pretangles in the subthalamic nucleus.

The substantia nigra usually shows moderate to severe nerve cell loss with extraneuronal neuromelanin and gliosis. Many of the remaining neurons contain ill-defined neurofibrillary inclusions, so-called ''corticobasal bodies'' (Gibb *et al*., 1989) (Fig. 13.12). These inclusions have a homogeneous or faintly whorled or amorphous morphology with enmeshed granules of melanin pigment. Immunocytochemical studies have shown that corticobasal bodies are indistinguishable from globose NFT of PSP, but they are less fibrillar. The specificity of the corticobasal body remains to be seen. The locus coeruleus and raphe nuclei have similar inclusions. In contrast to PSP, where neurons in the pontine base almost always have at least a few NFT, the pontine base is largely free of NFT in CBD. However, tau inclusions in glia and in cell processes are frequent in the pontine base in CBD (Dickson and D'Aversa, 1997). NFT are also detected in the tegmental gray matter.

In other subcortical regions the globus pallidus and putamen show nerve cell depletion with gliosis and occasional NFT. The nucleus basalis of Meynert has a few NFT. The red nucleus and subthalamic nucleus also show mild neuronal depletion and astrocytic gliosis and may have NFT. Thalamic nuclei may also be affected, particularly the ventrolateral nucleus. Mild neuronal depletion of the dentate nucleus occurs, but grumose degeneration is much less common than in PSP.

The Gallyas silver stain is a sensitive way of demonstrating pathology in neurons and glia in CBD (Horoupian and Chu, 1994; Matsumoto *et al*., 1996). This is due to the fact that the Gallyas stain, for reasons that are not clearly known, detects abnormal filamentous tau protein (Iqbal *et al*., 1991). Immunocytochemistry with monoclonal antibodies to tau protein gives comparable results (Mori *et al*., 1994; Wakabayashi *et al*., 1994; Feany and Dickson, 1995) and have wider usage given the commercial availability of tau antibodies. Tau immunostaining also requires less technical expertise. It has been claimed that glial lesions in PSP and CBD are distinguished more clearly with the Gallyas method than immunostaining (Komori, 1999), but section thickness needs to be taken into account. Typical preparations for Gallyas stains employ sections that are 10 times thicker than conventional histology sections.

In CBD the filaments have a paired helical appearance at the electron microscopic level, but the diameter is wider and the periodicity is longer (Ksiezak-Reding *et al*., 1996; Tracz *et al*., 1997). These structures have been referred to as twisted

ribbons. Similar filaments are found in some cases of FTDP-17, particularly those associated with overexpression of specific isoforms of tau containing exon 10 (see later). There is no evidence to suggest that specific isoforms of tau are overexpressed in CBD, but studies addressing this question are needed given the similarity of the lesions in CBD to FTDP-17.

C. Mixed and Transitional Pathology

While the majority of clinically well-characterized patients with PSP have distinctive postmortem findings, several reports suggest that the match is not always perfect (Collins *et al*., 1995; Gearing *et al*., 1994; Davis *et al*., 1985). Cases of PSP may present with dementia or rarely focal cortical syndromes that resemble CBD clinically. The pathologic substrate of the asymmetrical rigidity and apraxia syndrome typical of CBD is diverse and includes AD, PSP, Pick's disease, nonspecific frontotemporal dementia, and Creutzfeldt–Jakob disease (Boeve *et al*., 1999). Whereas ballooned neurons are not common in PSP and a useful histopathologic feature for differentiating PSP from CBD, there are reports of some cases of PSP with ballooned neurons (Mackenzie and Hudson, 1995; Mori *et al*., 1996; Mori and Oda, 1997). There are also reports of CBD without ballooned neurons (Kawasaki *et al*., 1996). In most of these reports, ballooned neurons are limited to limbic and paralimbic cortices. As mentioned previously, this distribution of ballooned neurons does not carry the diagnostic specificity as does ballooned neurons in non-limbic neocortices.

Some cases of PSP have transitional pathology with more widespread cortical pathology than typical PSP, where neurofibrillary degeneration is limited to the motor cortex. These cases can be difficult to differentiate from CBD with sparse ballooned neurons (Schneider *et al*., 1997). It is important to note that pathology in CBD is bilateral, but sampling the side with less pathology may also produce confusion. In particular, ballooned neurons may have a relatively restricted distribution and not be detected in less affected cortical areas. In case of doubt, it is best to sample the cortical areas with most atrophy or areas known to be most vulnerable to cortical pathology, particularly the superior frontal cortex.

Some cases of PSP have a paucity of neurofibrillary lesions. It is unclear if these cases are merely early pathologic stages of the disorder. They must be differentiated from cases of postencephalitic parkinsonism, a diagnosis that depends on an appropriate clinical history (Geddes *et al*., 1993).

Finally, PSP and CBD can be mixed with other pathologies (Lantos, 1994). Most common are infarcts, Alzheimer-type plaques, and Lewy bodies. These cases are best considered to be combinations of two or more separate disorders, although one could also argue that cases with mixed pathology represent distinct pathologic entities. It is also challenging to diagnose CBD when there are age-related amyloid plaques in the cortex as well as astrocytic plaques. Double staining for tau and amyloid will usually allow identification of what appear to be ''neuritic'' plaques without any amyloid deposits. The latter are astrocytic plaques. In contrast, Alzheimer-type plaques will have amyloid within the center of the tau-positive cell processes. Significant neurofibrillary degeneration in the limbic lobe is not typical of either PSP or CBD. When this is encountered it is likely due to mixed AD.

Another pathology that is common to the limbic lobe and that is relatively common in PSP and CBD is argyrophilic grain disease (Braak and Braak, 1989; Jellinger, 1998). Grains are tau-positive neuronal inclusions that have been localized to dendritic segments (Ikeda *et al*., 1995; Tolnay *et al*., 1998). Some of the tau-positive lesions in PSP, particularly CBD, may resemble grains. Unlike argyrophilic grain disease, the short granular processes (''grain-like'' lesions) in PSP and CBD are not restricted to the limbic lobe and certain hypothalamic nuclei (Braak and Braak, 1989). Nevertheless, grains can be detected in PSP and CBD (Martinez-Lage and Munoz, 1997; Jellinger, 1998). Another particularly difficult situation is the fact that most cases of AGD have BN, but in argyrophilic grain disease, ballooned neurons are limited to the limbic lobe, especially the amygdala (Tolnay and Probst, 1998).

IV. Tau Biochemistry

Given the presence of tau-immunoreactive neuronal and glial lesions in PSP and CBD it is not surprising that most recent biochemical studies have focused on tau protein abnormalities in PSP and CBD compared to normal control brains and abnormal tau proteins in AD (Ksiezak-Reding *et al*., 1994; Delacourte and Buée, 1997; Mailliot *et al*., 1998; Buée and Delacourte, 1999). The tau protein is a microtubule-associated phosphoprotein that promotes tubulin polymerization and stabilization of microtubules. It is present in neurons, preferentially in axons, and until recently was felt to be specific to neurons (Binder *et al*., 1985). More recent immunochemical evidence suggests that the tau protein is also expressed in glial cells, especially in pathologic conditions. It undergoes posttranslational modification, such as phosphorylation, which controls its functional state (reviewed in Buée and Delacourte, 1999). Phosphorylated tau is less efficient in promoting tubulin polymerization. The tau gene is located on chromosome 17, and it has 16 exons, 3 of which are alternatively spliced (Andreadis *et al*., 1992). In the amino-terminal half of the molecule are conserved 30 amino acid tandem repeats that are essential for interaction of tau with microtubules. One of the repeat domains is the product of exon 10 and is alternatively spliced. The various splice combinations generate six tau isoforms (Buée and Delacourte, 1999). In PSP and CBD exon 10-containing isoforms predominate (Sergeant *et al*., 1999).

In normal brain, tau is a natively unfolded, soluble protein that is heat stable. The pathological tau protein has altered solubility properties, being soluble in detergents, and it forms abnormal filamentous structures. Western blots of normal brain tissue homogenates show six bands in a range of 50–62 kDa. Homogenates of detergent-soluble tau protein from PSP and CBD migrate at a higher molecular weight due to increased phosphorylation. In addition the immunoblotting pattern is reduced to two major bands at about 64 and 68 kDa (Flament *et al*., 1991; Ksiezak-Reding *et al*., 1994; Buée and Delacourte, 1999) (Fig. 13.13). There are conflicting reports about whether exon 10 is expressed in the pathological tau protein of CBD (Ksiezak-Reding *et al*., 1994; Delacourte and Buée, 1997; Mailliot *et al*., 1998). This is due to the fact that the amino acid sequences in the repeat regions are very similar. Antibodies raised to exon 10 often cross-react with sequences

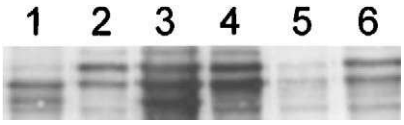

FIG. 13.13. Western blot of detergent-soluble fraction from several neurodegenerative disorders: Pick's disease (1), FTDP-17 (2), Alzheimer's disease (3), CBD (4), and PSP (5 and 6). Note that Pick's FTDP-17, CBD, and PSP have two prominent bands, whereas three are present in AD. Note also that the two bands in Pick's disease migrate at lower molecular weights than those in FTDP-17, CBD, and PSP. Western blots kindly provided by Drs. W.-K. Liu and S.-H. Yen.

in the other repeat regions, especially exons 9 and 11. The weight of current evidence suggests that the pathologic tau protein in CBD is generated preferentially from transcripts that contain exon 10. This is based on analysis not only with exon 10-specific antibodies, but also by immunoblotting tau preparations after extensive dephosphorylation. Analysis with antibodies specific to exon 3, another alternatively spliced exon, suggests that exon 3, in contrast to exon 10, is underrepresented in the pathological tau protein of CBD (Feany *et al*., 1995; Nishimura *et al*., 1997).

Given the abundance of tau pathology in glial cells in PSP and CBD, an unanswered question is how much the observed difference in tau isoform composition reflects tau derived from glial cells compared with tau from neurons. Interestingly, nonneuronal cells appear to express tau containing exon 10 (Vanier *et al*., 1998), and there is also exon 10 containing tau in white matter than gray matter (Janke *et al*., 1996). Another explanation for the selective involvement of specific splice forms of tau, namely tau containing four repeats, might be more abundant in the pathologic tau of PSP and CBD is that this form is overexpressed. Chambers *et al*., (1999) suggested that this may be the case, at least in some brain regions of PSP. This study needs to be confirmed in a larger series of cases and by sampling more areas that are vulnerable or relatively resistant to PSP and CBD.

Other disorders with neuronal and glial tau pathology share immunoblotting patterns with PSP and CBD. For example, Western blots of detergent-soluble tau protein from some cases of FTDP-17 have predominantly two bands (Ksiezak-Reding *et al*., 1994; Delacourte and Buée, 1997; Mailliot *et al*., 1998; Buée and Delacourte, 1999). Interestingly, abnormal tau in Pick's disease is also characterized by two major bands on Western blots, but they appear to be different from those in PSP and CBD (Buée and Delacourte, 1999). In Pick's disease the proteins migrate at estimated molecular weights of 55 and 64 kDa (Fig. 13.13). Evidence suggests that in Pick's disease pathological tau protein isoforms lack exon 10 (Delacourte *et al*., 1996; Sergeant *et al*., 1997), whereas those in PSP and CBD preferentially contain exon 10 (Spillantini and Goedert, 1998). This supports the concept that PSP and CBD may have common pathogenesis, despite differences in clinical and pathologic features.

Interestingly, tau protein isolated from the entorhinal cortex of PSP brains contained not the doublet characteristic of PSP, but the triplet found in AD material (Vermersch *et al*., 1994). These results support the suggestion that allocortical pathology in PSP is similar to that in AD.

V. Genetics

A. Clinical Studies

There are only a few reports of autopsy-proven familial PSP (Brown *et al*., 1993; Tetrud *et al*., 1996; de Yébenes *et al*., 1995; Gazeley and Maguire, 1996; Rojo *et al*., 1999). Nevertheless, it may be more common than previously thought given the heterogeneity in the clinical presentation of early-onset cases of PSP. Similarly, almost all reported cases of CBD have been sporadic, but there are rare familial reports of CBD (Brown *et al*., 1996). Whether these are actual cases of CBD or FTDP-17 remains to be determined by evaluation of these purported familial CBD cases for tau mutations. FTDP-17 has a number of clinical and pathologic features that are similar to CBD (Bugiani *et al*., 1999) and in some families to PSP (Reed *et al*., 1997). In particular, familial multisystem tauopathy (Spillantini *et al*., 1997, 1998b) and familial pallidopontonigral degeneration (Reed *et al*., 1998) have many histological, ultrastructural, and biochemical similarities. FTDP-17 has been shown to be caused by tau mutations in either coding or noncoding regions (Hutton *et al*., 1998). The location of the mutation, especially as it relates to exon 10 and the microtubule-binding region, contributes significantly to the clinical and pathologic phenotype and to the nature of the tau that accumulates in the fibrillary lesions (Hutton, *et al*., 1998). The mutations identified in tau directly affect its ability to interact with microtubules, suggesting a pathogenetic mechanism for neurodegeneration (Hong *et al*., 1998; Yen *et al*., 1999).

B. Tau Gene

A dinucleotide repeat polymorphism in the tau gene was reported to be associated with greater than chance frequency with PSP (Conrad *et al*., 1997). The number of dinucleotide repeats varied from 11 (a0) to 14 (a3). In PSP more than 95% of the cases had a0. The fact that a tau polymorphism was associated with PSP raised the possibility that specific forms of the tau gene might confer genetic risk for PSP, analogous to the apolipoprotein E genotype for AD (Mayeux *et al*., 1998). Given the fact that 75% of the normal population has the a0 polymorphism and yet PSP is such an uncommon disorder, it suggests that any risk conferred by the polymorphism must be minor. The increased frequency of this tau polymorphism in PSP has been confirmed in several studies among different ethnic groups (Oliva *et al*., 1998; Higgins *et al*., 1999; Hoenicka *et al*., 1999; Morris *et al*., 1999; Baker *et al*., 1999). Interestingly, a0 is more common in controls in Japan than in Western population and has been speculated to account for a possible increased frequency of PSP in Japanese patients (Conrad *et al*., 1998).

Additional studies of the genetic structure of the tau gene revealed a number of polymorphisms that were inherited in a nonrandom manner. Careful analysis suggested that the polymorphisms were inherited as extended haplotypes. Essentially, two ancestral forms of the tau gene have been identified: H1 and H2 (Baker *et al*., 1999). Most cases of typical PSP are H1 and a surprising large number of PSP patients are H1/H1 homozygous. There is some preliminary evidence to suggest greater variability in the disease phenotype in PSP cases that

are heterozygous (H1/H2), but this remains to be verified. Heterogeneity in phenotype can also be due to additional pathologies, most notably Alzheimer-type pathology. It is presently unclear whether CBD is also associated with an increased frequency of H1 haplotype. One report suggested that a0 polymorphism was not increased in CBD, but this study was based on a small sample size (Morris *et al*., 1999). Further limitations of studies of this type are the evolving clinical and pathologic criteria for CBD and the rarity of the disorder. Finding a genetic association will necessitate common diagnostic criteria, preferably autopsy confirmed, and a large sample size obtained from a number of cooperating centers. Such a multicenter study is currently in progress. If CBD should prove to have a similar genetic risk factor as PSP, it would add support to the relationships between PSP and CBD.

References

Agid, Y., Javoy-Agid, F., Ruberg, M., Pillon, B., Dubois, B., Duyckaerts, C., Hauw, J. J., Baron, J. C., and Scatton, B. (1987). Progressive supranuclear palsy: Anatomoclinical and biochemical considerations. *Adv. Neurol*. **45**, 191–206.

Andreadis, A., Brown, W. M., and Kosik, K. S. (1992). Structure and novel exons of the human tau gene. *Biochemistry* **31**, 10626–10633.

Arai N. (1987). ''Grumose degeneration'' of the dentate nucleus. A light and electron microscopic study in PSP and dentatorubropallidoluysian atrophy. *J. Neurol. Sci*. **90**, 131–145.

Arima, K., Uesugi, H., Fujita, I., Sakurai, Y., Oyanagi, S., Andoh, S., Izumiyama, Y., and Inose, T. (1994). Corticonigral degeneration with neuronal achromasia presenting with primary progressive aphasia: Ultrastructural and immunocytochemical studies. *J. Neurol. Sci*. **127**, 186–197.

Arima, K., Nakamura, M., Sunohara, N., Ogawa, M., Anno, M., Izumiyama, Y., Hirai, S., and Ikeda, K. (1997). Ultrastructural characterization of the tau-immunoreactive tubules in the oligodendroglial perikarya and their inner loop processes in progressive supranuclear palsy. *Acta Neuropathol*. **93**, 558–566.

Baker, M., Litvan, I., Houlden, H., Adamson, J., Dickson, D., Pérez-Tur, J., Hardy, J., Lynch, T., Bigio, E., and Hutton, M. (1999). Association of an extended haplotype in the tau gene with progressive supranuclear palsy. *Hum. Mol. Genet*. **8**, 711–715.

Bancher, C., Lassmann, H., Budka, H., Grundke-Iqbal, I., Iqbal, K., Wiche, G., Seitelberger, F., and Wisniewski, H. M. (1987). Neurofibrillary tangles in Alzheimer's disease and progressive supranuclear palsy: Antigenic similarities and differences. *Acta Neuropathol*. **74**, 39–46.

Baron, J. C., Mazière, B., Loch, C., Cambon, H., Sgouropoulos, P., Bonnet, A. M., and Agid, Y. (1986). Loss of striatal [^{76}Br]-bromospiperone binding sites demonstrated by positron tomography in progressive supranuclear palsy. *J. Cereb. Blood Flow Metab*. **6**, 131–136.

Bergeron, C., Pollanen, M. S., Weyer, L., Black, S. E., and Lang, A. E. (1996). Unusual clinical presentations of cortical-basal ganglionic degeneration. *Ann. Neurol*. **40**, 893–900.

Bergeron, C., Pollanen, M. S., Weyer, L., and Lang, A. E. (1997). Cortical degeneration in progressive supranuclear palsy. A comparison with cortical-basal ganglionic degeneration. *J. Neuropathol. Exp. Neurol*. **56**, 726–734.

Bergeron, C., Davis, A., and Lang, A. E. (1998). Corticobasal ganglionic degeneration and progressive supranuclear palsy presenting with cognitive decline. *Brain Pathol*. **8**, 355–365.

Bigio, E. H., Brown, D. F., and White, C. L., III (1999). Progressive supranuclear palsy with dementia: cortical pathology. *J. Neuropathol. Exp. Neurol*. **58**, 359–364.

Binder, L. I., Frankfurter, A., and Rebum, L. I. (1985). The distribution of tau in the mammalian central nervous system. *J. Cell Biol*. **101**, 1371–1378.

Blin, J., Vidailhet, M.-J., Pillon, B., Dubois, B., Fève, J.-R., and Agid, Y. (1992). Corticobasal degeneration: Decreased and asymmetrical glucose consumption as studied with PET. *Movement Disord*. **7**, 348–354.

Boeve, B. F., Maraganore, D. M., Parisi, J. E., Ahlskog, J. E., Graff-Radford, N., Caselli, R. J., Dickson, D. W., Kokmen, E., and Petersen, R. C. (1999). Pathologic heterogeneity in clinically diagnosed corticobasal degeneration. *Neurology* **53**, 795–800.

Bower, J. H., Dickson, D. W., Taylor, L., Maraganore, D. M., and Rocca, W. A. (1999a). Clinical misconceptions of the pathology underlying parkinsonism. *Neurology* **52**, A92 (abstr).

Bower, J. H., Maraganore, D. M., McDonnell, S. K., and Rocca, W. A. (1999b). Incidence and distribution of parkinsonism in Olmsted County, Minnesota, 1976–1990. *Neurology* **52**, 1214–1220.

Braak, H., and Braak, E. (1989). Cortical and subcortical argyrophilic grains characterize a disease associated with adult onset dementia. *Neuropathol. Appl. Neurobiol*. **15**, 13–26.

Braak, H., and Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol*. **82**, 239–259.

Braak, H., Braak, E., Grundke-Iqbal, I., and Iqbal, K. (1986). Occurrence of neuropil threads in the senile human brain and in Alzheimer's disease: A third location of paired helical filaments outside of neurofibrillary tangles and neuritic plaques. *Neurosci. Lett*. **65**, 351–355.

Braak, H., Jellinger, K., Braak, E., and Bohl, J. (1992). Allocortical neurofibrillary changes in progressive supranuclear palsy. *Acta Neuropathol*. **84**, 478–483.

Brooks, D. J., Ibanez, V., Sawle, G. V., Quinn, N., Lees, A. J., Mathias, C. J., Bannister, R., Marsden, C. D., and Frakowiak, R. S. J. (1990). Differing patterns of striatal ^{18}F-dopa uptake in Parkinson's disease multiple system atrophy and progressive supranuclear palsy. *Ann. Neurol*. **28**, 547–555.

Brooks, D. J., Ibanez, V., Sawle, G. V., Playford, E. D., Quinn, N., Mathias, C. J., Lees, A. J., Marsden, C. D., Bannister, R., and Frackowiak, R. S. (1992). Striatal D2 receptor status in patients with Parkinson's disease, striatonigral degeneration, and progressive supranuclear palsy, measured with ^{11}C-raclopride and positron emission tomography. *Ann. Neurol*. **31**, 184–192.

Brown, J., Lantos, P. L., Stratton, M., Roques, P., and Rossor, M. N. (1993). Familial progressive supranuclear palsy. *J. Neurol. Neurosurg. Psychiatry* **56**, 473–476.

Brown, J., Lantos, P. L., Roques, P., Fidani, L., and Rossor, M. N. (1996). Familial dementia with swollen achromatic neurons and corticobasal inclusion bodies: A clinical and pathological study. *J. Neurol. Sci*. **135**, 21–30.

Buée, L., and Delacourte, A. (1999). Comparative biochemistry of tau in progressive supranuclear palsy, corticobasal degeneration, FTDP-17 and Pick's disease. *Brain Pathol*. **9**, 681–693.

Bugiani, O., Mancardi, G. L., Brusa, A., and Ederli, A. (1979). The fine structure of subcortical neurofibrillary tangles in progressive supranuclear palsy. *Acta Neuropathol*. **45**, 147–152.

Bugiani, O., Murrell, J. R., Giaccone, G., Hasegawa, M., Ghigo, G., Tabaton, M., Morbin, M., Primavera, A., Carella, F., Solaro, C., Grisoli, M., Savoiardo, M., Spillantini, M. G., Tagliavini, F., Goedert, M., and Ghetti, B. (1999). Frontotemporal dementia and corticobasal degeneration in a family with a P301S mutation in tau. *J. Neuropathol. Exp. Neurol*. **58**, 667–677.

Chambers, C. B., Lee, J. M., Troncoso, J. C., Reich, S., and Muma, N. A. (1999). Overexpression of four-repeat tau mRNA isoforms in progressive supranuclear palsy but not in Alzheimer's disease. *Ann. Neurol*. **46**, 325–332.

Chin, S. S., and Goldman, J. E. (1996). Glial inclusions in CNS degenerative diseases. *J. Neuropathol. Exp. Neurol*. **55**, 499–508.

Clark, A. W., Manz, H. J., White, C.L., III Lehmann, J., Miller, D., and Coyle, J. T. (1986). Cortical degeneration with swollen chromatolytic neurons: Its relationship to Pick's disease. *J. Neuropathol. Exp. Neurol*. **45**, 268–284.

Collins, S. J., Ahlskog, J. E., Parisi, J. E., and Maraganore, D. M. (1995). Progressive supranuclear palsy: Neuropathologically based diagnostic clinical criteria. *J. Neurol. Neurosurg. Psychiatry* **58**, 167–173.

Conrad, C., Andreadis, A., Trojanowski, J. Q., Dickson, D. W., Kang, D., Chen, X., Wiederholt, W., Hansen, L., Masliah, E., Thal, L. J., Katzman, R., Xia, Y., and Saitoh, T. (1997). Genetic evidence for the involvement of τ in progressive supranuclear palsy. *Ann. Neurol*. **41**, 277–281.

Conrad, C., Amano, N., Andreadis, A., Xia, Y., Namekataf, K., Oyama, F., Ikeda, K., Wakabayashi, K., Takahashi, H., Thal, L. J., Katzman, R., Shackelford, D. A., Matsushita, M., Masliah, E., and Sawa, A. (1998). Differences in a dinucleotide repeat polymorphism in the tau gene between Caucasian and Japanese populations: Implication for progressive supranuclear palsy. *Neurosci. Lett*. **250**, 135–137.

Constantinidis, J., Richard, J., and Tissot, R. (1974). Pick's disease Histological and clinical correlations. *Eur. Neurol*. **11**, 208–217.

Cruz-Sanchez, F. F., Rossi, M. L., Cardozo, A., Deacon, P., and Tolosa, E. (1992a). Clinical and pathological study of two patients with progressive supranuclear palsy and Alzheimer's changes. Antigenic determinants that distinguish cortical and subcortical neurofibrillary tangles. *Neurosci. Lett*. **136**, 43–46.

Cruz-Sanchez, F. F., Rossi, M. L., Cardozo, A., Picardo, A., and Tolosa, E. (1992b). Immunohistological study of grumose degeneration of the dentate nucleus in progressive supranuclear palsy. *J. Neurol. Sci*. **110**, 228–231.

D'Antona, R., Baron, J. C., Sanson, Y., Serdaru, M., Viader, F., Agid, Y., and Cambier, J. (1985). Subcortical dementia: frontal cortex hypometabolism detected by positron tomography in patients with progressive supranuclear palsy. *Brain* **108**, 785–799.

Davis, D. G., Wang, H. Z., and Markesbery, W. R. (1992). Image analysis of neuropil threads in Alzheimer's disease, Pick's disease, diffuse Lewy body disease and progressive supranuclear palsy. *J. Neuropathol. Exp. Neurol*. **51**, 594–600.

Davis, P. H., Bergeron, C., and McLachlan, R. (1985). Atypical presentation of progressive supranuclear palsy. *Ann. Neurol*. **17**, 337–343.

Delacourte, A., and Buée, L. (1997). Normal and pathological Tau proteins as factors for microtubule assembly. *Int. Rev. Cytol*. **171**, 167–224.

Delacourte, A., Robitaille, Y., Sergeant, N., Buée, L., Hof, P. R., Wattez, A., Laroche-Cholette, A., Mathieu, J., Chagnon, P., and Gauvreau, D. (1996). Specific pathological Tau protein variants characterize Pick's disease. *J. Neuropathol. Exp. Neurol*. **55**, 159–168.

de Yébenes, Y. G., Sarasa, J. L., Daniel, S. E., and Lees, A. J. (1995). Familial progressive supranuclear palsy: Description of a pedigree and review of the literature. *Brain* **118**, 1095–1103.

Dickson, D. W. (1998). Pick's disease: A modern approach. *Brain Pathol*. **8**, 339–354.

Dickson, D. W. (1999a). Tau and synuclein and their role in neuropathology. *Brain Pathol*. **9**, 657–661.

Dickson, D. W. (1999b). Neuropathologic differentiation of progressive supranuclear palsy and corticobasal degeneration. *J. Neurol*. **246**(Suppl. 2), 6–15.

Dickson, D. W., and D'Aversa, T. (1997). Pontine cytoskeletal pathology in the differential diagnosis of non-Alzheimer dementias. *J. Neuropathol. Exp. Neurol*. **56**, 592.

Dickson, D. W., Yen, S.-H., Suzuki, K. I., Davies, P., Garcia, J. H., and Hirano, A. (1986). Ballooned neurons in select neurodegenerative diseases contain phosphorylated neurofilament epitopes. *Acta Neuropathol*. **71**, 216–223.

Doi, T., Iwasa, K., Makifuchi, T., and Takamori, M. (1999). White matter hyperintensities on MRI in a patient with corticobasal degeneration. *Acta Neurol. Scand*. **99**, 199–201.

Doody, R. S., and Jankovic, J. (1992). The alien hand and related signs. *J. Neurol., Neurosurg. Psychiatry* **55**, 806–810.

Dubois, B., Pillon, B., Legault, F., Agid, Y., and Lhermitte, F. (1988). Slowing of cognitive processing in progressive supranuclear palsy: A comparison with Parkinson's disease. *Arch. Neurol. (Chicago)* **45**, 1194–1199.

Feany, M. B., and Dickson, D. W. (1995). Widespread cytoskeletal pathology characterizes corticobasal degeneration. *Am. J. Pathol*. **146**, 1388–1396.

Feany, M. B., and Dickson, D. W. (1996). Neurodegenerative disorders with extensive tau pathology: A comparative study and review. *Ann. Neurol*. **40**, 139–148.

Feany, M. B., Ksiezak-Reding, H., Liu, W.-K., Vincent, I., Yen, S.-H., and Dickson, D. W. (1995). Epitope expression and hyperphosphorylation of tau protein in corticobasal degeneration: Differentiation from progressive supranuclear palsy. *Acta Neuropathol*. **90**, 37–43.

Feany, M. B., Mattiace, L. A., and Dickson, D. W. (1996). Neuropathologic overlap of progressive supranuclear palsy, Pick's disease and corticobasal degeneration. *J. Neuropathol. Exp. Neurol*. **55**, 53–67.

Flament, S., Delacourte, A., Verny, M., Hauw, J.-J., and Javoy-Agid, F. (1991). Abnormal tau proteins in progressive supranuclear palsy. *Acta Neuropathol*. **81**, 591–596.

Foster, N. L., Gilman, S., Berent, S., Morin, E. M., Brown, M. B., and Koeppe, R. A. (1988). Cerebral hypometabolism in progressive supranuclear palsy studied with positron emission tomography. *Ann. Neurol*. **24**, 399–406.

Foster, N. L., Wilhelmsen, K., Sima, A. A. F., Jones, M. Z., D'Amato, C. J., Gilman, S., and Conference Participants. (1997). Frontotemporal dementia and parkinsonism linked to chromosome 17: A consensus conference. *Ann. Neurol*. **41**, 706–715.

Gazeley, S., and Maguire, J. A. (1996). Familial progressive supranuclear palsy. *Clin. Neuropathol*. **15**, 215–220.

Gearing, M., Olson, D. A., Watts, R. L., and Mirra, S. S. (1994). Progressive supranuclear palsy: Neuropathologic and clinical heterogeneity. *Neurology* **44**, 1015–1024.

Geddes, J. F., Hughes, A. J., Lees, A. J., and Daniel, S. E. (1993). Pathological overlap in cases of parkinsonism associated with neurofibrillary tangles. A study of recent cases of postencephalitic parkinsonism and comparison with progressive supranuclear palsy and Guamanian parkinsonism-dementia complex. *Brain* **116**, 281–302.

Gibb, W. R. G., Luthert, P. J., and Marsden, C. D. (1989). Corticobasal degeneration. *Brain* **112**, 1171–1192.

Gimenez-Roldan, S., Mateo, D., Benito, C., Grandas, F., and Perez-Gilabert, Y. (1994). Progressive supranuclear palsy and corticobasal ganglionic degeneration: Differentiation by clinical features and neuroimaging techniques. *J. Neural Transm. Suppl*. **42**, 79–90.

Golbe, L. I. (1993). Progressive supranuclear palsy. *In* "Parkinsonian Syndromes" (M. B. Stern and W. C. Koller, eds.), pp. 227–247. 1 Dekker, New York.

Golbe, L. I., Davis, P. H., Schoenberg, B. S., and Duvoisin, R. C. (1988). Prevalence and natural history of progressive supranuclear palsy. *Neurology* **38**, 1031–1034.

Grafman, J., Litvan, I., Gomez, C., and Chase, T. N. (1990). Frontal lobe function in progressive supranuclear palsy. *Arch. Neurol. (Chicago)* **47**, 553–558.

Greenberg, S. C., and Davies, P. (1990). A preparation of Alzheimer paired helical filaments that displays distinct tau proteins by polyacrylamide gel electrophoresis. *Proc. Natl. Acad. Sci. U.S.A*. **87**, 5827–5831.

Halliday, G. M., Davies, L., McRitchie, D. A., Cartwright, H., Pamphlett, R., and Morris, J. G. (1995). Ubiquitin-positive achromatic neurons in corticobasal degeneration. *Acta Neuropathol*. **90**, 68–75.

Hardy, J., and Gwinn-Hardy, K. (1998). Genetic classification of primary neurodegenerative disease. *Science* **282**, 1075–1079.

Hauw, J.-J., Verny, M., Delaère, P., Cervera, P., He, Y., and Duyckaerts, C. (1990). Constant neurofibrillary changes in the neocortex in progressive supranuclear palsy: Basic differences with Alzheimer's disease and aging. *Neurosci. Lett*. **119**, 182–186.

Hauw, J.-J., Daniel, S. E., Dickson, D., Horoupian, D. S., Jellinger, K., Lantos, P. L., McKee, A., Tabaton, M., and Litvan, I. (1994). Preliminary NINDS neuropathologic criteria for Steele-Richardson-Olszewski syndrome (progressive supranuclear palsy). *Neurology* **44**, 2015–2019.

Higgins, J. J., Adler, R. L., and Loveless, J. M. (1999). Mutational analysis of the tau gene in progressive supranuclear palsy. *Neurology* **53**, 1421–1424.

Hoenicka, J., Perez, M., Perez-Tur, J., Barabash, A., Godoy, M., Vidal, L., Astarloa, R., Avila, J., Nygaard, T., and de Yébenes, J.G. (1999). The tau gene A0 allele and progressive supranuclear palsy. *Neurology* **53**, 1219–1225.

Hof, P. R., Delacourte, A., and Bouras, C. (1992). Distribution of cortical neurofibrillary tangles in progressive supranuclear palsy: A quantitative analysis of six cases. *Acta Neuropathol*. **84**, 45–51.

Hong, M., Zhukareva, V., Vogelsberg-Ragaglia, V., Wszolek, Z., Reed, L., Miller, B. I., Geschwind, D. H., Bird, T. D., McKeel, D., Goate, A., Morris, J. C., Wilhelmsen, K. C., Schellenberg, G. D., Trojanowski, J. Q., and Lee, V. M. (1998). Mutation-specific functional impairments in distinct tau isoforms of hereditary FTDP-17. *Science* **282**, 1914–1917.

Horoupian, D. S., and Chu, P. L. (1994). Unusual case of corticobasal degeneration with tau/Gallyas-positive neuronal and glial tangles. *Acta Neuropathol*. **88**, 592–598.

Hutton, M., Lendon, C. L., Rizzu, P., Baker, M., Froelich, S., Houlden, H., Pickering-Brown, S., Chakraverty, S., Isaacs, A., Grover, A., Hackett, J., Abramson, J., Lincoln, S., Dickson, D., Davies, P., Petersen, R. C., Stevens, M., de Graaf, E., Wauters, E., van Baren, J., Hillebrand, M., Joosen, M., Kwon, J., Nowotny, J., Che, L. K., Norton, J., Morris, J. C., Reed, L. A., Trojanowski, J., Basun, H., Lannfelt, L., Neystat, M., Fahn, S., Dark, F., Tannenberg, T., Dodd, P. R., Hayward, N., Kwok, J. B. J., Schofield, P. R., Andreadis, A., Snowden, J., Crauford, D., Neary, D., Owen, F., Oostra, B. A., Hardy, J., Goate, A., van Swieten, J., Mann, D., Lynch, T., and Heutink, P. (1998). Association of missense and 5'-splice-site mutations in tau with the inherited dementia (FTDP-17). *Nature (London)* **393**, 702–705.

Ikeda, K., Akiyama, H., Haga, C., Kondo, H,. Arima, K., and Oda, T. (1994). Argyrophilic thread-like structure in corticobasal degeneration and supranuclear palsy. *Neurosci. Lett*. **174**, 157–159.

Ikeda, K., Akiyama, H., Kondo, H., and Haga, C. (1995). A study of dementia with argyrophilic grains. Possible cytoskeletal abnormality in dendrospinal portion of neurons and oligodendroglia. *Acta Neuropathol*. **89**, 409–414.

Ikeda, K., Akiyama, H., Iritani,S., Kase, K., Arai, T., Niizato, K., Kuroki, N., and Kosaka, K. (1996). Corticobasal degeneration with primary progressive aphasia and accentuated cortical lesion in superior temporal gyrus: Case report and review. *Acta Neuropathol*. **92**, 534–539.

Iqbal, K., Grundke-Iqbal, I., Smith, A. J., George, L., Tung, Y.-C., and Zaidi, T. (1989). Identification and localization of tau peptide to paired helical filaments of Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A*. **86**, 5646–5650.

Iqbal, K., Braak, E., Braak, H., Zaidi, T., and Grundke-Iqbal, I. (1991). A silver impregnation method for labeling both Alzheimer paired helical filaments and their polypeptides separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Neurobiol. Aging* **12**, 357–361.

Ishino, H., and Otsuki, S. (1976). Frequency of Alzheimer's neurofibrillary tangles in the cerebral cortex in progressive supranuclear palsy (subcortical argyrophilic dystrophy). *J. Neurol Sci*. **28**, 309–316.

Iwatsubo, T., Hasegawa, M., and Ihara, Y. (1994). Neuronal and glial tau-positive inclusions in diverse neurologic diseases share common phosphorylation characteristics. *Acta Neuropathol*. **88**, 129–136.

Janke, C., Holzer, M., Klose, J., and Arendt, T. (1996). Distribution of isoforms of the microtubule-associated protein tau in grey and white matter areas of human brain: A two-dimensional gel electrophoretic analysis. *FEBS Lett*. **379**, 222–226.

Jellinger, K. (1971). Progressive supranuclear palsy (subcortical argyrophilic dystrophy). *Acta Neuropathol*. **19**, 347–352.

Jellinger, K. A. (1998). Dementia with grains (argyrophilic grain disease). *Brain Pathol*. **8**, 377–386.

Jellinger, K. A., and Bancher, C. (1992). Neuropathology. *In* "Progressive Supranuclear Palsy: Clinical and Research Approaches" (I. Litvan and Y. Agid, eds.), pp. 44–88. Oxford University Press, New York.

Jicha, G. A., Rockwood, J. M., Berenfeld, B., Hutton, M., and Davies, P. (1999). Altered conformation of recombinant frontotemporal dementia-17 mutant tau proteins. *Neurosci. Lett*. **260**, 153–156.

Kawasaki, K., Iwanaga, K., Wakabayashi, K., Yamada, M., Nagai, H., Idezuka, J., Homma, Y., and Ikuta, F. (1996). Corticobasal degeneration with neither argyrophilic inclusions nor tau abnormalities: A new subgroup? *Acta Neuropathol*. **91**, 140–144.

Knopman, D. S., Mastri, A. R., Frey, F., Sung, J. H., and Rustan, T. (1990). Dementia lacking distinctive histologic features: A common non-Alzheimer degenerative dementia. *Neurology* **40**, 251–256.

Komori, T. (1999). Tau-positive glial inclusions in progressive supranuclear palsy, corticobasal degeneration and Pick's disease. *Brain Pathol*. **9**, 663–679.

Komori, T., Arai, N., Oda, M., Nakayama, H., Murayama, S., Amano, N., Shibata, N., Kobayashi, M., Sasaki, S., and Yagishita, S. (1997). Morphologic difference of neuropil threads in Alzheimer's disease, corticobasal degeneration and progressive supranuclear palsy: A morphometric study. *Neurosci. Lett*. **233**, 89–92.

Komori, T., Arai, N., Oda, M., Nakayama, H., Mori, H., Yagishita, S., Takahashi, T., Amano, N., Murayama, S., Murakami, S., Shibata, N., Kobayashi, M., Sasaki, S., and Iwata, M. (1998). Astrocytic plaques and tufts of abnormal fibers do not coexist in corticobasal degeneration and progressive supranuclear palsy. *Acta Neuropathol*. **96**, 401–408.

Ksiezak-Reding, H., Morgan, K., Mattiace, L. A., Davies, P., Liu, W.-K., Yen, S.-H., Weidenheim, K. M., and Dickson, D. W. (1994). Ultrastructure and biochemical composition of paired helical filaments in corticobasal degeneration. *Am. J. Pathol*. **145**, 1496–1508.

Ksiezak-Reding, H., Tracz, E., Yang, L. S., Dickson, D. W., Simon, M., and Wall, J. S. (1996). Ultrastructural instability of paired helical filaments from corticobasal degeneration as examined by scanning transmission electron microscopy. *Am. J. Pathol*. **149**, 639–651.

Lang, A. E., Riley, D. E., and Bergeron, C. (1994). Cortical-basal ganglionic degeneration. *In* "Neurodegenerative Diseases" (D. B. Calne, ed.), pp. 877–894. Saunders, Philadelphia.

Lantos, P. L. (1994). The neuropathology of progressive supranuclear palsy. *J. Neural Transm. Suppl*. **42**, 137–152.

Lantos, P. L. (1998). The definition of multiple system atrophy: A review of recent developments. *J. Neuropathol. Exp. Neurol*. **57**, 1099–1111.

Leenders, K. L., Frackowiak, R. S. J., and Lees, J. (1988). Steele-Richardson-Olszewski syndrome: Brain energy metabolism blood flow and fluorodopa uptake measured by positron emission tomography. *Brain* **111**, 615–630.

Litvan, I., and Agid, Y. (1992). "Progressive Supranuclear Palsy: Clinical and Research Approaches." Oxford University Press, New York.

Litvan, I., Grafman, J., Gomez, C., and Chase, T. N. (1989). Memory impairment in patients with progressive supranuclear palsy. *Arch. Neurol. (Chicago)* **46**, 765–767.

Litvan, I., Hauw, J. J., Bartko, J. J., P. L., Daniel, S. E., Horoupian, D. S., McKee, A., Dickson, D., Bancher, C., Tabton, M., Jellinger, K., and Anderson, D. W. (1996). Validity and reliability of the preliminary NINDS neuropathologic criteria for progressive supranuclear palsy and related disorders. *J. Neuropathol. Exp. Neurol*. **55**, 97–105.

Litvan, I., Agid, Y., Goetz, C., Jankovic, J., Wenning, G. K., Brandel, J. P., Lai, E. C., Verny, M., Ray-Chaudhuri, K., McKee, A., Jellinger, K., Pearce, R. K., and Bartko, J. J. (1997a). Accuracy of the clinical diagnosis of corticobasal degeneration: A clinicopathologic study. *Neurology* **48**, 119–125.

Litvan, I., Campbell, G., Mangone, C. A., Verny, M., McKee, A., Chaudhuri, K. R., Jellinger, K., Pearce, R. K., and D'Olhaberriague, L. (1997b). Which clinical features differentiate progressive supranuclear

palsy (Steele-Richardson-Olszewski syndrome) from related disorders? A clinicopathological study. *Brain* **120**, 65–74.

Litvan, I., Grimes, D. A., Lang, A. E., Jankovic, J., McKee, A., Verny, M., Jellinger, K., Chaudhuri, K. R., and Pearce, R. K. (1999). Clinical features differentiating patients with postmortem confirmed progressive supranuclear plasy and corticobasal degeneration. *J. Neurol*. **246** (suppl. 2), 1–5.

Litvan, I., Goetz, C. G., and Lang, A. E. (2000). ''Corticobasal Degeneration and Related Disorders, Advances in Neurology,'' Vol. 82. Lippincott/Williams and Wilkins, Philadelphia.

Lowe, J., Errington, D. R., Lennox, G., Pike, I., Spendlove, I., Landon, M., and Mayer, R. J. (1992). Ballooned neurons in several neurodegenerative diseases and stroke contain alpha B crystallin. *Neuropathol. Appl. Neurobiol*. **18**, 341–350.

Mackenzie, I. R., and Hudson, L. P. (1995). Achromatic neurons in the cortex of progressive supranuclear palsy. *Acta Neuropathol*. **90**, 615–619.

Maher, E. R., Smith, E. M., and Lees, A. J. (1985). Cognitive deficits in the Steele-Richardson-Olszewski syndrome (progressive supranuclear palsy). *J. Neurol. Neurosurg. Psychiatry* **48**, 1234–1239.

Mailliot, C., Sergeant, N., Bussière, T., Caillet-Boudin, M. L., Delacourte, A., and Buée, L. (1998). Phosphorylation of specific sets of tau isoforms reflects different neurofibrillary degeneration processes. *FEBS Lett*. **433**, 201–204.

Martinez-Lage, P., and Munoz, D. G. (1997). Prevalence and disease associations of argyrophilic grains of Braak. *J. Neuropathol. Exp. Neurol*. **56**, 157–164.

Matsumoto, S., Udaka, F., Kameyama, M., Kusaka, H., Ito, H., and Imai, T. (1996). Subcortical neurofibrillary tangles, neuropil threads, and argentophilic glial inclusions in corticobasal degeneration. *Clin. Neuropathol*. **15**, 209–214.

Mayeux, R., Saunders, A. M., Shea, S., Mirra, S., Evans, D., Roses, A. D., Hyman, B. T., Crain, B., Tang, M. X., and Phelps, C. H. (1998). Utility of the apolipoprotein E genotype in the diagnosis of Alzheimer's disease. Alzheimer's Disease Centers Consortium on Apolipoprotein E and Alzheimer's Disease. *N. Engl. J. Med*. **338**, 506–511.

Mizusawa, H., Yen, S.-H., Hirano, A., and Llena, J. (1989). Pathology of the dentate nucleus in progressive supranuclear palsy: A histological immunohistochemical and ultrastructural study. *Acta Neuropathol*. **78**, 419–428.

Mori, H., and Oda, M. (1997). Ballooned neurons in corticobasal degeneration and progressive supranuclear palsy. *Neuropathology* **17**, 248–252.

Mori, H., Nishimura, M., Namba, Y., and Oda, M. (1994). Corticobasal degeneration: A disease with widespread appearance of abnormal tau and neurofibrillary tangles and its relation to progressive supranuclear palsy. *Acta Neuropathol*. **88**, 113–121.

Mori, H., Oda, M., and Mizuno, Y. (1996). Cortical ballooned neurons in progressive supranuclear palsy. *Neurosci. Lett*. **209**, 109–112.

Morris, H. R., Janssen, J. C., Bandmann, O., Daniel, S. E., Rossor, M. N., Lees, A. J., and Wood, N. W. (1999). The tau gene A0 polymorphism in progressive supranuclear palsy and related neurodegenerative diseases. *J. Neurol. Neurosurg. Psychiatry* **66**, 665–667.

Nishimura, M., Namba, Y., Ideda, K., and Oda, M. (1992). Glial fibrillary tangles with straight tubules in the brains of patients with progressive supranuclear palsy. *Neurosci. Lett*. **143**, 35–38.

Nishimura, T., Ikeda, K., Akiyama, H., Kondo, H., Kato, M., Li, F., Iseki, E., and Kosaka, K. (1995). Immunohistochemical investigation of tau-positive structures in the cerebral cortex of patients with progressive supranuclear palsy. *Neurosci. Lett*. **201**, 123–126.

Nishimura, T., Ikeda, K., Akiyama, H., Arai, T., Kondo, H., Okochi, M., Furiya, Y., Mori, H., Oda, T., Kato, M., and Iseki, E. (1997). Glial tau-positive structures lack the sequence encoded by exon 3 of the tau protein gene. *Neurosci. Lett*. **224**, 169–172.

Oliva, R., Tolosa, E., Ezquerra, M., Molinuevo, J. L., Valldeoriola, F., Burguera, J., Calopa, M., Villa, M., and Ballesta, F. (1998). Significant changes in the tau A0 and A3 alleles in progressive supranuclear palsy and improved genotyping by silver detection. *Arch. Neurol (Chicago)*. **55**, 1122–1124.

Oyanagi, K., Takahashi, H., Wakabayashi, K., and Ikuta, F. (1988). Selective decrease of large neurons in the neostriatum in progressive supranuclear palsy. *Brain Res*. **458**, 218.

Paulus, W., and Selim, M. (1990). Corticonigral degeneration with neuronal achromasia and basal neurofibrillary tangles. *Acta Neuropathol*. **81**, 89–94.

Poorkaj, P., Bird, T. D., Wijsman, E., Nemens, E., Garruto, R. M., Anderson, L., Andreadis, A., Wiederholt, W. C., Raskind, M., and Schellenberg, G. D. (1998). Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Ann. Neurol*. **43**, 815–825.

Powell, H. C., London, G. W., and Lampert, P. W. (1974). Neurofibrillary tangles in progressive supranuclear palsy: Electron microscopic observations. *J. Neuropathol. Exp. Neurol*. **33**, 98–106.

Probst, A., Langui, D., Lautenschlager, C., Ulrich, J., Brion, J. P., and Anderton, B. H. (1988). Progressive supranuclear palsy: Extensive neuropil threads in addition to neurofibrillary tangles. *Acta Neuropathol*. **77**, 61–68.

Rajput, A. H., Offord, K. P., Beard, C. M., and Kurland, L. T. (1984). Epidemiology of parkinsonism: Incidence classification and mortality. *Ann. Neurol*. **16**, 278–282.

Rajput, A. H., Rozdilsky, B., and Rajput, A. (1991). Accuracy of clinical diagnosis in parkinsonism—a prospective study. *Can. J. Neurol. Sci*. **18**, 275–278.

Rebeiz, J. J., Kolodny, E. H., and Richardson, E. P., Jr. (1967). Corticodentatonigral degeneration with neuronal achromasia: A progressive disorder of late adult life. *Trans. Am. Neurol. Assoc*. **92**, 23–26.

Rebeiz, J. J., Kolodny, E. H., and Richardson, E. P., Jr. (1968). Corticodentatonigral degeneration with neuronal achromasia. *Arch. Neurol. (Chicago)* **18**, 20–33.

Reed, L. A., Grabowski, T. J., Schmidt, M. L., Morris, J. C., Goate, A., Solodkin, A., Van Hoesen, G. W., Schelper, R. L., Talbot, C. J., Wragg, M. A., and Trojanowski, J. Q. (1997). Autosomal dominant dementia with widespread neurofibrillary tangles. *Ann. Neurol*. **42**, 564–572.

Reed, L. A., Schmidt, M. L., Wszolek, Z. K., Balin, B. J., Soontornniyomkij, V., Lee, V. M., Trojanowski, J. Q., and Schelper, R. L. (1998). The neuropathology of a chromosome 17-linked autosomal dominant parkinsonism and dementia (''pallido-ponto-nigral degeneration''). *J. Neuropathol. Exp. Neurol*. **57**, 588–601.

Riley, D. E., Lang, A. E., Lewis, A., Resch, L., Ashby, P., Hornykiewcz, O., and Black, S. (1990). Cortical-basal ganglionic degeneration. *Neurology* **40**, 1203–1212.

Rojo, A., Pernaute, R. S., Fontan, A., Ruiz, P. G., Honnorat, J., Lynch, T., Chin, S., Gonzalo, I., Rabano, A., Martinez, A., Daniel, S., Pramsteller, P., Morris, H., Wood, N,. Lees, A., Tabernero, C., Nyggard, T., Jackson, A. C., Hanson, A., and de Yébenes, J. G. (1999). Clinical genetics of familial progressive supranuclear palsy. *Brain* **122**, 1233–1245.

Roy, S., Datta, C. K., Hirano, A., Ghatak, N. R., and Zimmerman, H. M. (1974). Electron microscopic study of neurofibrillary tangles in Steele-Richardson-Olszewski syndrome. *Acta Neuropathol*. **29**, 175–179.

Sawle, G. V., Brooks, D. J., Marsden, C. D., and Frackowiak, R. S. J. (1991). Corticobasal degeneration. A unique pattern of regional cortical oxygen hypometabolism and striatal fluorodopa uptake demonstrated by positron emission tomography. *Brain* **114**, 541–556.

Schmidt, M. L., Lee, V. M.-Y., Hurtig, H., and Trojanowski, J. Q. (1988). Properties of antigenic determinants that distinguish neurofibrillary tangles in progressive supranuclear palsy and Alzheimer's disease. *Am. J. Pathol*. **59**, 460–466.

Schmidt, M. L., Huang, R., Martin, J. A., Henley, J., Mawal-Dewan, M., Hurtig, H. I., Lee, V. M., and Trojanowski, J. Q. (1996). Neurofibrillary tangles in progressive supranuclear palsy contain the same tau epitopes identified in Alzheimer's disease PHF-tau. *J. Neuropathol. Exp. Neurol*. **55**, 534–539.

Schneider, J. A., Watts, R. L., Gearing, M., Brewer, R. P., and Mirra, S. S. (1997). Corticobasal degeneration: Neuropathologic and clinical heterogeneity. *Neurology* **48**, 959–969.

Sergeant, N., David, J. P., Lefranc, D., Vermersch, P., Wattez, A., and Delacourte, A. (1997). Different distribution of phosphorylated tau protein isoforms in Alzheimer's and Pick's diseases. *FEBS Lett*. **412**, 578–582.

Sergeant, N., Wattez, A., and Delacourte, A. (1999). Neurofibrillary degeneration in progressive supranuclear palsy and corticobasal degeneration: Tau pathologies with exclusively ''exon 10'' isoforms. *J. Neurochem*. **72**, 1243–1249.

Smith, T. W., Lippa, C. F., and de Girolami, U. (1992). Immunocytochemical study of ballooned neurons in cortical degeneration with neuronal achromasia. *Clin. Neuropathol*. **11**, 28–35.

Soliveri, P., Monza, D., Paridi, D., Radice, D., Grisoli, M. Testa, D., Savoiardo, M., and Girotti, F. (1999). Cognitive and magnetic resonance imaging aspects of corticobasal degeneration and progressive supranuclear palsy. *Neurology* **53**, 502–507.

Spillantini, M. G., and Goedert, M. (1998). Tau protein pathology in neurodegenerative diseases. *Trends Neurosci*. **21**, 428–433.

Spillantini, M. G., Goedert, M., Crowther, R. A., Murrell, J. R., Farlow, M. R., and Ghetti, B. (1997). Familial multiple system tauopathy with presenile dementia: A disease with abundant neuronal and glial tau filaments. *Proc. Natl. Acad. Sci. U.S.A*. **94**, 4113–4118.

Spillantini, M. G., Bird, T. D., and Ghetti, B. (1998a). Frontotemporal dementia and Parkinsonism linked to chromosome 17: A new group of tauopathies. *Brain Pathol*. **8**, 387–402.

Spillantini, M. G., Murrell, J. R., Goedert, M., Farlow, M. R., Klug, A., and Ghetti, B. (1998b). Mutation in the tau gene in familial multisystem tauopathy with presenile dementia. *Proc. Natl. Acad. Sci. U.S.A*. **95**, 7737–7741.

Steele, J. C., Richardson, J. C., and Olszewski, J. (1964). Progressive supranuclear palsy: A heterogenous degeneration involving the brain stem basal ganglia and cerebellum with vertical gaze and pseudobulbar palsy nuchal dystonia and dementia. *Arch. Neurol*. **10**, 333–339.

Tagliavini, F., Pilleri, G., Bouras, C., and Constantinidis, J. (1984). The basal nucleus of Meynert in patients with progressive supranuclear palsy. *Neurosci. Lett*. **44**, 37–42.

Takahashi, T., Amano, N., Hanihara, T., Nagatomo, H., Yagishita, S., Itoh, Y., Yamaoka, K., Toda, H., and Tanabe, T. (1996). Corticobasal degeneration: widespread argentophilic threads and glia in addition to neurofibrillary tangles. Similarities of cytoskeletal abnormalities in corticobasal degeneration and progressive supranuclear palsy. *J. Neurol. Sci*. **138**, 66–77.

Tellez-Nagel, I., and Wisniewski, H. M. (1973). Ultrastructure of neurofibrillary tangles in Steele-Richardson-Olszewski syndrome. *Arch. Neurol (Chicago)*. **29**, 324–327.

Terry, R. D. (1963). The fine structure of neurofibrillary tangles in Alzheimer's disease. *J. Neuropathol. Exp. Neurol*. **22**, 629–642.

Tetrud, J. W., Golbe, L. I., Forno, L. S., and Farmer, P. M. (1996). Autopsy-proven progressive supranuclear palsy in two siblings. *Neurology* **46**, 931–934.

Tolnay, M., and Probst, A. (1998). Ballooned neurons expressing alpha B-crystallin as a constant feature of the amygdala in argyrophilic grain disease. *Neurosci. Lett*. **246**, 165–168.

Tolnay, M., Mistl, C., Ipsen, S., and Probst, A. (1998). Argyrophilic grains of Braak: occurrence in dendrites of neurons containing hyperphosphorylated tau protein. *Neuropathol. Appl. Neurobiol*. **24**, 53–59.

Tracz, E., Dickson, D. W., Hainfeld, J. F., and Ksiezak-Reding, H. (1997). Paired helical filaments in corticobasal degeneration: the fine fibrillary structure with NanoVan. *Brain Res*. **773**, 33–44.

Tsuchiya, K., Ikeda, K., Uchihara, T., Oda, T., and Shimada, H. (1997). Distribution of cerebral cortical lesions in corticobasal degeneration: A clinicopathological study of five autopsy cases in Japan. *Acta Neuropathol*. **94**, 416–424.

Uchihara, T., Mitani, K., Mori, H., Kondo, H., Yamada, M., and Ikeda, K. (1994). Abnormal cytoskeletal pathology peculiar to corticobasal degeneration is different from that of Alzheimer's disease or progressive supranuclear palsy. *Acta Neuropathol*. **88**, 379–383.

Vanier, M. T., Neuville, P., Michalik, L., and Launay, J. F. (1998). Expression of specific tau exons in normal and tumoral pancreatic acinar cells. *J. Cell Sci*. **111**, 1419–1432.

Vermersch, P., Robitaille, Y., Berneir, L., Wattez, A., Gauvreau, D., and Delacourte, A. (1994). Biochemical mapping of neurofibrillary degeneration in a case of progressive supranuclear palsy: Evidence for general cortical involvement. *Acta Neuropathol*. **87**, 572–577.

Verny, M., Jellinger, K. A., Hauw, J. J., Bancher, C., Litvan, I., and Agid, Y. (1996). Progressive supranuclear palsy: A clinicopathological study of 21 cases. *Acta Neuropathol*. **91**, 427–431.

Wakabayashi, K., Oyanagi, K., Makifuchi, T., Ikuta, F., Honna, A., Honna, Y., Horikawa, Y., and Tokiguche, S. (1994). Corticobasal degeneration: Etiopathological significance of the cytoskeletal alterations. *Acta Neuropathol*. **87**, 545–553.

Wenning, G. K., Litvan, I., Jankovic, J., Granata, R., Mangone, C. A., McKee, A., Poewe, W., Jellinger, K., Ray Chaudhuri, K., D'Olhaberriague, L., and Pearce, R. K. (1998). Natural history and survival of 14 patients with corticobasal degeneration confirmed at postmortem examination. *J. Neurol. Neurosurg. Psychiatry* **64**, 184–189.

Yagishita, S., Itoh, Y., Amano, N., Nakano, T., and Saitoh, A. (1979). Ultrastructure of neurofibrillary tangles in progressive supranuclear palsy. *Acta Neuropathol*. **48**, 27–30.

Yamada, T., and McGeer, P. L. (1995). Oligodendroglial microtubular massess: An abnormality observed in some human neurodegenerative diseases. *Neurosci. Lett*. **120**, 163–166.

Yamada, T., McGeer, P. L., and McGeer, E. G. (1992). Appearance of paired nucleated tau-positive glia in patients with progressive supranuclear palsy brain tissue. *Neurosci. Lett*. **135**, 99–102.

Yamada, T., Calne, D. B., Akiyama, H., McGeer, E. G., and McGeer, P. L. (1993). Further observations on tau-positive glia in the brains with progressive supranuclear palsy. *Acta Neuropathol*. **85**, 308–315.

Yamaguchi, H., Nakazato, Y., Shoji, M., Ihara, Y., and Hirai, S. (1990). Ultrastructure of the neuropil threads in the Alzheimer brain: Their dendritic origin and accumulation in the senile plaques. *Acta Neuropathol*. **80**, 368–374.

Yamaguchi, H., Fukuyama, H., Nagahama, Y., Katsumi, Y., Dong, Y., Hayashi, T., Konishi, J., and Kimura, J. (1998). Atrophy of the corpus callosum, cortical hypometabolism, and cognitive impairment in corticobasal degeneration. *Arch. Neurol. (Chicago)* **55**, 609–614.

Yen, S. H., Hutton, M., DeTure, M., Ko, L. W., and Nacharaju, P. (1999) Fibrillogenesis of tau: insights from tau missense mutations in FTDP-17. *Brain Pathol*. **9**, 695–705.

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Lawrence Hansen and Eliezer Masliah

14

Neurobiology of Disorders with Lewy Bodies

Lewy body disease (LBD) and Alzheimer's disease (AD) continue to be the most common disorders causing dementia in the elderly population. Disorders with Lewy bodies comprise a diverse group of diseases. A consensus paper by McKeith and others has proposed comprehensive criteria in an attempt to reconcile the disagreements within the current terminology. Although the precise cause of these disorders is currently unknown, neuropathologically the common denominator is the degeneration of neuronal populations within the nigral dopaminergic system and mesial temporal regions, LB formation, extensive neuritic degeneration, and spongiform vacuolization. Previous studies have shown that cognitive alterations in these disorders are associated with synaptic damage. Injury to the synapse may be associated with an altered function of synaptic proteins. Among them, studies have shown that abnormal aggregation and accumulation of synaptic proteins such as α-synuclein (or the precursor of the non-Aβ component of AD amyloid, NACP) may be associated with plaque formation in AD and Lewy body formation in LBD. Further reinforcing the argument that α-synuclein plays a major role in the pathogenesis of these disorders, work has shown that mutations which alter the conformation of this molecule are associated with familial forms of LBD and that α-synuclein is a major component of Lewy bodies. The mechanisms by which altered function or aggregation of α-synuclein leads to neurodegeneration are not completely clear; however, new evidence points to a potential role for this molecule in synaptic damage and neurotoxicity via amyloid fibril formation and mitochondrial dysfunction. This chapter reviews data linking α-synuclein to the pathogenesis of LBD.

I. Introduction

Lewy body disease (LBD) is a leading cause of dementia, second only to Alzheimer's disease (AD) (Hansen and Crain, 1995), and it accounts for 15–25% of the cognitively impaired elderly, most of whom were diagnosed as AD during life. Previous studies have shown that cognitive alterations in these disorders are associated with synaptic damage (DeKosky and Scheff, 1990; Terry *et al*., 1991). Injury to the synapse might be associated with the altered function of synaptic proteins (Masliah, 1998a). Among them, studies have pointed to α-synuclein, or the precursor of the non-Aβ component of AD amyloid (NACP), as a major player in the pathogenesis of AD and LBD (Masliah, 1998b) (Fig. 14.1). α-Synuclein was first isolated by Maroteaux *et al*. (1988) by expression screening of a Torpedo cDNA library using an antiserum against a purified synaptic vesicle. The term synuclein was proposed because immunoreactivity was observed in the presynaptic terminal and nucleus. Later on, Nakajo (1990) and Tobe (1992) *et al*. purified a presynaptic phosphoprotein (PNP14) very similar to synuclein from bovine brain; PNP14 is now known as β-synuclein.

Synuclein biology became particularly interesting in 1993 when Saitoh and colleagues identified α-synuclein as NACP, which was copurified with amyloid from the brains of AD patients (Ueda *et al*., 1993). Then, NACP is the human homologue to murine α-synuclein; however, for practical purposes we will refer to NACP as α-synuclein; which was also found to be a highly abundant synaptic protein in the mammalian brain (Iwai *et al*., 1995). Subsequently, Jakes and Goedert purified both α-synuclein and β-synuclein from human brain using monoclonal antibodies raised against neurons displaying granulovacuolar degeneration (Jakes *et al*., 1994) (Fig. 14.1). During the same time, Clayton and colleagues identified synelfin, the avian homologue of α-synuclein, whose expression was downregulated dramatically during a critical period for song learning in the canary (George *et al*., 1995), suggesting that synelfin may play a critical role in neural plasticity. More recently, γ-synuclein was isolated as the D3 synuclein-like molecule, which was expressed predominantly in peripheral sympathetic neurons (Akopian and Wood, 1995). γ-Synuclein was later cloned from the EST library as the breast cancer-specific gene (BCSG1) whose expression was observed in the invasive types of breast cancer (Ji *et al*., 1997). Finally, and most importantly, in 1997, Polymeropoulos and colleagues identified a missense substitution of alanine to threonine at 53 (A53T) of α-synuclein in Italian and Greek families of autosomal dominant types of Parkinson's disease (PD), and Kruger *et al*. (1998) identified a missense mutation alanine to proline at 30 (A30P) of

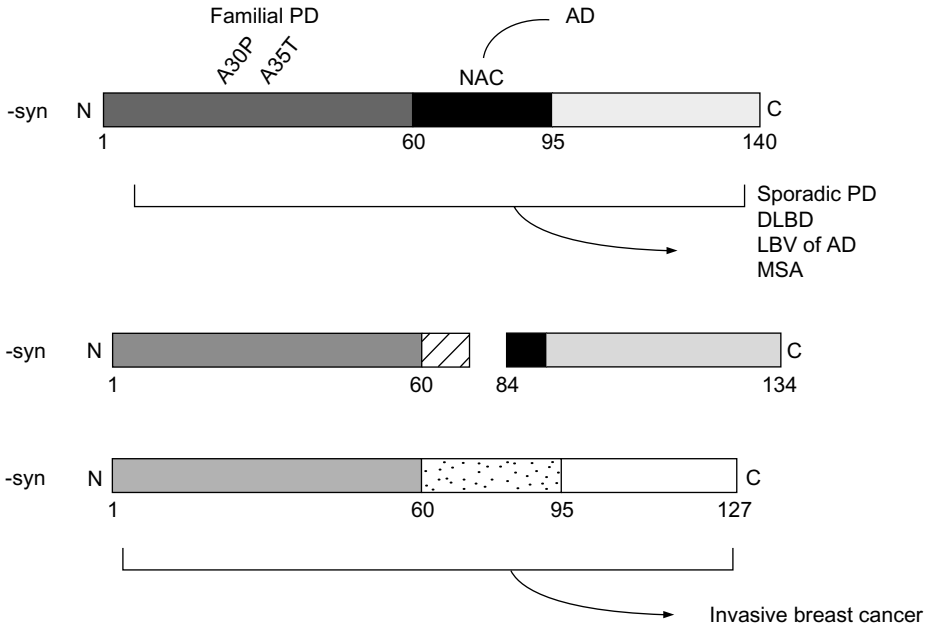

FIG. 14.1. Synuclein family of proteins and their relationship to neurodegenerative disorders. AD, Alzheimer's disease; DLBD, diffuse Lewy body disease; LBV, Lewy body variant; MSA, multiple system atrophy; PD, Parkinson's disease.

α-synuclein in a German family. Subsequent neuropathological and biochemical studies established that abnormal aggregates of α-synuclein are major components of inclusion bodies and dystrophic neurites in LBD (Wakabayashi *et al*., 1995; Spillantini *et al*., 1997; Takeda *et al*., 1998b). In this context, the main objective of this chapter is to review the pathogenesis of LBD and its relationship to α-synuclein.

II. Nosology of Disorders with Lewy Bodies

Patients with LBD present clinically with parkinsonism, hallucinations, and rapidly progressive dementia (Salmon and Galasko, 1996). The presentation and severity of these symptoms are highly variable and their relationship with specific neuropathological alterations is under investigation. Disorders with Lewy bodies comprise a diverse group of diseases, including a Lewy body variant of AD (LBV) (Hansen *et al*., 1990), diffuse Lewy body disease (DLBD) (Dickson *et al*., 1989), and PD (Gaspar and Graf, 1984). The so-called LBV has also been referred to by others as combined AD and PD or senile dementia of the Lewy body type (Dickson *et al*., 1987; Perry *et al*., 1990). No fewer than 18 different labels have been affixed to LBD characterized by dementia and varying degrees of motor parkinsonism (see Table 14.1). Because such demented PD patients will invariably have at least a few neocortical as well as subcortical LBs (Hughes *et al*., 1992), they could literally be construed as examples of LBD. Most investigators, however, did not apply terms such as ''diffuse Lewy body disease'' (Dickson *et al*., 1987) or ''Lewy body dementia'' (Perry *et al*., 1997) to clinically diagnosed PD patients who developed dementia, but rather to patients presenting with dementia who may have been mildly parkinsonian and whose brains were found to harbor Lewy bodies.

In an attempt to resolve some of these controversies, an international consortium published in 1996 consensus guidelines for the clinical and pathologic diagnosis of LBD (McKeith *et al*., 1996). This group recommended that if dementia occurs within 12 months of the onset of extrapyramidal motor symptoms, the patient should be assigned to a

TABLE 14.1 Nosologic Diversity in Lewy Body Disease

Lewy body disease, group A, diffuse type
Lewy body disease, group B, transitional type
Lewy body disease, group C, brain stem type
Lewy body disease, cerebral type
Lewy body dementia
Diffuse Lewy body disease
Diffuse Lewy body disease, common form, with plaques $\pm$ tangles
Diffuse Lewy body disease, pure form, with no senile changes
Alzheimer's disease and Parkinson's disease
Alzheimer's disease and Parkinson's disease—related changes
Alzheimer's disease with incidental Lewy bodies
Alzheimer's disease with concomitant Lewy body disease
Lewy bodies in Alzheimer's disease
Senile dementia of the Lewy body type
Lewy body variant of Alzheimer's disease
Alzheimer's disease with Lewy bodies
Parkinson's disease in Alzheimer's disease
Diffuse cortical Lewy body disease

TABLE 14.2 Clinical Features of Dementia with Lewy Bodies

Progressive cognitive decline
Fluctuating cognition with variations in attention and alertness
Visual hallucinations, usually repeated and detailed
Spontaneous motor parkinsonism, typically mild
Repeated falls
Transient loss of consciousness
Neuroleptic sensitivity
Systematized delusions
Hallucinations in other modalities

primary diagnosis of possible LBD. If the clinical history of parkinsonism is longer than 12 months, PD with dementia will usually be a more appropriate diagnostic label. Thus clinically defined, LBD was endorsed as a generic term to include cases referred to previously as DLBD (Dickson *et al*., 1987), senile dementia of the Lewy body type (Ince *et al*., 1991), LBV (Hansen, 1997; Hansen and Samuel, 1997), and AD plus PD (Mirra *et al*., 1993; Hulette, 1995). The LBD rubric, which neuropathologically requires only the presence of brain stem and neocortical Lewy bodies, was chosen as an all-encompassing designator with the understanding that it acknowledged the presence of Lewy bodies without specifying their relative importance in symptom formation with respect to other degenerative (i.e., AD) or vascular pathology that might be simultaneously present (Table 14.2).

More recently, the genetic basis for these disorders has begin to be elucidated (Table 14.3) (Hashimoto and Masliah, 1999). This adds a new level of complexity to the problem, but holds the promise of reclassifying these heterogeneous disorders according to the molecular alterations. α-Synuclein, which has now been implicated in the pathogenesis of the Lewy body, is an incompletely understood protein found primarily in neurons, where it is localized predominately at axon terminals or synapses (Trojanowski and Lee, 1998). α-Synuclein is the same protein previously designated as NACP (Ueda *et al*., 1993). Nigral and neocortical Lewy bodies and dystrophic nigral neurites in PD and DLB contain α-synuclein immunoreactivity (Irizarry *et al*., 1998). An Ala 53 Thr mutation in the α-synuclein gene has been found associated with PD in four chromosome 4-linked families with autosomal dominant PD (Polymeropoulos *et al*., 1997); however, sporadic cases of PD are not associated with linkage to this gene. Because both neurofilaments and α-synuclein have been detected immunohistochemically within Lewy bodies, it seems reasonable to speculate that aberrant interactions of one with the other might lead to the abnormal accumulation of these proteins into pathological Lewy bodies (Trojanowski and Lee, 1998).

TABLE 14.3 Genetic Risk Factors for Parkinson's Disease

Gene	Chromosome	Inheritance
α-Synuclein (PARK1)	4	Autosomal dominant
Parkin (PARK2)	6	Autosomal dominant
PARK3	2	Autosomal dominant
4p haplotype	4	Incomplete penetrance
UCH-L1	4	Autosomal dominant

III. Neuropathology of Disorders with Lewy Bodies

Neuropathologically, the common denominator is the degeneration of neuronal populations within the nigral dopaminergic system and mesial temporal regions, Lewy body formation, extensive neuritic degeneration, and spongiform vacuolization (Hansen *et al*., 1989). The substantia nigra displays significant pallor associated with neuronal loss (Fig. 14.2A, see color insert). Lewy bodies are intracytoplasmic eosinophilic neuronal inclusions (Fig. 14.2B), and in pigmented neurons of the brain stem they typically appear as dense eosinophilic cores surrounded by less densely staining peripheral halos (Fig. 14.2B). Neocortical Lewy bodies are more subtle and appear in the perikarya of pyramidal neurons in layers V and VI as spherical, homogeneous, slightly eosinophilic inclusions lacking a concentric laminar structure (Fig. 14.2C). Neocortical LBs are preferentially found in the cingulate cortex, insular region, temporal cortex, and occasionally in the frontal cortex and hippocampus and have long been underreported because they are so subtle with hematoxylin and eosin stains (Hansen, 1997). Ubiquitin antibodies stain many of the neuronal inclusions associated with neurodegenerative diseases, including Lewy bodies (Fig. 14.2D), Pick bodies, and neurofibrillary tangles (Love *et al*., 1988). Ubiquitin is a highly conserved protein in evolution and its function is to target other proteins for breakdown by cytosolic proteases. Lewy bodies are formed, at least in part, from altered neuronal cytoskeletal elements, and immunohistochemical studies of these inclusions have identified neurofilament, microtubular, and paired helical filament epitopes (Iwatsubo *et al*., 1996). Electron microscopic examination of nigral Lewy bodies discloses a dense central core of circular profiles surrounded by a peripheral rim of radiating 7- to 20-nm filaments (Kosaka, 1978). Neocortical Lewy bodies are composed of amorphous material and 10- to 20-nm filaments with irregular contours (Kosaka, 1978). The filamentous ultrastructure of the Lewy body and its immunohistochemical profile have been interpreted as suggesting that disturbed neurofilament metabolism or transportation is at the core of Lewy body formation (Iwatsubo *et al*., 1996).

A minority of LBD brains have only Lewy body-related pathology, which consists of neuron loss and gliosis with Lewy body formation in the remaining neurons in the substantia nigra, locus coeruleus, and nucleus basalis of Meynart. Neocortical Lewy bodies occur in medium-sized pyramidal neurons of the deeper cortical layers, especially in the limbic cortex (Wakabayashi *et al*., 1995). Many cases also display ubiquitin, neurofilament, and α-synuclein positive Lewy neurites in CA2–3 of the hippocampal formation, the amygdala, the nucleus basalis of Meynert, and in brain stem nuclei (Dickson *et al*., 1991; Kim *et al*., 1995) (Fig. 14.3A, see color insert). Dementia with Lewy bodies brains with only Lewy

body pathology can be construed as pure DLBD and pathologically resemble idiopathic PD, which has been shown to feature neocortical Lewy bodies in almost all cases and Lewy neurites in many (Churchyard and Lees, 1997). It may be that such pure DLBD patients are less demented that those whose brains have both LB and AD (Salmon and Galasko, 1996; Samuel *et al*., 1997a), but unequivocal dementia does occur in brains with only Lewy bodies and Lewy neurites. Dementia in LBD correlates with neocortical Lewy body counts. Such correlations have been found in studies of LBV (Samuel *et al*., 1996), LBD (Perry *et al*., 1997; Samuel *et al*., 1997a), DLBD, and even PD with dementia. Lewy neurites in the hippocampus may contribute substantively to cognitive impairment, but they are also found in some nondemented PD patients (Churchyard and Lees, 1997). Neuropsychiatric complications in neuropathologically pure PD overlap those of LBD, DLBD, LBV, and senile dementia of the Lewy body type and may in fact differ only in the timing of their onset relative to motor parkinsonism (Churchyard and Lees, 1997).

Many LBD brains display a striking spongiform vacuolization of the neuropil, which strongly resembles that seen in Creutzfeldt–Jakob disease at both light and electron microscopic levels of magnification (Hansen *et al*., 1989). This vacuolar abnormality is negative for anti-prion antibodies and is nontransmissible. Spongiform vacuolization is usually confined to the entorhinal cortex, temporal neocortex, amygdala, and sometimes the cingulate gyrus (Fig. 14.3B, see color insert). Recent findings indicate that these vacuoles may derive from the degeneration of terminal axons of large pyramidal neurons resident in layers IIIC and V of the transentorhinal cortex and with disappearance of their ubiquitin-containing granular processes (Iseki *et al*., 1997).

The neuropathologic substrate of dementia in clinically diagnosed PD is itself a complicated topic. About one-third of demented PD patients have AD pathology, 10% have widely distributed Lewy bodies and the remaining 55% have only the typical pathology of PD (Hughes *et al*., 1992). The severity of the dementia in these disorders is associated with the density of Lewy bodies and in AD cases with the Braak stage and apolipoprotein E (APOE) genotype. Synaptic injury and altered neurotransmitter (acetylcholine and DOPA) release may also be important. In this regard, neocortical cholinergic activity (choline acetyltransferase) is depleted far more severely in LBD than in AD (Perry *et al*., 1997). The extent of neocortical choline acetyltransferase loss correlates well with dementia in LBD (Samuel *et al*., 1997a) and is attributable to neuron loss in the nucleus basalis of Meynert. Other cholinergic deficits affect intrinsic striatal cholinergic neurons in the caudate nucleus and the reticular nucleus in the thalamus, which receives cholinergic projections from both the basal forebrain and brain stem pedunculopontine nuclei. Loss of striatal dopamine in LBD is comparable to that seen in PD and greatly exceeds any such loss in AD (Langlais *et al*., 1993). It is reasonable to attribute motor parkinsonism, and perhaps visual hallucinations, in LBD to disruptions of striatal dopaminergic input. Finally, the role of programmed cell death (namely apoptosis) in DLBD is currently being explored; however, results are inconclusive at this point (Lassmann *et al*., 1995).

IV. Contribution of Alzheimer's Pathology to Disorders with Lewy Bodies

As indicated in the previous section, most, but not all, DLB brains have more AD pathology than age-matched controls (Ince *et al*., 1991; Gentleman *et al*., 1992; Gearing *et al*., 1995; Kosaka and Iseki, 1996; McKenzie *et al*., 1996; Perry *et al*., 1997; Samuel *et al*., 1996, 1997a; Hansen, 1997). This fact is invariably entangled with questions about how much AD pathology is necessary to warrant a diagnosis of AD and how much can be attributed to normal aging, or so-called ''pathologic aging.'' If neocortical amyloid is one hallmark for a neuropathologic diagnosis of AD, then most LBD brains have concomitant AD. Most LBD brains not only have as much neocortical amyloid as AD specimens when measured immunocytochemically (Gentleman *et al*., 1992), but typically have as many neuritic plaques as AD brains (McKenzie *et al*., 1996) and far more than age-matched controls. Therefore they meet 1985 NIA criteria for AD (Khachaturian, 1985), and because many of the plaques are neuritic, albeit usually lacking tau-positive neurites (Samuel *et al*., 1997b), they also typically qualify as ''definite'' or ''probable'' AD according to criteria from the consortium to establish a registry for AD (Mirra *et al*., 1993). Apolipoprotein E (APOE) $\epsilon 4$ is overrepresented in LBD, just as it is in AD, and is related to increased amyloid plaque pathology in both conditions (Gearing *et al*., 1995; Olichney *et al*., 1996; St. Clair, 1997). Apolipoprotein E $\epsilon 4$ is not increased in PD or in neuropathologically pure DLBD lacking amyloid plaques (St. Clair, 1997).

Some authorities are dismissive of this amyloid overlap in LBD and AD, as they interpret such pathology as so-called ''pathological aging'' (Dickson *et al*., 1987, 1991). These experts contend that only abundant microtubule-associated protein tau abnormalities in the neocortex constitute conclusive evidence of AD (Strong *et al*., 1995), and therefore insist upon the presence of numerous neocortical neurofibrillary tangles, neuropil threads, or tau-positive swollen neurites in neuritic plaques for a diagnosis of AD. Such criteria run contrary to the recent Consensus Recommendation for the Post-Mortem Diagnosis of AD from the National Institute on Aging and the Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of AD, which was of the opinion that ''any Alzheimer lesions should be considered as pathological'' (Hyman and Trojanowski, 1997). Even if only tau pathology in LBD is ruled admissible as evidence for concomitant AD, the situation is made more complicated by the presence of more tau pathology in the medial temporal lobes of most, but not all, DLB brains than in age-matched controls (Ince *et al*., 1991; Samuel *et al*., 1996, 1997a; Hansen, 1997; Hansen and Samuel, 1997). Most, but not all, LBD brains occupy higher Braak stages of AD neurofibrillary pathology (Braak and Braak, 1991) than age-matched controls, but lower stages than brains with pure AD. Because Braak staging is based on entorhinal and hippocampal neurofibrillary tangles and neuropil threads, it seems that most, but not all, LBD brains have more tau pathology than controls, but less than AD. Correlational studies in LBD with dementia have shown statistically strong relationships between cognitive impairment and Braak stages of AD pathology (Jellinger,

1996; Samuel *et al*., 1996, 1997a). Still, as stated earlier, a minority of LBD brains lack AD pathology altogether and yet come from clinically demented patients.

V. α-Synuclein in Lewy Body Disease

The discovery of missense mutations in α-synuclein gene led to extensive neuropathological studies that culminated in the identification of α-synuclein in Lewy bodies and Lewy neurites. Spillantini *et al*. (1997) found that Lewy bodies and Lewy neurites in sporadic PD and DLBD cases are immunoreactive with antibodies against α-synuclein. Both cortical and subcortical Lewy bodies were strongly immunoreactive with anti-α-synuclein antibodies, and this immunoreactivity was observed with several antibodies recognizing different regions of α-synuclein, including both N- and C-terminals (Spillantini *et al*., 1998,; Takeda *et al*., 1998a,b) (Fig. 14.3C, see color insert), indicting that full-length and/or partially truncated α-synuclein is accumulated in Lewy bodies. Semiquantification by immunohistochemical procedures has revealed that staining for α-synuclein was more extensive than that for ubiquitin (Spillantini *et al*., 1998). Until then, the most sensitive marker for Lewy bodies and Lewy neurites was ubiquitin (Kuzuhara *et al*., 1988) and, to a lesser extent, neurofilaments (Schmidt *et al*., 1991). In contrast, neither β- nor α-synuclein immunoreactivity was found in Lewy bodies and other amyloid lesions in various neurodegenerative disorders (Spillantini *et al*., 1998). These results were subsequently confirmed by a number of reports. Takeda *et al*. (1998b) showed that α-synuclein immunoreactivity was detected in all variants of LBD, but not in neurofibrillary tangles, neuropil threads, Pick bodies, ballooned neurons, and glial tangles (most of which were tau positive). Moreover, α-synuclein immunoreactivity has also been found in other neurodegenerative disorders, including multiple systemic atrophy (Arima *et al*., 1998; Wakabayashi *et al*., 1998) and amyotrophic lateral sclerosis (Mezey *et al*., 1998), creating a new category of so-called α-synucleopathies (Hardy and Gwinn-Hardy, 1998).

Iwatsubo and colleagues have prepared monoclonal antibodies using highly purified Lewy bodies from brains of DLBD patients and corroborated biochemically that α-synuclein was accumulated in Lewy bodies (Iwatsubo *et al*., 1996; Baba *et al*., 1998). Furthermore, their results suggested that a partially truncated α-synuclein also exists in Lewy bodies (Baba *et al*., 1998), although the mechanism of fragmentation was unclear. The altered conformation of the structure may be essential for the aggregation and accumulation of α-synuclein in Lewy bodies and other cellular sites. Supporting this contention, immunoelectron microscopic studies have shown that the pattern of immunogold labeling obtained by the C-terminal antibody is distinct from that of the N-terminal antibody (Spillantini *et al*., 1998). Furthermore, α-synuclein immunoreactivity was enhanced significantly by formic acid and proteinase K treatment, suggesting that the structure α-synuclein may be dynamically changed, associated with altered epitopes of antibodies (Takeda *et al*., 1998a). In fact, utilizing this similar approach to reveal hidden epitopes, we have found that the anti-C-terminal antibody against α-synuclein immunostained after proteinase K (but not formic acid) treatment, neurofibrillary tangles of AD, progressive supranuclear palsy, and corticobasal degeneration and glial inclusions of progressive supranuclear palsy and corticobasal degeneration, as well as Pick bodies (Takeda *et al*., 1999). In contrast, in LBD, plaques (Fig. 14.3D, see color insert), astroglial cells, and granular neurons were immunostained only after formic acid pretreatment with the anti-NACP antibody. The N-terminal region antibody recognized only the lesions of LBD cases, but not of other neurodegenerative disorders (Fig. 14.3). These results support the view that different fragments of α-synuclein may play an important role in the pathogenesis of several neurodegenerative disorders.

α-Synuclein belongs to the synuclein family of peptides, which also includes β- and γ-synucleins (Clayton and George, 1998) (Fig. 1). α-Synuclein is a 140 amino acid protein with little or no secondary structure (Weinreb *et al*., 1996), which is divided into three domains: (1) the N-terminal region composed of an incompletely repeated KTKEGV motif, (2) the NAC domain in the middle of region that is extremely hydrophobic, and (3) the C-terminal region with a negative charge (Clayton and George, 1998). α-Synuclein is encoded by a gene located on chromosome 4. Interestingly, both the A53T and the A30P mutations lie in the N-terminal region, suggesting that the N-terminal domain may be critical for amyloidogenesis (Fig. 14.1).

α- and β-synuclein have been shown to inhibit specifically the activity of phospholipase D2 in a cell-free system (Jenco *et al*., 1998), raising the possibility that a conserved function shared by synucleins may be involved in certain specific signal transduction pathways. In this context, it is noteworthy that α-synuclein conferred increased invasive property to breast cancer cells (Jia *et al*., 1999) and also selectively degraded neurofilaments in primary cultured neurons (Buchman *et al*., 1998), suggesting that synucleins may influence the integrity of cytoskeleton networks. Finally, synphilin-1 was identified as a cytosolic-binding protein of α-synuclein using a yeast two-hybrid method. Further characterization of this molecule may provide clues as to the physiological function of α-synuclein (Engelender *et al*., 1999).

VI. α-Synuclein as a Genetic Risk Factor for Parkinson's Disease

The great majority of PD cases are probably sporadic, and strong environmental factors inducing oxidative stress may play an important role (Markesbery and Carney, 1999). However, a small percentage of PD cases are familial and inherited in either an autosomal dominant or recessive manner (Veldman *et al*., 1998). Progress in genetic research in PD has provided new insights into the involvement of specific genes in familial PD (Table 14.3). In 1996, Polymeropoulos and colleagues identified a linkage of markers at chromosome 4q21 to the autosomal dominant type of PD in a large Italian family, mapped the gene locus sucessfully, and identified an A to G mutation at position 209 in exon 4 of the α-synuclein gene, resulting in a missense substitution of alanine to threonine (A53T) in this family and also in three Greek families

(Polymeropoulos *et al*., 1997). In 1998, Kruger and colleagues identified another missense mutation, alanine to proline (A30P), in a German family. Because both of these cases were autosomal dominant, it was predicted that a possible gain of function of α-synuclein may cause these rare familial type of PD (Table 14.3).

Although α-synuclein mutations have not been found in subsequent cohorts of cases with sporadic PD (Chan *et al*., 1998; Farrer *et al*., 1998; Group, 1998; Vaughan *et al*., 1998), the α-synuclein pathway has been regarded as being situated at the center of the pathogenic pathways leading to sporadic LBD. This could be similar, in a way, to the role of amyloid precursor protein (APP) in the pathogenesis of AD. Despite the fact that only a small percentage of familial AD cases exhibit APP mutations, Aβ protein is regarded as a crucial player in the amyloidogenesis of AD. In this regard, Kruger *et al*. (1999) have found a highly significant difference between sporadic PD patients and control individuals regarding the combination of APOE4 and allele 1 of the α-synuclein promoter polymorphism, suggesting a role for interactions or combined actions of these proteins in the pathogenesis of sporadic PD.

Other genes responsible for familial PD have been identified, including parkin (PARK2) (Kitada *et al*., 1998), UCH-L1 (Leroy *et al*., 1998)), and 4p haplotype (Farrer *et al*., 1998), which cause autosomal recessive, penetrate incompletely, and autosomal dominant inheritances, respectively (Table 14.3). It was also reported that chromosome 2p13 was linked to certain autosomal dominant PD (Gasser *et al*., 1998) and designated PARK3 (Table 14.3), although the corresponding gene has not been identified. It is important to determine whether these risk factors are involved in the α-synuclein pathogenic pathway. For example, because UCH-L1 is a thiol protease, functional disturbance of this protein due to gene mutation might result in the altered proteolysis, which may affect aggregation and ubiquitination of α-synuclein. Notably, it has been reported that UCH-L1 is immunoreactive in Lewy bodies (Lowe *et al*., 1990). Finally, previous genetic studies have indicated that the presence of a mutant allele on the CYP2D6 gene (Saitoh *et al*., 1995) and/or the APOE gene (Seeman *et al*., 1978) might confer increased susceptibility to LBD; however, controversy still exists as to the significance of these findings. In summary, these studies suggest that genetic alterations in synaptic proteins with amyloidogenic potential might lead to LBD via oxidative stress and conformational changes in the molecules that might lead to abnormal aggregation (Hashimoto and Masliah, 1999).

VII. Modulators of α-Synuclein Aggregation in Lewy Body Disease

In LBD, the protein level of α-synuclein is not so high in substantia nigra where dopaminergic neurons are predominantly degraded. Thus, it is possible to predict that such a discrepancy may reflect the regional availability of other modulators, which directly or indirectly affect the aggregation of α-synuclein. In AD, it has been well characterized that the aggregation of Aβ protein is potently stimulated by several factors, including apoE, α_1-antichymotrypsin, and proteoglycans (Ma *et al*., 1994; Snow *et al*., 1994; Wisniewski *et al*., 1994). These molecules have been shown to be coaccumulated with Aβ protein in the extracellular space or senile plaques, leading to the concept of pathological chaperones during neurodegeneration (Wisniewski *et al*., 1994). In this context, it is reasonable to hypothesize that certain molecules may act as pathological chaperones for the aggregation of α-synuclein and might be coaccumulated with α-synuclein in Lewy bodies in the PD brain. In this regard, it has been shown that the aggregation of α-synuclein *in vitro* is modulated by various factors, such as Aβ protein (Yoshimoto *et al*., 1995; Jensen *et al*., 1997; Paik *et al*., 1998), NACP (Paik *et al*., 1998), and lipids (Davidson *et al*., 1998). It is noteworthy that in Down syndrome and many familial AD cases with both presenilins (1 and 2), APP mutations exhibit LB formation in certain brain areas associated with the enhanced aggregation of α-synuclein, suggesting that the Aβ protein may be involved in the intracellular aggregation of α-synuclein in these types of AD (Lippa *et al*., 1998). Although it has been described previously that amyloid precursor protein, but not Aβ protein, was detectable in Lewy bodies of PD and DLBD (Arai *et al*., 1992), more detailed biochemical and immunocytochemical analyses will be necessary to clarify this issue.

α-Synuclein may be proteolytically degraded to give rise to short fragments containing the NACP region, which tend to be more prone to be aggregated than the full-length α-synuclein and to be part of the amyloid plaques in AD and LBD (Fig. 14.3D). Such short fragments might become the core of aggregation and further induce aggregation of α-synuclein. Indeed, the degraded product of α-synuclein was detected in highly purified Lewy bodies from the brain of LBD patients. Furthermore, it was shown that an α-synuclein mutant deleted with the C-terminal region was aggregated more easily than the wild type (Crowther *et al*., 1998). Apparently, lipids seem to participate in the regulation of α-synuclein aggregation. Immunoelectron microscopic studies showed that some gold particles decorated synaptic vesicles (Iwai *et al*., 1995) in the nervous system and secretory vesicles and plasma membrane in platelets (Hashimoto *et al*., 1997). Consistent with this, synelfin, the avian homologue of α-synuclein (George *et al*., 1995), was shown to be associated with membranous fractions. A further study showed that α-synuclein binds to phospholipids associated with a certain conformational change of structure (Davidson *et al*., 1998). Thus, the altered composition of lipids of membranes in neurodegeneration may be a prerequisite for the aggregation of α-synuclein.

At the present time, it has yet to be determined whether these molecules could explain the mechanism by which the aggregation of α-synuclein occurs preferentially in the LBD brain. Because dozen of molecules have already been identified as constitutes of LBs (Pollanen *et al*., 1993), some of them might turn out to be candidates for regional specific factors for the aggregation of α-synuclein in the PD brain.

Iron may also play an essential role in the elevated oxidative stress conditions in the PD brain (Youdim *et al*., 1989), as the substantia nigra contains elevated levels of iron, which can act as a free radical catalyst via the Fenton reaction. In this line, our data showed that α-synuclein was aggregated preferentially in the presence of ferric ion (Hashimoto *et al*., 1999a). Although ferrous ion was not effective by itself, it potently aggregated α-synuclein in the presence of hydrogen peroxide.

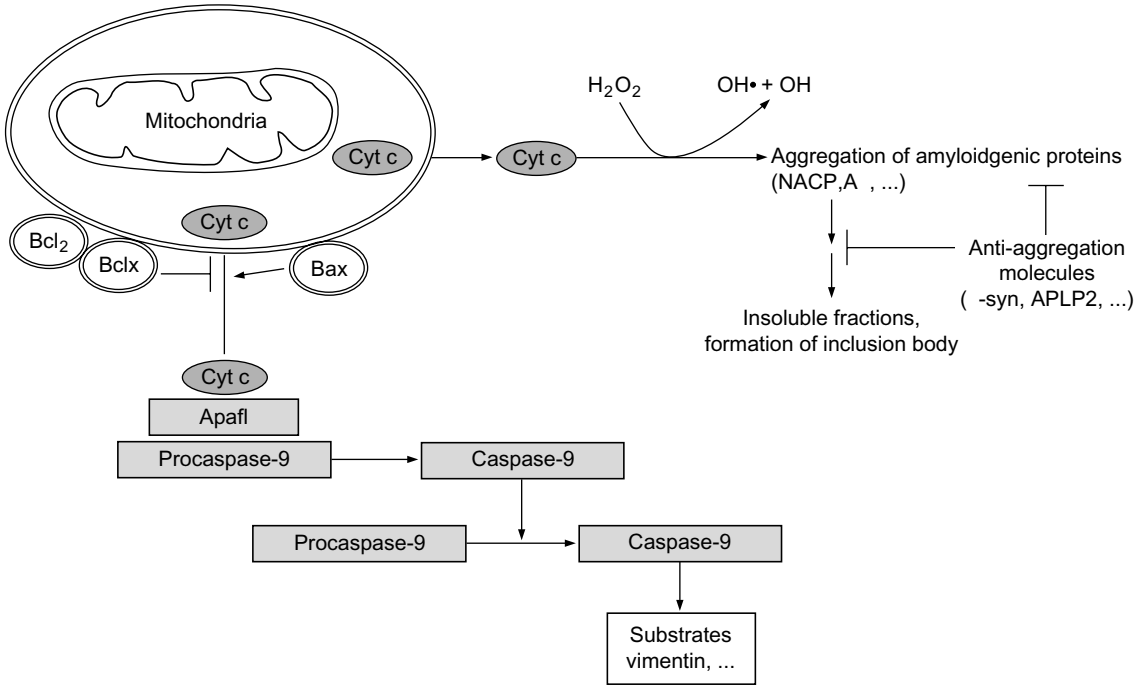

FIG. 14.4. Possible mechanisms of cell death in Lewy body disease.

The aggregates of α-synuclein displayed both Thioflavine-S reactivity and Congo red birefringence, reminiscent of amyloid-like fibrils. These results suggest that an aberrant accumulation of ferric ion might act as a risk factor for the aggregation of α-synuclein in the PD brain. Indeed, it has been suspected that the shift from ferrous to ferric ion may be a risk factor for PD. Riederer *et al*. (1989) showed that the ratio of ferric to ferrous ion in the substantia nigra of the patient with advanced PD was remarkably increased compared to controls. Furthermore, it was shown that neuromelanin, which acts as an endogenous chelator of ferrous/ferric ions in substantia nigra, differentially regulates the rate of hydroxyl radical production depending on the redox state of the iron ion (Pilas *et al*., 1988). Therefore, if the iron-catalyzed oxidative reaction plays a significant role in α-synuclein aggregation *in vivo*, either an antioxidant or a iron chelator could be used as a possible therapeutic strategy. Other metal ions may also contribute to the aggregation of α-synuclein in neurodegeneration. For example, aluminum ion potently stimulates the aggregation of α-synuclein (Paik *et al*., 1997), and copper ion induces aggregation of α-synuclein in the presence of a specific cross-linker. Finally, it has been suggested that the mitochondrial heme protein cytochrome C may participate in the aggregation of α-synuclein in the PD brain (Hashimoto *et al*., 1999b). *In vitro*, recombinant α-synuclein was coinduced to be aggregated in the presence of cytochrome C/hydrogen peroxide, which was inhibited by *N*-acetyl-L-cysteine. In the brains of patients with LBD, cytochrome C immunoreactivity was detected in Lewy bodies in substantia nigra, whereby approximately half of the α-synuclein-immunoreactive Lewy bodies were also cytochrome C-immunoreactive. The cytochrome C staining was specifically observed in Lewy bodies in substantia nigra, but not in other lesions, such as cortical Lewy bodies, senile plaques, and neurofibrillary tangles. Cytochrome C is localized in the intramembrane of mitochondria and is released upon apoptotic stimuli into cytoplasm where it triggers apoptotic cascades (Green and Reed, 1998). Taken together, these results suggest that the aggregation of α-synuclein may be closely related to mitochondrial dysfunction and apoptosis in neurodegeneration in LBD (Hashimoto and Masliah, 1999) (Fig. 14.4).

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References

Akopian, A. N., and Wood, J. N. (1995). Peripheral nervous system-specific genes identified by subtractive cDNA cloning. *J. Biol. Chem.* **270**, 21264–21270.

Arai, H., Lee, V.-Y., Hill, W. D., Greenberg, B. D., and Trojanowski, J. Q. (1992). Lewy bodies contain beta-amyloid precursor proteins of Alzheimer's disease. *Brain Res.* **585**, 386–390.

Arima, K., Ueda, K., Sunohara, N., Arakawa, K., Hirai, S., Nakamura, M., Tonozuka-Euhara, H., and Kawai, M. (1998). NACP/alpha-synuclein immunoreactivity in fibrillary components of neuronal and oligodendroglial cytoplasmic inclusions in the pontine nuclei in multiple system atrophy. *Acta Neuropathol.* **96**, 439–444.

Baba, M., Nakajo, S., Tu, P.-H., Tomita, T., Nakaya, K., Lee, V. M.-Y., Trojanowski, J. Q., and Iwatsubo, T. (1998). Aggregation of α-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. *Am. J. Pathol.* **152**, 879–884.

Braak, H., and Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol*. **82**, 239–259.

Buchman, V. L., Hunter, H., Pinon, L., Thompson, J., Privalova, E. M., Ninkina, N. N., and Davies, A. M. (1998). Persyn, a member of the synuclein family, has a distinct pattern of expression in the developing nervous system. *J. Neurosci*. **18**, 9335–9341.

Chan, P., Tanner, C. M., Jiang, X., and Langston, J. W. (1998). Failure to find the alpha-synuclein gene missense mutation (G209A) in 100 patients with younger onset Parkinson's disease. *Neurology* **50**, 513–514.

Churchyard, A., and Lees, A. J. (1997). The relationship between dementia and direct involvement of the hippocampus and the amygdala in Parkinson's disease. *Neurology* **49**, 1570–1576.

Clayton, D. F., and George, J. M. (1998). The synucleins: A family of proteins involved in synaptic function, plasticity, neurodegeneration and disease. *Trends Neurosci*. **21**, 249–254.

Crowther, R. A., Jakes, R., Spillantini, M. G., and Goedert, M. (1998). Synthetic filaments assembled from C-terminally truncated alpha-synuclein. *FEBS Lett*. **436**, 309–312.

Davidson, W. S., Jonas, A., Clayton, D. F., and George, J. M. (1998). Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes. *J. Biol. Chem*. **273**, 9443–9449.

DeKosky, S. T., and Scheff, S. W. (1990). Synapse loss in frontal cortex biopsies in Alzheimer's disease: Correlation with cognitive severity. *Ann. Neurol*. **27**, 457–464.

Dickson, D. W., Davies, P., Mayeux, R., Crystal, H., Horoupian, D. S., Thompson, A., and Goldman, J. E. (1987). Diffuse Lewy body disease. Neuropathological and biochemical studies of six patients. *Acta Neuropathol*. **75**, 8–15.

Dickson, D. W., Crystal, H., Mattiace, L. A., Kress, Y., Schwagerl, A., Ksiezak-Reding, H., Davies, P., and Yen, S.-H. C. (1989). Diffuse Lewy body disease: Light and electron microscopic immunocytochemistry of senile plaques. *Acta Neuropathol*. **78**, 572–584.

Dickson, D. W., Ruan, D., Crystal, H., Mark, M. H., Davies, P., Kress, Y., and Yen, S.-H. (1991). Hippocampal degeneration differentiates diffuse Lewy body disease (DLBD) from Alzheimer's disease: Light and electron microscopic immunocytochemistry of CA2-3 neurites specific to DLBD. *Neurology* **41**, 1402–1409.

Engelender, S., Kaminsky, Z., Guo, X., Sharp, A. H., Amaravi, R. K., Kleiderlein, J. J., Margolis, R. L., Troncoso, J. C., Lanahan, A. A., Worley, P. F., Dawson, V. L., Dawson, T. M., and Ross, C. A. (1999). Synphilin-1 associates with alpha-synuclein and promotes the formation of cytosolic inclusions. *Nat. Genet*. **22**, 110–114.

Farrer, M., Wavrant-De Vrièze, F., Crook, R., Boles, L., Perez-Tur, J., Hardy, J., Johnson, W. G., Steele, J., Maraganore, D., and Gwinn, K. (1998). Low frequency of alpha-synuclein mutations in familial Parkinson's disease. *Ann. Neurol*. **43**, 394–397.

Gaspar, P., and Graf, F. (1984). Dementia in idiopathic Parkinson's disease. *Acta Neuropathol*. **64**, 43–52.

Gasser, T., Muller-Myhsok, B., Wszolek, Z. K., Oehlmann, R., Calne, D. B., Bonifati, V., Bereznai, B., Fabrizio, E., Vieregge, P., and Horstmann, R. D. (1998). A susceptibility locus for Parkinson's disease maps to chromosome 2p13. *Nat. Genet*. **18**, 262–265.

Gearing, M., Schneider, J. A., Rebeck, G. W., Hyman, B. T., and Mirra, S. S. (1995). Alzheimer's disease with and without co-existing Parkinson's disease changes: Apolipoprotein E genotype and neuropathologic correlates. *Neurology* **45**, 1985–1990.

Gentleman, S. M., Williams, B., Royston, M. C., Jagoe, R., Clinton, J., Perry, R. H., Ince, P. G., Allsop, D., Polak, J. M., and Roberts, G. W. (1992). Quantification of βA4 protein deposition in the medial temporal lobe: A comparison of Alzheimer's disease and senile dementia of the Lewy body type. *Neurosci. Lett*. **142**, 9–12.

George, J. M., Jin, H., Woods, W. S., and Clayton, D. F. (1995). Characterization of a novel protein regulated during the critical period for song learning in the zebra finch. *Neuron* **15**, 361–372.

Green, D. R., and Reed, J. C. (1998). Mitochondria and apoptosis. *Science* **281**, 1309–1312.

Hansen, L. A. (1997). The Lewy body variant of Alzheimer disease. *J. Neural Transm*. **51**, 111–121.

Hansen, L. A., and Crain, B. J. (1995). Making the diagnosis of mixed and non-Alzheimer's dementias. *Arch. Pathol. Lab. Med*. **119**, 1023–1031.

Hansen, L. A., and Samuel, W. (1997). Criteria for Alzheimer's disease and the nosology of dementia with Lewy bodies. *Neurology* **48**, 126–132.

Hansen, L. A., Masliah, E., and Terry, R. D. (1989). A neuropathological subset of Alzheimer's disease with concomittant Lewy body disease and spongiform change. *Acta Neuropathol*. **78**, 194–201.

Hansen, L. A., Salmon, D., Galasko, D., Masliah, E., Katzman, R., DeTeresa, R., Thal, L., Pay, M. M., Hofstetter, R., and Klauber, M. (1990). The Lewy body variant of Alzheimer's disease: A clinical and pathologic entity. *Neurology* **40**, 1–7.

Hardy, J., and Gwinn-Hardy, K. (1998). Genetic classification of primary neurodegenerative disease. *Science* **282**, 1075–1079.

Hashimoto, M., and Masliah, E. (1999). Alpha-synuclein in Lewy body disease and Alzheimer's disease. *Brain Pathol*. **9**, 707–720.

Hashimoto, M., Yoshimoto, M., Sisk, A., Hsu, L. J., Sundsmo, M., Kittel, A., Saitoh, T., Miller, A., and Masliah, E. (1997). NACP, a synaptic protein involved in Alzheimer's disease, is differentially regulated during megakaryocyte differentiation. *Biochem. Biophys. Res. Commun*. **237**, 611–616.

Hashimoto, M., Hsu, L. J., Xia, Y., Takeda, A., Sundsmo, M., and Masliah, E. (1999a). Oxidative stress induces amyloid-like aggregate formation of NACP/α-synuclein in vitro. *NeuroReport* **10**, 717–721.

Hashimoto, M., Takeda, A., Hsu, L. J., Takenouchi, T., and Masliah, E. (1999b). Role of cytochrome c as a stimulator of α-synuclein aggregation in Lewy body disease. *J. Biol. Chem*. **274**, 28849–28852.

Hughes, A. J., Daniel, S. E., Kilford, L., and Lees, A. J. (1992). Accuracy of clinical diagnoses of idiopathic Parkinson's disease: A clinico-pathological study of 100 cases. *J. Neurol. Neurosurg. Psychiatry* **55**, 181–184.

Hulette, C. (1995). The consortium to establish a registry for Alzheimer's disease (CERAD). Part IX. A perspective clinical neuropathologic study of parkinson's features, in Alzheimer's disease. *Neurology* **45**, 1991–1995.

Hyman, B. T., and Trojanowski, J. Q. (1997). Consensus recommendations for the postmortem diagnosis of Alzheimer disease from the National Institute on Aging and the Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer disease. *J. Neuropathol. Exp. Neurol*. **56**, 1095–1097.

Ince, P., Irving, D., MacArthur, F., and Perry, R. H. (1991). Quantitative neuropathologic study of Alzheimer-type pathology in the hippocampus: Comparison of senile dementia of Alzheimer type, senile dementia of Lewy body type, Parkinson's disease and non-demented eldery control patients. *J. Neurol. Sci*. **106**, 142–152.

Irizarry, M. C., Growdon, W., Gómez-Isla, T., Newell, K., George, J. M., Clayton, D. F., and Hyman, B. T. (1998). Nigral and cortical Lewy bodies and dystrophic nigral neurites in Parkinson's disease and cortical Lewy body disease contain α-synuclein immunoreactivity. *J. Neuropathol. Exp. Neurol*. **57**, 334–337.

Iseki, E., Li, F., Odawara, T., and Kosaka, K. (1997). Close relationship between spongiform change and ubiquitin-positive granular structures in diffuse Lewy body disease. *J. Neurol. Sci*. **146**, 53–57.

Iwai, A., Yoshimoto, M., Masliah, E., and Saitoh, T. (1995). Non-Aβ component of Alzheimer's disease amyloid (NAC) is amyloidogenic. *Biochemistry* **34**, 10139–10145.

Iwatsubo, T., Yamaguchi, H., Fujimuro, M., Yokosawa, H., Ihara, Y., Trojanowski, J. Q., and Lee, V.-M. (1996). Purification and characterization of Lewy bodies from brains of patients with diffuse Lewy body disease. *Am. J. Pathol*. **148**, 1517–1529.

Jakes, R., Spillantini, M. G., and Goedert, M. (1994). Identification of two distinct synucleins from human brain. *FEBS Lett*. **345**, 27–32.

Jellinger, K. A. (1996). Structural basis of dementia in neurodegenerative disorders. *J. Neural Transm*. **47**, 1–29.

Jenco, J. M., Rawlingson, A., Daniels, B., and Morris, A. J. (1998). Regulation of phospholipase D2: Selective inhibition of mammalian phospholipase D isoenzymes by α- and β- synucleins. *Biochemistry* **37**, 4901–4909.

Jensen, P. H., Hojrup, P., Hager, H., Nielsen, M. S., Jacobsen, L., Olesen, O. F., Gliemann, J., and Jakes, R. (1997). Binding of Aβ to α- and β-synucleins: Identification of segments in α-synuclein/NAC precursor that bind Aβ and NAC. *Biochem. J.* **323**, 539–546.

Ji, H., Liu, Y. E., Wang, M., Liu, J., Xiao, G., Joseph, B. K., Rosen, C., and Shi, Y. E. (1997). Identification of a breast cancer-specific gene, BCSG1, by direct differential cDNA sequencing. *Cancer Res*. **57**, 759–764.

Jia, T., Liu, Y. E., Liu, J., and Shi, Y. E. (1999). Stimulation of breast cancer invasion and metastasis by synuclein gamma. *Cancer Res*. **59**, 742–747.

Khachaturian, Z. S. (1985). Diagnosis of Alzheimer's disease. *Arch. Neurol. (Chicago)* **42**, 1097–1105.

Kim, H., Gearing, M., and Mirra, S. S. (1995). Ubiquitin positive CA2-3 neurites in hippocampus coexist with cortical Lewy bodies. *Neurology* **45**, 1768–1770.

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y., and Shimizu, N. (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature (London)* **392**, 605–608.

Kosaka, K. (1978). Lewy bodies in cerebral cortex. Report of three cases. *Acta Neuropathol*. **42**, 127–134.

Kosaka, K., and Iseki, E. (1996). Dementia with Lewy bodies. *Curr. Opin. Neurol*. **9**, 271–275.

Kruger, R., Kuhn, W., Muller, T., Woitalla, D., Graeber, M., Kosel, S., Przuntek, H., Epplen, J. T., Schols, L., and Reiss, O. (1998). Ala30Pro mutation in the gene encoding α-synuclein in Parkinsons's disease. *Nat. Genet*. **18**, 106–108.

Kruger, R., Vieira-Saecker, A. M., Kuhn, W., Berg, D., Muller, T., Kuhnl, N., Fuchs, G. A., Storch, A., Hungs, M., Woitalla, D., Przuntek, H., Epplen, J. T., Schols, L., and Riess, O. (1999). Increased susceptibility to sporadic Parkinson's disease by a certain combined alpha-synuclein/apolipoprotein E genotype. *Ann. Neurol*. **45**, 611–617.

Kuzuhara, S., Mori, H., Izumiyama, N., Yoshimura, M., and Ihara, Y. (1988). A light and electron microscopic immunocytochemical study. *Acta Neuropathol*. **75**, 345–353.

Langlais, P. J., Thal, L., Hansen, L., Galasko, D., Alford, M., and Masliah, E. (1993). Neurotransmitters in basal ganglia and cortex of Alzheimer's disease with and without Lewy bodies. *Neurology* **43**, 1927–1934.

Lassmann, H., Bancher, C., Breitschopf, H., Wegiel, J., Bobinski, M., Jellinger, K., and Wisniewski, H. M. (1995). Cell death in Alzheimer's disease evaluated by DNA fragmentation in situ. *Acta Neuropathol*. **89**, 35–41.

Leroy, E., Boyer, R., Auburger, G., Leube, B., Ulm, G., Mezey, E., Harta, G., Brownstein, M. J., Jonnalagada, S., Chernova, T., Dehejia, A., Lavedan, C., Gasser, T., Steinbach, P. J., Wilkinson, K. D., and Polymeropoulos, M. H. (1998). The ubiquitin pathway in Parkinsons's disease. *Nature (London)* **395**, 451–452.

Lippa, C. F., Fujiwara, H., Mann, D. M., Giasson, B., Baba, M., Schmidt, M. L., Nee, L. E., O'Connell, B., Pollen, D. A., St. George-Hyslop, P., Ghetti, P., Ghetti, B., Nochlon, D., Bird, T. D., Cairns, N. J., Lee, V.-M., Iwatsubo, T., and Trojanowski, J. Q. (1998). Lewy bodies contain altered alpha-synuclein in brains of many familial Alzheimer's disease patients with mutations in presenilin and amyloid precursor protein genes. *Am. J. Pathol*. **153**, 1365–1370.

Love, S., Saitoh, T., Quijada, S., Cole, G. M., and Terry, R. D. (1988). Alz-50, ubiquitin and tau immunoreactivity of neurofibrillary tangles, Pick bodies, and Lewy bodies. *J. Neuropathol. Exp. Neurol*. **47**, 393–405.

Lowe, J., McDermott, H., Landon, M., Mayer, R. J., and Wilkinson, K. D. (1990). Ubiquitin carboxyl-terminal hydrolase (PGP 9.5) is selectively present in ubiqutinated inclusion bodies characteristic of human neurodegenerative diseases. *J. Pathol*. **161**, 153–160.

Ma, J., Yee, A., Brewer, H. B. J., Das, S., and Potter, H. (1994). Amyloid-associated proteins α1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer β-protein into filaments. *Nature (London)* **372**, 92–94.

Markesbery, W. R., and Carney, J. M. (1999). Oxidative alterations in Alzheimer's disease. *Brain Pathol.* 9, 133–146.

Maroteaux, L., Campanelli, J. T., and Scheller, R. H. (1988). Synuclein: A neuron-specific protein localized to the nucleus and presynaptic nerve terminal. *J. Neurosci*. **8**, 2804–2815.

Masliah, E. (1998a). Mechanisms of synaptic pathology in Alzheimer's disease *J. Neural. Transm. Suppl*. **53**, 147–158.

Masliah, E. (1998b). The role of synaptic proteins in neurodegenerative disorders. *Neurosci. News* **1**, 14–20.

McKeith, I. G., Galasko, D., Kosaka, K., Perry, E. K., Dickson, D. W., Hansen, L. A., Salmon, D. P., Lowe, J., Mirra, S. S., Byrne, E. J., Quinn, N. P., Edwardson, J. A., Ince, P. G., Bergeron, C., Burns, A., Miller, B. L., Lovestone, S., Collerton, D., Jansen, E., de Vos, R., Wilcock, G. K., Jellinger, K. A., and Perry, R. H. (1996). Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB). *Neurology* **47**, 1113–1124.

McKenzie, J. E., Edwards, R. J., Gentleman, S. M., Ince, P. G., Perry, R. H., Royston, M. C., and Roberts, G. W. (1996). A quantitative comparison of plaque types in Alzheimer's disease and senile dementia of the Lewy body type. *Acta Neuropathol*. **91**, 526–529.

Mezey, E., Dehejia, A., Harta, G., Papp, M., Polymeropoulos, M., and Brownstein, M. (1998). Alpha synuclein in neurodegenerative disorders: Murderer or accomplice? *Nat. Med*. **4**, 755–757.

Mirra, S. S., Hart, M. N., and Terry, R. D. (1993). Making the diagnosis of Alzheimer's disease. A primer for practicing pathologists. *Arch. Pathol. Lab. Med*. **117**, 132–144.

Nakajo, S. (1990). Purification and characterization of a novel brain-specific 14-kDa protein. *J. Neurochem*. **55**, 2031–2038.

Olichney, J. M., Hansen, L. A., Galasko, D., Saitoh, T., Hofstetter, C. R., Katzman, R., and Thal, L. J. (1996). The spolipoprotein E ϵ4 allele is associated with increased neuritic plaques and cerebral amyloid angiopathy in Alzheimer's disease and Lewy body variant. *Neurology* **47**, 190–196.

Paik, S. R., Lee, J. H., Kim, D. H., Chang, C. S., and Kim, J. (1997). Aluminum-induced structural alterations of the precursor of the non-A beta component of Alzheimer's disease amyloid. *Arch. Biochem. Biophys*. **344**, 325–334.

Paik, S. R., Lee, J. H., Kim, D. H., Chang, C. S., and Kim, Y. S. (1998). Self-oligomerization of NACP, the precursor protien of the non-amyloid beta/A4 protein (A beta) component of Alzheimer's disease amyloid, observed in the presence of a C-terminal A beta fragment (residues 25-35). *FEBS Lett*. **421**, 73–76.

Perry, R. H., Irving, D., Blessed, G., Fairbairn, A., and Perry, E. K. (1990). Senile dementia of Lewy body type. A clinically and neuropathologically distinct form of Lewy body dementia in the elderly. *J. Neurol. Sci*. **95**, 119–139.

Perry, R. H., McKeith, I. G., and Perry, E. K. (1997). Lewy body dementia—clinical, pathological and neurochemical interconnections. *J. Neural. Transm. Suppl.* **51**, 95–109.

Pilas, B., Sarna, T., Kalyanaraman, B., and Swartz, H. M. (1988). The effect of melanin on iron associated decomposition of hydrogen peroxide. *Free Rad. Biol. Med*. **4**, 285–293.

Pollanen, M. S., Dickson, D. W., and Bergeron, C. (1993). Pathology and biology of the Lewy body. *J. Neuropathol. Exp. Neurol*. **52**, 183–191.

Polymeropoulos, M. H., Higgins, J. J., Golbe, L. I., Johnson, W. G., Ide, S. E., Di Iorio, G., Sangers, G., Stenroos, E. S., Pho, L. T., Schaffer, A. A., Lazzarini, A. M., Nussbaum, R. L., and Duvoisin, R. C. (1996). Mapping of a gene for Parkinson's disease to chromosome 4q21–q23. *Science* **274**, 1197–1199.

Polymeropoulos, M. H., Lavedant, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E. S.,

Chandrasekharappa, S., Athanassiadou, A., Papapetropulos, T., Johnson, W. G., Lazzarini, A. M., Duvoisin, R. C., Di Iorio, G., Golbe, L. I., and Nussbaum, R. L. (1997). Mutation in the α-synuclein gene identified in families with Parkinson's disease. *Science* **276**, 2045–2047.

Riederer, P., Sofic, E., Rausch, W.-D., Schmidt, B., Reynolds, G. P., and Youdim, M. B. (1989). Transition metals, ferritin, glutathione, and ascorbic acid in Parkinsonian brains. *J. Neurochem.* **52**, 515–520.

Saitoh, T., Xia, Y., Chen, X., Masliah, E., Galasko, D., Shults, C., Thal, L. J., Hansen, L. A., and Katzman, R. (1995). The CYP2D6B mutant allele is overrepresented in the Lewy body variant of Alzheimer's disease. *Ann. Neurol*. **37**, 110–112.

Salmon, D. P., and Galasko, D. (1996). Neuropsychological aspects of Lewy body dementia. *In* "Dementia with Lewy Bodies" (E. K. Perry, I. G. McKeith, and R. H. Perry, eds.), pp. 99–113. Cambridge University Press, London.

Samuel, W., Galasko, D., Masliah, E., and Hansen, L. A. (1996). Neocortical Lewy counts correlate with dementia in the Lewy body variant of Alzheimer's disease. *J. Neuropathol. Exp. Neurol*. **55**, 44–52.

Samuel, W., Alford, M., Hofstetter, R., and Hansen, L. A. (1997a). Dementia with Lewy bodies versus pure Alzheimer disease: Differences in cognition, neuropathology, cholinergic dysfunction, and synapse density. *J. Neuropathol. Exp. Neurol*. **56**, 499–508.

Samuel, W., Crowder, R., Hofstetter, R., and Hansen, L. A. (1997b). Neuritic plaques in the Lewy body variant of Alzheimer disease lack paired helical filaments. *Neurosci. Lett*. **223**, 73–76.

Schmidt, M. L., Murray, J., Lee, V. M.-Y., Hill, W. D., Wertkin, A., and Trojanowski, J. Q. (1991). Epitope map of neurofilament protein domains in cortical and peripheral nervous system Lewy bodies. *Am. J. Pathol*. **139**, 53–65.

Seeman, P., Tedesco, J. L., Lee, T., Chau-Wong, M., Muller, P., Bowles, J., Whitaker, P. M., McManus, C., Tittler, M., Weinreich, P., Friend, W. C., and Brown, G. M. (1978). Dopamine receptors in the central nervous system. *Fed. Proc., Fed. Am. Soc. Exp. Biol*. **37**, 131–136.

Snow, A. D., Seikiguchi, R., Nochlin, D., Fraser, P., Kimata, K., Mizutani, A., Arai, M., Schreier, W. A., and Morgan, D. G. (1994). An important role of heparan sulfate proteoglycan (Perlecan) in a model system for the deposition and persistence of fibrillar A beta-amyloid in rat brain. *Neuron* **12**, 219–234.

Spillantini, M. G., Schmidt, M. L., Lee, V.-Y., Trojanowski, J. Q., Jakes, R., and Goedert, M. (1997) α-Synuclein in Lewy bodies. *Nature (London)* **388**, 839–840.

Spillantini, M. G., Crowther, R. A., Jakes, R., Hasegawa, M., and Goedert, M. (1998). alpha-synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with Lewy bodies. *Proc. Natl. Acad. Sci. U.S.A*. **95**, 6469–6473.

St. Clair, D. (1997). Apolipoprotein E gene in Parkinson's disease, Lewy body dementia, and Alzheimer's disease. *In* "Dementia in Parkinsonism," pp. 161–165. Springer-Verlag, New York.

Strong, C., Anderton, B. H., Perry, R. H., Perry, E. K., Ince, P. G., and Lovestone, S. (1995). Abnormally phosphorylated tau protein in senile dementia of Lewy body type and Alzheimer disease: Evidence that the disorders are distinct. *Alzheimer Dis. Assoc. Disord*. **9**, 128–222.

Takeda, A., Hashimoto, M., Mallory, M., Sundsmo, M., Hansen, L., Sisk, A., and Masliah, E. (1998a). Human NACP/α-synuclein distribution in Lewy body disease. *Lab. Invest*. **78**, 1169–1177.

Takeda, A., Mallory, M., Sundsmo, M., Honer, W., Hansen, L., and Masliah, E. (1998b). Abnormal accumulation of NACP/α-synuclein in neurodegenerative disorders. *Am. J. Pathol*. **152**, 367–372.

Takeda, A., Hashimoto, M., Mallory, M., Sundsmo, M., Hansen, L., and Masliah, E. (1999). C-terminal α-synuclein immunoreactivity in structures other than Lewy bodies in neurodegenerative disorders. *Acta Neuropathol*. (in press).

Terry, R. D., Masliah, E., Salmon, D. P., Butters, N., DeTeresa, R., Hill, R., Hansen, L. A., and Katzman, R. (1991). Physical basis of cognitive alterations in Alzheimer disease: Synapse loss is the major correlate of cognitive impairment. *Ann. Neurol*. **30**, 572–580.

The French Parkinsons's Disease Genetics Study Group (1998). Alpha-synuclein gene and Parkinson's disease. *Science* **279**, 1116–1117.

Tobe, T., Nakajo, S., Tanaka, A., Mitoya, A., Omata, K., Nakaya, K., Tomita, M., and Nakamura, Y. (1992). Cloning and characterization fo the cDNA encoding a novel brain-specific 14 kDa protein. *J. Neurochem*. **59**, 1624–1629.

Trojanowski, J. Q., and Lee, V. M. (1998). Aggregation of neurofilament and alpha-synuclein proteins in Lewy bodies: Implications for pathogenesis of Parkinson disease and Lewy body dementia. *Arch. Neurol. (Chicago)* **55**, 151–152.

Ueda, K., Fukushima, H., Masliah, E., Xia, Y., Iwai, A., Otero, D., Kondo, J., Ihara, Y., and Saitoh, T. (1993). Molecular cloning of a novel component of amyloid in Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A*. **90**, 11282–11286.

Vaughan, J., Durr, A., Tassin, J., Bereznai, B., Gasser, T., Bonifati, V., De Michele, G., Fabrizio, E., Volpe, G., Bandmann, O., Johnson, W. G., Golbe, L. I., Breteler, M., Meco, G., Agid, Y., Brice, A., Marsden, C. D., and Wood, N. W. (1998). The alpha-synuclein Ala53Thr mutation is not a common cause of familial Parkinsons's disease: A study of 230 European cases. *Ann. Neurol*. **44**, 270–273.

Veldman, B. A., Wijn, A. M., Knoers, N., Praamstra, P., and Horstink, M. W. (1998). Genetic and environmental risk factors in Parkinson's disease. *Clin. Neurol. Neurosurg*. **100**, 15–26.

Wakabayashi, K., Hansen, L. A., and Masliah, E. (1995). Cortical Lewy body-containing neurons are pyramidal cells: Laser confocal imaging of double-immunolabeled sections with anti-ubiquitin and SMI32. *Acta Neuropathol*. **89**, 404–408.

Wakabayashi, K., Yoshimoto, M., Tsuji, S., and Takahashi, H. (1998). α-Synuclein immunoreactivity in glial cytoplasmic inclusions in multiple system atrophy. *Neurosci. Lett*. **249**, 180–182.

Weinreb, P. H., Zhen, W., Poon, A. W., Conway, K. A., and Lansbury, P. T., Jr. (1996). NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded. *Biochemistry* **35**, 13709–13715.

Wisniewski, T., Castano, E. M., Golabek, A., Vogel, T., and Frangione, B. (1994). Acceleration of Alzheimer's fibril formation by apolipoprotein E in vitro. *Am. J. Pathol*. **145**, 1030–1035.

Yoshimoto, M., Iwai, A., Kang, D., Otero, D., Xia, Y., and Saitoh, T. (1995). NACP, the precursor protein of non-amyloid β/A4 protein (Aβ) component of Alzheimer disease amyloid, binds Aβ and stimulates Aβ aggregation. *Proc. Natl. Acad. Sci. U.S.A*. **92**, 9141–9145.

Youdim, M. B., Ben-Schachar, D., and Riederer, P. (1989). Is Parkinson's disease a progressive sidereosis of substantia nigra resulting in iron and melanin induced neurodegeneration? *Acta Neurol. Scand*. **126**, 47–54.

Daniel P. Perl

15

Amyotrophic Lateral Sclerosis/ Parkinsonism–Dementia Complex of Guam

I. Introduction

Guam is the largest of the Marianas islands, an archipelago consisting of 15 relatively small islands in the Western Pacific. The Marianas are part of Micronesia, a broader group of Pacific islands that, in addition to the Marianas, consist of the Caroline, the Marshall, and the Gilbert islands. Guam is the southernmost island of the Marianas chain and, although it is a relatively small island, it represents the largest land mass in the Western Pacific. Guam is approximately 30 miles long and varies from 4 to 9 miles wide (with a total area of approximately 212 square miles). Located 3500 miles west of Hawaii, 1500 miles south of Tokyo, and 1500 miles east of Manila, Guam occupies a central and strategic location in the Western Pacific (Fig. 15.1). In 1898, as part of the settlement of the Spanish–American War, the United States obtained Guam as a territorial possession. In an era of coal-burning, ocean-going ships, Guam served as a valuable site for steamships needing to take on fresh water and other vital supplies. Its strategic central location in the Western Pacific, from a military perspective, was also an important feature. The U.S. Navy administered the island through the first half of the 20th century. In 1950, with the passage of the Organic Act of Guam, the inhabitants of Guam were finally granted citizenship to the United States. In addition, the residents of Guam were allowed to elect a local governor and an island legislature, as well as a nonvoting representative in the U.S. Congress. They vote in the American presidency elections, serve is the U.S. military, and pay federal taxes (although all collected federal tax revenues remain on the island for local use).

The 1990, the population of Guam was 123,203, of which 43,000 inhabitants were identified as Chamorros, 30,043 as Filipino, 22,318 as migrants from the U.S. mainland, and 6166 as other Micronesians, mostly originating from the Caroline islands. The Chamorros are a local indigenous population that has continuously inhabited Guam since approximately 1500 B.C., when they are believed to have originated from the Malay Archipelago. The Chamorros of Guam first encountered European explorers when Ferdinando Magellan, the Portuguese explorer, landed near the village of Umatac in southern Guam in 1521. Magellan and his crew were in the process of circumnavigating the world and stopped, after a long journey across the Pacific, in Guam to take on supplies. Following his leaving Guam, Magellan took his fleet to the Philippines, where he was unfortunately killed in a violent native dispute.

The presence of a high incidence focus of neurodegenerative disease on Guam was first brought to medical attention by Dr. Harry M. Zimmerman, a pathologist who was assigned in 1944 to Guam by the U.S. Navy. On December 8, 1941, the day after the Japanese attack on Pearl Harbor, Japanese forces also occupied Guam. For most of World War II, the Japanese military held Guam in a rather brutal occupation. However, in the summer of 1944, U.S. military forces retook the island from the Japanese. Due to the relatively large size and strategic position of the island Guam played a significant role in the latter stages of the Pacific campaign, serving as a major staging area and take-off site for bombing attacks of the major cities of Japan. In the final year of World War II, large numbers of military personnel to support these operations were assigned to Guam, including Dr. Zimmerman. During his stay on Guam, Dr. Zimmerman identified a number of cases of amyotrophic lateral sclerosis (ALS) among the native population who were being cared for in the local hospital. As a pathologist with neuropathology training, he subsequently confirmed the diagnosis at autopsy on two affected individuals. Zimmerman recognized that ALS was a relatively uncommon disorder and that this represented an inordinately high prevalence of the disease. He therefore wrote to his naval supervisors in Washington, DC with his observations and wondered if this might represent an unusual genetic focus of ALS in an island-bound and presumably inbred population. Apparently his message was taken seriously, as after the end of the war a series of clinicians and epidemiologists were sent to Guam to investigate the situation further. Based on their initial findings, a major commitment of the National Institutes of Health (NIH) was made to determine the nature of the disorder and, in

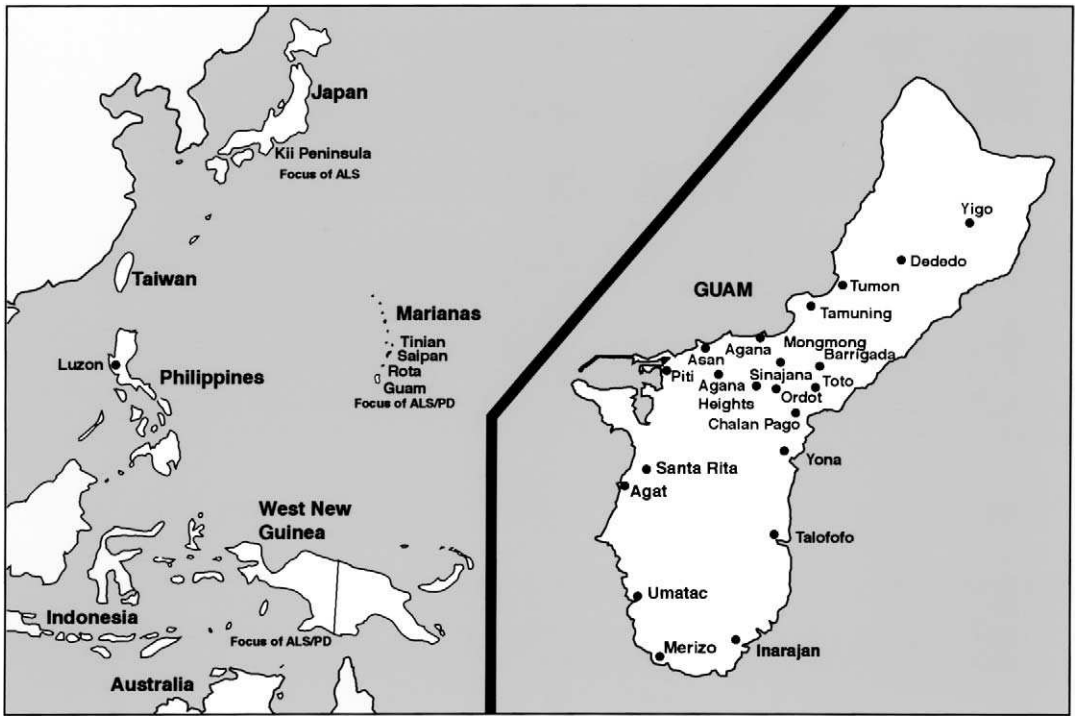

FIG. 15.1. Map of the Western Pacific demonstrating Guam and the location of other foci of neurodegeneration on the Kii Peninsula in Japan and in the Irian Jaya in southwestern New Guinea. Insert shows the island of Guam.

particular, its etiologic basis. This commitment evolved into the establishment of an NIH-associated research station, which was maintained on Guam for approximately 30 years by the National Institute for Neurological and Communication Diseases and Stroke (NINCDS). This research station was eventually abandoned in 1984, but efforts to unravel the etiology of this remarkable focus of age-related neurodegenerative disease continue to this day.

Initial research studies on the island supported the observations of Dr. Zimmerman, namely that there was a high prevalence of amyotrophic lateral sclerosis on Guam (Arnold *et al*., 1953). However, in the process of doing door-to-door surveys of all neurologic diseases on the island, it was soon noted that a second form of neurodegenerative disease was also highly prevalent among the native Guamanian Chamorros. This disorder had components of parkinsonism and was accompanied by relatively severe dementia, reminiscent of Alzheimer's disease, and was subsequently called parkinsonism–dementia complex of Guam. It was later recognized that both ALS and parkinsonism–dementia complex had been present on Guam for many years prior to its ''discovery'' by Dr. Zimmerman. At the time of Dr. Zimmerman's stay on Guam, both ALS and parkinsonism–dementia complex were referred to by the Chamorros by specific terms in their native Chamorro language as ''lytico'' and ''bodig,'' respectively. The term ''lytico'' is said to originate from the Spanish ''paralytico,'' referring to the progressive paralysis associated with anterior horn cell degeneration in ALS. The origin of the term ''bodig'' is more obscure, but is defined loosely as ''slow'' or ''lazy'' and refers to the parkinsonism–dementia form of the disorder. Remarkably, archival death certificate records maintained by U.S. Navy personnel dated from the first decade of the 20th century survived the extensive destruction of facilities on Guam during the hostilities of World War II. These meticulously detailed records carefully document virtually every adult death from that era and contain numerous entries with diagnoses of amyotrophic lateral sclerosis, as well as other entries with ''presenile degeneration of the brain and spinal cord.'' Furthermore, folklore accounts suggest that the disease had been present on Guam at least as early as the beginning of the 19th century. It is clear that whatever causes this remarkable outbreak of neurodegenerative disease has been present on Guam for hundreds of years and preceded the arrival of Western industrialized society to the island.

II. Clinical Features

Both ALS and parkinsonism–dementia complex of Guam were initially described as separate and distinct clinical entities, which were highly endemic among the native Chamorro population living on Guam. Clinical and epidemiologic approaches to the study of neurologic disease on Guam have attempted to consider them as two different nosologic entities. Nevertheless, as will be discussed later, there is a considerable amount of evidence, both clinical and neuropathologic, for overlap between the two diseases. Many researchers with extensive experience in the clinical and neuropathologic evaluation of Guam cases have noted such overlap and suggested that this may actually represent a single disorder with a broad spectrum of neurodegeneration, ranging

from relatively ''pure'' ALS to parkinsonism with dementia to an exclusively dementing form of the illness. These concepts will be discussed further later. Nevertheless, the three major forms, namely ALS, parkinsonism–dementia, and so-called ''Marianas dementia,'' will be described separately.

III. Amyotrophic Lateral Sclerosis of Guam (or the Marianas Form of ALS)

Initial observations indicated that Guamanian patients with ALS were clinically indistinguishable from patients encountered with the sporadic form of the disorder elsewhere in the world (Kurland and Mulder, 1954; Kurland *et al*., 1956). On Guam, the disease typically begins in an insidious fashion with complaints of weakness, clumsiness, and/or unexplained weight loss. Early signs include muscle weakness, atrophy accompanied by widespread fasciculations, and hyperreflexia. The disease progresses inexorably, and within a year or more, prominent muscular atrophy, weakness, and a flaccid paralysis are present. In the absence of identifiable muscular wasting, spasticity is seen at presentation in approximately 10–15% of cases. In later stages of the illness, prominent dysphagia and dysarthria are noted as the level of paralysis proceeds in an ascending fashion. As will be discussed later, despite the fact that such patients show a rather widespread and extensive distribution of neurofibrillary tangle (NFT) formation, dementia has been noted clinically in only about 10% of ALS cases seen on Guam. Death is typically produced by aspiration and/or hypostatic pneumonia. Affected individuals generally survive for approximately 2–4 years following initial diagnosis. However, occasional patients are seen with more rapid progression of the illness, whereas others are encountered with unusually prolonged periods of survival (Uebayashi, 1984; Rogers-Johnson *et al*., 1986). Those patients with prolonged survival typically progress in a slower fashion and their disease progression may actually plateau without further deterioration in neurologic status over a period of many years. Indeed, rare individuals with the Guamanian form of ALS have been followed clinically for over 15 or more years and the diagnosis has been verified neuropathologically at autopsy.

IV. Parkinsonism–Dementia Complex of Guam

The parkinsonism–dementia complex of Guam was first recognized clinically by Kurland and Mulder and was subsequently defined as a clinicopathologic entity in the seminal papers by Hirano and colleagues (1961a,b). An earlier account of the disorder was apparently published in 1936 (Yase *et al*., 1978). The major features of the disease include parkinsonism in association with a progressive loss of cognitive function. The parkinsonian features include rigidity, bradykinesia, and gait disturbance. A 4–7 Hz pill-rolling tremor at rest of the hands may be noted in some patients, but is rarely incapacitating. A large percentage of patients also show a form of impairment of ocular motility that is reminiscent of patients with progressive supranuclear palsy (Lepore *et al*., 1988). As the disease progresses, bradykinesia and rigidity increase in severity and become a major cause of disability.

The dementia that accompanies the disorder includes severe memory impairment, disorientation, and difficulty with reasoning and simple calculations. In about 30% of cases, the dementia represents the presenting symptom with the parkinsonian features being encountered later in the course of the disease (Elizan *et al*., 1966). Personality changes, in the form of agitation, apathy, irritability, and, in some cases, aggressiveness, may be encountered in the course of the illness and have been noted in approximately one-third of cases. This is an unremittingly progressive and invariably fatal disorder that typically leads to death within 4–6 years after the initial diagnosis. Although initial therapy with levodopa may produce some mild improvement in the parkinsonian features (Schnur *et al*., 1971), eventually the Guam patients soon become refractory to its effects.

V. Marianas Dementia

In recent years, door-to-door surveys of neurologic disease on Guam have identified a significant number of Chamorros with dementia, in the absence of overt accompanying signs of amyotrophy or extrapyramidal disturbances. Such individuals have been mostly women, living in the southern villages of the island and in their early to mid-60s. Several such patients have now been followed longitudinally, and although some have gone on to develop parkinsonian features, others have shown a progressive course with progressive cognitive loss in the absence of any superimposed parkinsonian signs or motor weakness. We have tentatively referred to this entity as ''Marianas dementia,'' pending more detailed clinical, neuropsychological, and neuropathological characterization. A limited number of autopsies have been performed on such patients, and neuropathologic examination has revealed the typical changes associated with ALS/parkinsonism–dementia complex (Perl *et al*., 1994). These fascinating cases suggest that ''lytico-bodig'' may also have an onset and clinical course characterized by a progressive dementing syndrome, in the absence of amyotrophy and/or parkinsonism, and that this may represent an additional clinical profile of this disorder.

VI. Neuropathologic Features

A. Grossly Visible Features

The brains of patients with both ALS and parkinsonism–dementia complex almost invariably show prominent generalized cerebral atrophy accompanied by severely dilated lateral ventricles. The brain weight of severely affected end-stage cases is frequently less than 1000 g, and brains that weigh less than 900 g are also noted occasionally. In patients with parkinsonism–dementia complex, normally pigmented nuclei of the brain stem, namely the substantia nigra and locus ceruleus, are virtually completely depigmented on gross inspection (Fig. 15.2). Patients with ALS may show discoloration of the lateral columns of the spinal cord that is apparent

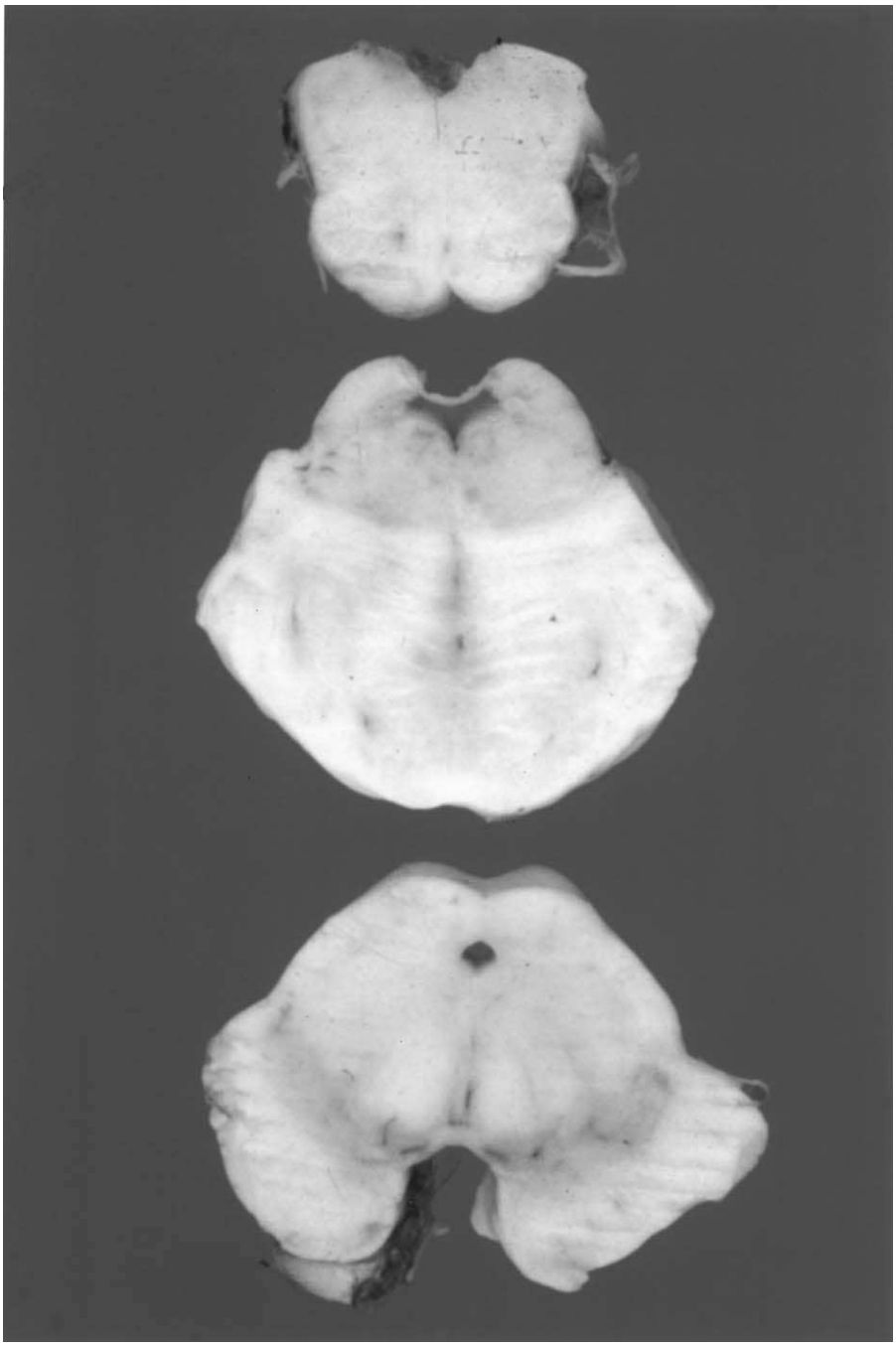

FIG. 15.2. Gross appearance of the upper brain stem of a case of parkinsonism–dementia complex of Guam. Note the lack of pigmentation of the substantia nigra.

with the naked eye. Prominent atrophy of the ventral spinal roots is also recognized readily.

VII. Microscopic Features

A. Neurofibrillary Tangles

The neuropathologic hallmark of both the Guamanian form of ALS and parkinsonism–dementia complex is the presence of severe widespread NFT formation. In the initial neuropathologic examination of cases of ALS from Guam, these NFTs failed to be identified. It was Dr. Nathan Malamud, a neuropathologist at the University of California, San Francisco, who first noted NFTs in the Guam ALS cases (Malamud *et al.*, 1961). These lesions were characterized further in the seminal papers of Hirano and colleagues (Hirano *et al.*, 1961a,b, 1967b; Hirano and Zimmerman, 1962). Severe NFT involvement is typically encountered in the hippocampus, entorhinal cortex, amygdala, and neocortex. In many cases, the

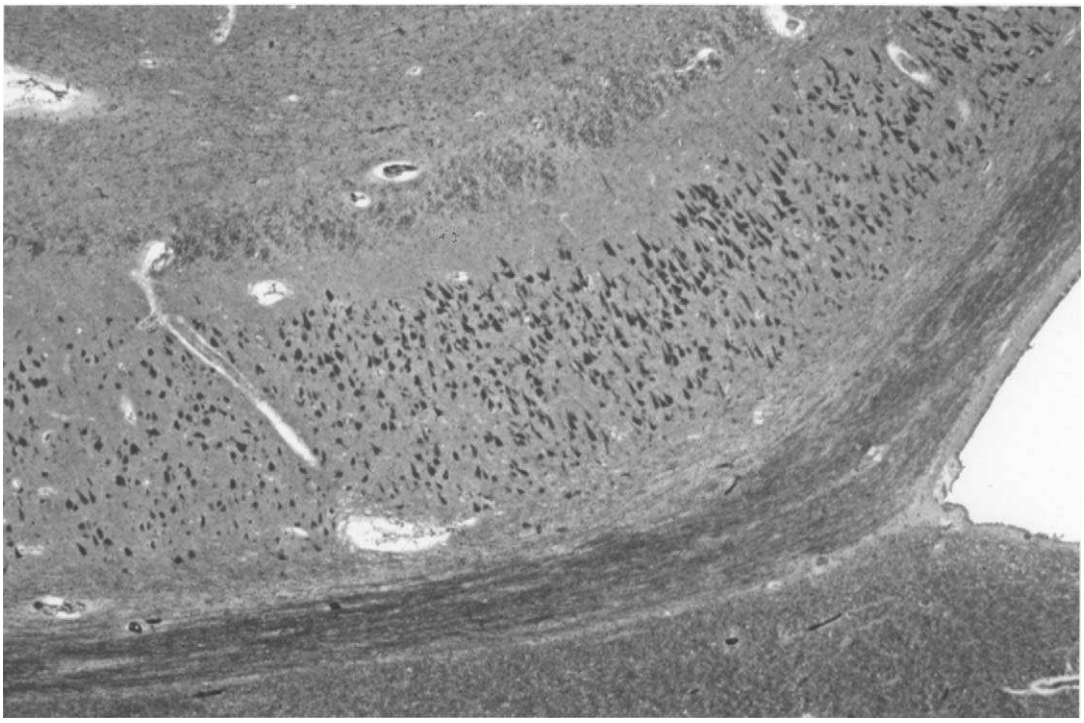

FIG. 15.3. Microscopic appearance of the CA1 region of the hippocampus, parkinsonism–dementia complex of Guam showing virtual complete involvement by neurofibrillary tangles. (Modified Bielschowski stain.)

CA1 region of the hippocampus will show almost complete involvement with virtually every pyramidal neuron containing a neurofibrillary tangle (Fig. 15.3). When NFT formation is particularly severe in the hippocampus, it is generally accompanied by extensive neuronal loss and the appearance of many extracellular ''ghost'' tangles as well as a prominent reactive astrocytic response.

It is interesting to note that patients with Guamanian ALS may show rather extensive hippocampal NFT formation at autopsy, yet were considered to have essentially intact cognitive function during life. It should be noted that in the later stages of ALS, such patients may have exhibited severe dysphonia, bordering on aphonia, and that this inherent inability to communicate may clearly preclude the proper assessment of cognitive function. Nevertheless, such patients do appear, within their physical limitations, to be able to follow and carry out verbal commands. Such observations appear to suggest that severe involvement of the CA1 of the hippocampus by NFTs may not be associated with severe cognitive losses, particularly involving recent memory processing, as has been assumed to be operative in cases of Alzheimer's disease (AD).

In patients with marked parkinsonian features, extensive NFT formation is noted in brain stem regions, including the periaqueductal gray matter, substantia nigra, and locus coeruleus. In such regions the NFT takes on a globoid appearance, reflecting the rounded shape of the soma, as opposed to the flame-shaped configuration taken by NFTs within pyramidal neurons (Fig. 15.4). Cases with particularly severe NFT formation may display such lesions in the dentate nucleus of the cerebellum (Fig. 15.5), inferior olivary nuclei, and even anterior horn cells in the spinal cord.

Immunohistochemical and electron microscopic studies have shown the NFTs encountered in patients with ALS/parkinsonism–dementia complex to be virtually identical to those encountered in cases of AD seen elsewhere in the world (Hirano *et al*., 1968; Hirano, 1973; Shankar *et al*., 1989). Ultrastructural studies have shown that while the NFTs of the Guam cases contain paired-helical filaments, they commonly also show additional straight filaments. NFTs react with antibodies directed against tau protein (Buée-Scherrer *et al*., 1995a,b), apolipoprotein E (Buée *et al*., 1996), lactotransferrin (Leveugle *et al*., 1994), β-amyloid peptide (Ito *et al*., 1991), and ubiquitin, all of which are constituents of NFTs in AD cases. Buée-Scherrer and co-workers (1995a,b) demonstrated that PHF-tau derived from cases of ALS/parkinsonism–dementia complex are abnormally phosphorylated and have biochemical and immunological properties that are identical to the PHF-tau of AD. Studies have shown that the PHF-tau associated with ALS/parkinsonism–dementia complex is phosphorylated at the identical amino acid residues as is the PHF-tau of AD (Mawal-Dewan *et al*., 1996).

We have shown (Hof *et al*., 1991; 1994b) that the pattern of neocortical involvement by NFTs in cases of ALS/parkinsonism–dementia complex differs significantly from what is seen in cases of Alzheimer's disease. In cases of ALS/parkinsonism–dementia complex, NFTs of the neocortex are situated most densely in layers II–III when compared to the extent of involvement in layer V (Fig. 15.6). This pattern of involvement differs distinctly from what is seen in cases of AD, where NFT formation is more severe in the infragranular layer (layer V), compared to more superficial aspects of the neocortex (layers II–III). The pattern of neocortical NFT formation demonstrated in cases of ALS/parkinsonism–dementia complex with layer

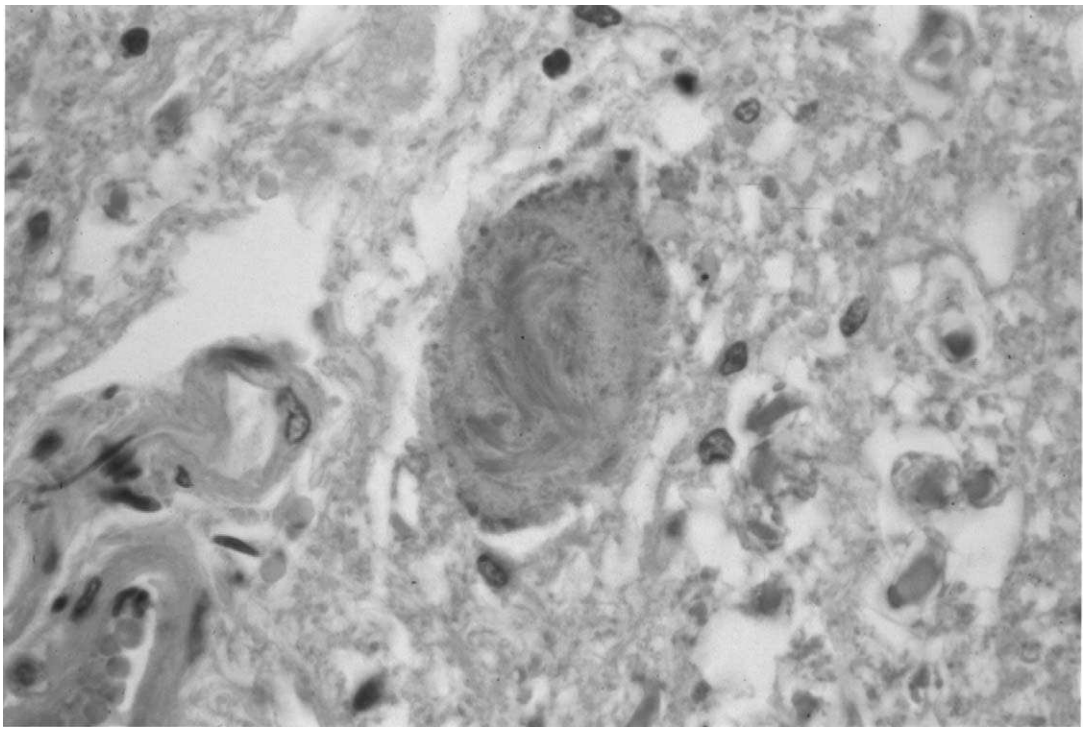

FIG. 15.4. Microscopic appearance of globoid neurofibrillary tangle in pigmented neuron of the substantia nigra, parkinsonism–dementia complex of Guam. (Hematoxylin and eosin stain.)

II–III predominance is also a characteristic feature of cases of postencephalitic parkinsonism (Hof *et al*., 1992b), posttraumatic dementia (dementia pugilistica) (Hof *et al*., 1992c), and progressive supranuclear palsy (Hof *et al*., 1992a). As in cases of AD, in ALS/parkinsonism–dementia complex those neurons containing the calcium-binding proteins calbindin, calretinin, and parvalbumin tend to be spared from involvement in NFT formation (Hof *et al*., 1994a).

FIG. 15.5. Microscopic appearance of neurofibrillary tangle in neuron of dentate nucleus of cerebellum, parkinsonism–dementia complex of Guam. (Modified Bielschowski stain.)

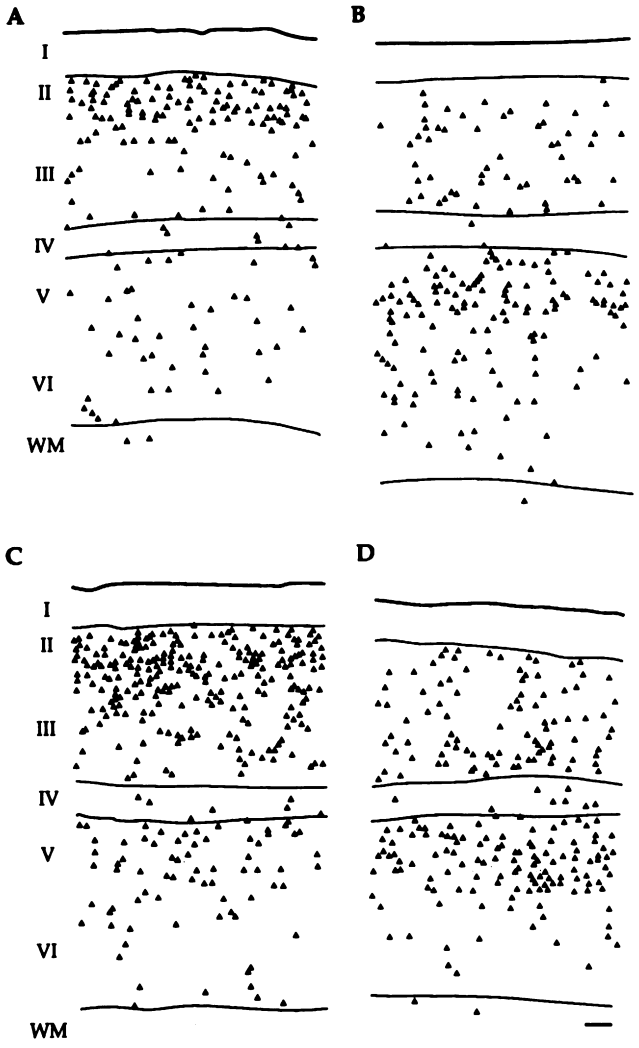

FIG. 15.6. Computer-generated maps of NFT distribution in a case of parkinsonism–dementia complex of Guam (A,C) and an Alzheimer's disease case (B,D). Maps A and B represent area 9 (prefrontal cortex) and maps C and D represent area 21 (mid-temporal gyrus). Note the difference in NFT (triangles) distribution within layers II–III and V–VI between the cases. Cortical layers are indicated by Roman numerals. Scale bar = 100 μm.

VIII. β-Amyloid Accumulation in ALS/Parkinsonism–Dementia Complex of Guam

In the classic neuropathologic descriptions of ALS and parkinsonism–dementia complex, Malamud and Hirano specifically noted that despite the presence of large numbers of NFTs, there was a distinct lack of senile plaque formation. Indeed, they noted "there is no senile plaque formation, although occasionally there are a few faintly argyrophylic coagulated structures, resembling early senile plaques" (Hirano *et al*., 1961b) and "another interesting feature was the presence of senile plaques in the hippocampus in 3 of the 22 cases [of Guam ALS], but even in these cases only 2 or 3 plaques were observed per slide" (Malamud *et al*., 1961). Guam cases that had been considered by classic neuropathologic staining techniques to be senile plaque free were studied by immunohistochemistry and remained negative for evidence of parenchymal amyloid accumulation, despite the use of formic acid pretreatment (Gentleman *et al*., 1991). However, about 20% of more recently autopsied cases indicated the presence of some degree of parenchymal amyloid accumulations. These deposits are mostly in the form of diffuse plaques and typically lack accompanying dystrophic neuritic changes. While it is true that neuritic plaques may be encountered in some cases of ALS/parkinsonism–dementia complex of Guam, in such cases the density of plaques seen is typically small. These cases should not be interpreted as representing examples of Alzheimer's disease among the native Chamorro population as the pattern of neocortical and brain stem NFT formation follows that of the Guam disorder and not AD.

In our experience, amyloid deposition in association with cerebral blood vessels is also extremely uncommon in Guamanian brain specimens. When it is present, these vascular deposits are generally quite scanty. The explanation for this observation remains unclear. Studies of cases of ALS/parkinsonism–dementia complex have failed to show evidence of mutations in exons 16 and 17 of the amyloid precursor protein (sites that have previously been associated with cases of familial AD) (Chartier-Harlin *et al*., 1993) or of tau (Pérez-Tur *et al*., 1999). Studies of 12 cases of ALS/parkinsonism–dementia complex showed an $\epsilon 3$ allele frequency of 91.7%, as opposed to 79.2% for 12 Guamanian controls (Waring *et al*., 1994). Only one patient and one control had an $\epsilon 3/\epsilon 4$ genotype and these represented the only $\epsilon 4$ isoforms identified in this study. Comparable data were obtained in another study (Buée *et al*., 1996). Although these data are scant, this study suggests that the apolipoprotein E phenotype does not represent a significant predisposing factor in the development of neurodegenerative disease among the at-risk Chamorro population, nor does it explain the distinct absence of cerebral amyloid deposits.

IX. Hirano Bodies (Eosinophilic Rod-like Inclusions)

In Hirano's original descriptions of the neuropathologic features of ALS/parkinsonism–dementia complex, he noted the presence of numerous eosinophilic rod-like inclusion bodies in the CA1 region of the hippocampus (Fig. 15.7). These prominent inclusions, encountered immediately adjacent to the pyramidal neurons, had apparently not been described previously and were initially considered to be unique and specific for the Guam disease. Subsequently, Hirano noted the presence of identical inclusions in the hippocampus of cases of AD, Pick's disease, and Creutzfeldt–Jakob disease. When seen in these settings, the number of such inclusions is typically less than is usually encountered in Guam cases. Rare examples of the rod-like inclusions may also be encountered in the brains of elderly nondemented controls elsewhere in the world (Gibson and Tomlinson, 1977; Hirano, 1994).

It appears that over the decades countless neuropathologists had examined the brains of numerous cases of AD but had failed to recognize these rather prominent inclusion bodies, although it must be noted that in most cases they are present in

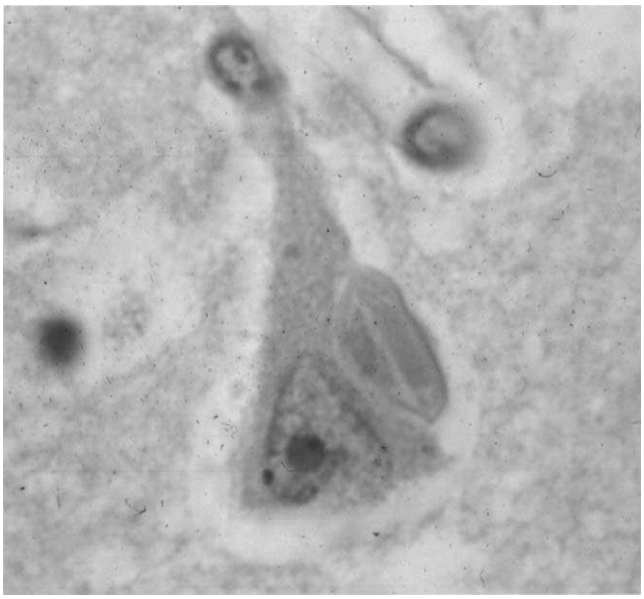

FIG. 15.7. Microscopic appearance of eosinophilic rod-like inclusion (Hirano body), hippocampus, parkinsonism–dementia complex of Guam. (Hematoxylin and eosin stain.)

relatively small numbers. However, when Hirano was presented with numerous examples of such inclusions in the specimens examined on Guam, they were readily noted and subsequently began to be characterized. Using their high density in Guam-derived cases, Hirano described the ultrastructural appearance of these eosinophilic rod-like inclusions, demonstrating their unique ''herring bone.'' Using immunohistochemical approaches, it has subsequently been shown that they contain actin and actin-associated proteins, tau (Goldman, 1983; Galloway *et al*., 1987a,b), and the middle molecular weight neurofilament protein (Schmidt *et al*., 1989b). These eosinophilic rod-like inclusions are now referred to by most neuropathologists as ''Hirano bodies'' in honor of his contributions in first characterizing this lesion. The recognition of the Hirano body in cases of Guam ALS/parkinsonism–dementia complex represents an example of how, through the presence of such a high density of lesions in such cases, important insights have been gained in our understanding of AD, and its related conditions.

X. Granulovacuolar Degeneration

Granulovacuolar degeneration is a poorly understood alteration encountered in the hippocampus of cases of AD. It consists of multiple vacuoles, each with a single internal deeply basophilic granule, which are located in the perikaryal cytoplasm of pyramidal neurons in the boundary zone of the CA1 and CA2 regions of the hippocampus. They are seen in small numbers in the elderly in association with normal cognitive function and, when present in relatively large numbers, especially in the posterior portion of the hippocampus, correlate highly with a diagnosis of AD (Tomlinson and Kitchener, 1972; Ball and Lo, 1977). Granulovacuolar degeneration may be extremely prominent in the hippocampus of cases of ALS/parkinsonism–dementia complex. In affected cases, one sees large numbers of involved neurons, and although there are no specific quantitative data, within each involved neuron the number of vacuoles present appears more numerous and the size of the individual granules appears larger. Hirano again took advantage of the extent of involvement by granulovacuolar degeneration to use Guamanian tissues to demonstrate for the first time the ultrastructural appearance of this lesion (Hirano *et al*., 1968).

XI. Other Features

In cases associated with prominent parkinsonian clinical manifestations, the degree of cell loss in the substantia nigra and locus ceruleus can be almost complete. This is accompanied by evidence of incontinent neuromelanin pigment as well as examples of phagocytosed neuromelanin granules by local macrophages and a significant astrocytic gliosis. The few remaining pigmented neurons will typically contain globoid forms of NFTs. The degree of nigral degeneration can be so severe in end-stage cases with prolonged survival that it may be difficult to identify any remaining intact neurons. In general, Lewy bodies are not encountered in cases of parkinsonism–dementia complex. However, in a large retrospective study, approximately 10% of Guam parkinsonism cases did show evidence of Lewy body formation in either the substantia nigra or the locus coeruleus. Our experience in examining many Guamanian cases is quite similar. However, it should be noted that when Lewy bodies are identified in the Guam cases, they are usually few in number and other pigmented neurons will always show evidence of the classic globoid NFTs. In the Guam cases with Lewy bodies, these inclusions are immunoreactive to ubiquitin and α-synuclein. Despite complete autopsy examination of virtually all patients from Guam who had suffered from parkinsonian symptoms over a period of over 40 years, examples of Lewy body Parkinson's disease are extremely rare. Indeed, the author is aware of only a single such case being documented at autopsy in a Chamorro native from Guam. The explanation for this absence of what is a relatively common disorder elsewhere in the world remains unknown.

Neuropil threads, a prominent feature of AD are relatively sparse in cases of ALS/parkinsonism–dementia complex (Wakayama *et al*., 1993). However, similar thread-like structures have been reported in the white matter of the spinal cord, especially in patients who exhibit more prominent amyotrophic features (Umahara *et al*., 1994).

Using antibodies raised against the protein core of heparin sulfate proteoglycans, severe morphologic abnormalities of the cerebral microvasculature were noted in brain specimens of parkinsonism–dementia complex patients (Buée *et al*., 1994). The distribution of these vascular lesions suggested that there is an association between microvascular abnormalities and a high density of NFT formation. Similar lesions have been seen in cases of AD, where they were thought to be related to sites of vascular and/or parenchymal β-amyloid deposition. The Guam cases, with their typical absence of amyloid deposition,

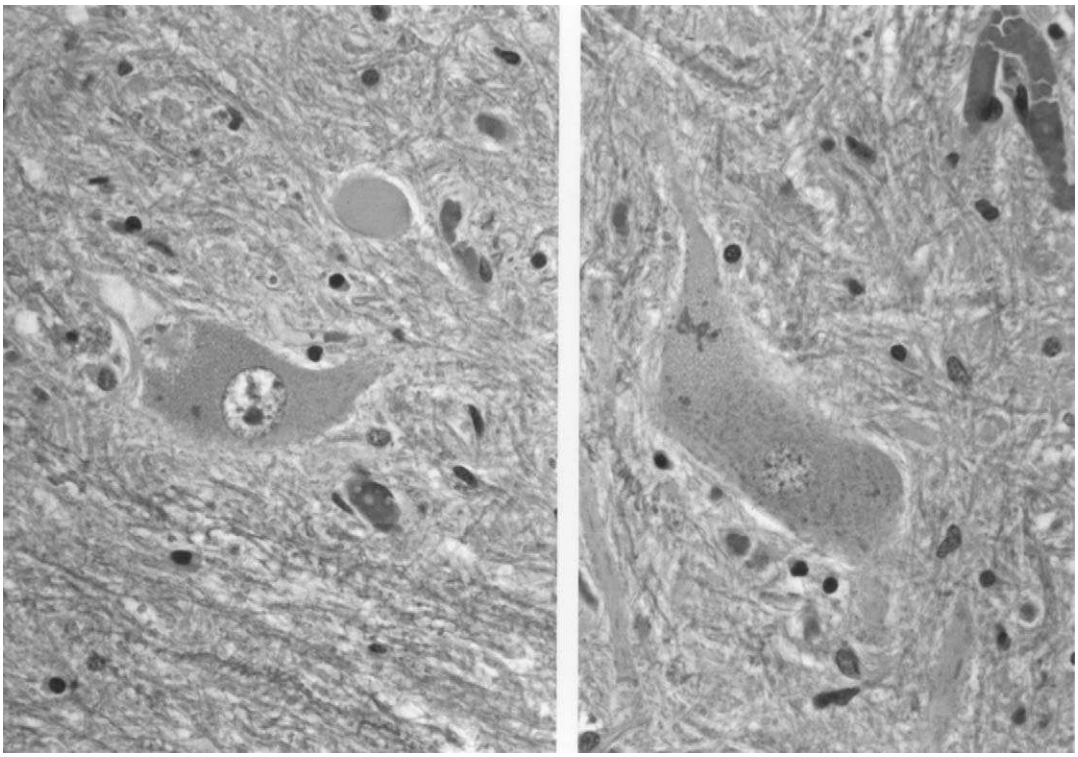

FIG. 15.8. Microscopic appearance of eosinophilic cytoplasmic inclusions (Bunina bodies), anterior horn cells, amyotrophic lateral sclerosis of Guam. (Hematoxylin and eosin stain.)

provided an opportunity to demonstrate that amyloid deposition was not required for the formation of these vascular abnormalities.

In cases of Guamanian ALS there is typically a prominent loss of anterior horn cells. The remaining anterior horn cells may be shrunken, often with pyknotic nuclei, or else swollen with perikaryal skein-like accumulations of ubiquitinated phosphorylated neurofilaments. As in classic ALS, the intervening anterior horn cells may also have a normal histologic appearance. In many Guam patients the remaining anterior horn cells may display small eosinophilic inclusions in their cytoplasm, which were first described by Bunina (1962) (Fig. 15.8). These are frequently referred to as "Bunina bodies." Similar to cases of sporadic ALS seen elsewhere in the world (Mourelatos *et al*., 1990), Guam ALS cases show fragmentation of the Golgi apparatus (Mourelatos *et al*., 1994). Upper motor neuron involvement is virtually always present in the Guam cases and results in prominent myelin loss in both lateral and anterior corticospinal tracts (Fig. 15.9). In contrast to many cases of familial ALS (Hirano *et al*., 1967a), the posterior columns of the cases of Guam ALS remain intact.

XII. Neuropathologic Studies of Neurologically Intact Guamanian Chamorros

In 1979, Anderson and co-workers reported the results of a study in which they sought to examine neuropathologically a number of Guamanian Chamorros who had died and come to autopsy but during life were considered to be free of evidence of neurologic dysfunction. The study included a total of 69 individuals, and their premortem clinical status was based on a review of available clinical records and, when possible, interviews with surviving relatives. This study revealed that 29% of those who died between the ages of 30 and 40 years and 40% of those dying between 40 and 50 years showed significant numbers of NFTs in the hippocampus and neocortex. The authors presumed those Guamanians who showed evidence of premature NFT formation were in a preclinical stage of ALS/parkinsonism–dementia complex but had not accumulated a sufficient burden of lesions for symptoms to be detected clinically. Because, at the time, ALS/parkinsonism–dementia complex was responsible for 10–20% of adult deaths and because it is likely that the disease takes many years to develop fully, the finding of relatively large numbers of individuals in the early stages of the disorder is not unexpected. Nevertheless, the extent of the problem that this study uncovered is striking. Using a similar strategy, Chen (1981) reported similar data by examining asymptomatic cases seen in the Guam medical examiner's office.

Studies of this type suggest that the brain has a considerable capacity to compensate for the accumulation of significant numbers of NFTs without the patient demonstrating overt symptomatology. Indeed, the Anderson study is remarkable, as some of the patients showed a striking density of NFTs, yet appeared to be free of discernible functional impairment. However, it should be recognized that the subjects reported in this study had not undergone detailed neurologic or

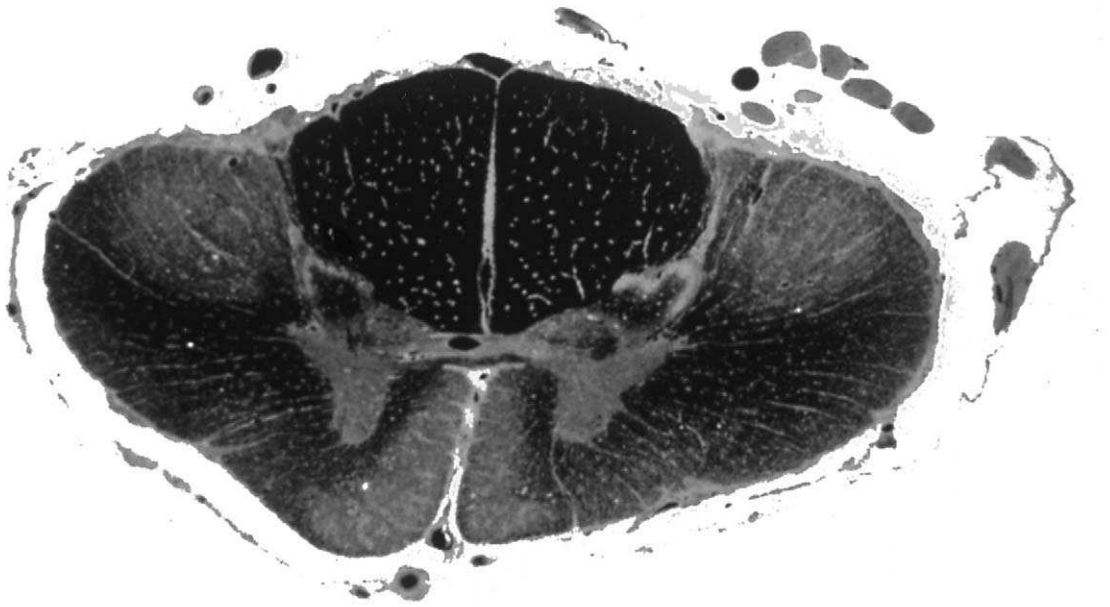

FIG. 15.9. Microscopic appearance of spinal cord showing prominent demyelination of lateral and anterior corticospinal tracts, amyotrophic lateral sclerosis of Guam. (Myelin stain.)

neuropsychological evaluation, and deficits in functioning could have been overlooked by their treating physicians and family members.

Snow and colleagues (1990) reported an important study regarding the functional integrity of the striatonigral dopaminergic system among Guamanian Chamorros. They used positron emission tomography (PET) with ^{18}F-6-fluorodopa on Guamanian subjects with both ALS/parkinsonism–dementia complex and Guam controls. This technique allows visualization and quantification of the integrity of the dopaminergic input into the striatum in living patients. With this approach they were able to assess the relative extent of damage to the substantia nigral neurons on these subjects. Parkinsonism–dementia complex cases showed a profoundly decreased fluorodopa uptake consistent with postmortem studies. Interestingly, of the eight normal Chamorro controls, two demonstrated reduced fluorodopa uptake. The degree of impaired fluorodopa uptake in these two subjects was intermediate between what was found in the parkinsonism–dementia complex cases and that of normal controls from Vancouver, British Columbia. On neurologic examination, these two individuals showed no observable extrapyramidal features and were considered to be in a preclinical phase of parkinsonism–dementia complex. The authors of the study anticipated that these two subjects would, sometime in the future, develop overt parkinsonian symptoms. At present, no follow-up data are available to determine if that prediction came true.

Finally, we have performed a study that was similar to that performed by Anderson in which we evaluated brains of Guamanian Chamorros who had died and come to autopsy, yet were free of a diagnosis of ALS/parkinsonism–dementia complex. In our study we also noted a substantial number of relatively young individuals who showed evidence of widespread NFT formation. In some cases the extent of involvement was quite striking. The pattern of involvement by NFTs paralleled that encountered in diagnosed cases of ALS/parkinsonism–dementia complex, including predominant neocortical involvement in layers II–III as opposed to layer V. Furthermore, it was noted that the more severe the NFT involvement, the more closely the pattern of NFTs approached that of the diagnosed disorder. Accordingly, we concluded that such cases did indeed represent examples of an early phase of the disorder and that the extent of lesion formation had not been sufficient to attract clinical attention and diagnosis. These findings suggest that the extent of ALS/parkinsonism–dementia complex among the Chamorro population on Guam represents a more pervasive problem than have been recognized by clinical/epidemiologic survey conducted over the years.

XIII. Epidemiology

Extensive epidemiologic surveys of neurologic disease among the inhabitants of Guam and its neighboring islands have been conducted over the years since the initial studies of Kurland and Mulder in the 1950s. These studies established that the incidence of ALS on Guam was 50 to 100 times greater than is seen in the continental United States. A similarly high incidence of the disease has been noted in the next nearest island to Guam, Rota. Rota, a much smaller and

more isolated island, is populated by approximately 1500 Chamorros. For the period 1947–1953, the average annual incidence rate for Chamorros living on Guam per 100,000 population was twice as high for men when compared to women (85 and 40, respectively) with a mean age of onset of 44 years in both sexes. ALS accounted for approximately 10% of all deaths on the island, a figure that was 50 times greater than reported for the continental United States. Subsequent studies for the period 1945–1972 indicated a steady decline in the age-adjusted incidence rate for ALS on Guam. These data included rates for men of 67/100,000 population in 1950–1954 to 21/100,000 in 1970–1972 and of 43 and 12 for women. By 1980–1982, Garruto and co-workers (1985) found the age-adjusted incidence rates to be less than 5/100,000 for both men and women. A study (Waring, 1994) reviewing data from 1954 to 1989 showed that the median age of onset of ALS among Guamanian Chamorros increased from 48 years to 52 years in men and from 42 years to 52 years in women. Furthermore, comparing the annual age-adjusted incidence of ALS per 100,000 for 1950–1954 to 1985–1989, the rates for men decreased from 65 to 10 and for women from 30 to 4. The decrease for both sexes together was 47/100,000 population to 7.

In 1961, with the identification of parkinsonism–dementia complex of Guam as a distinct clinicopathologic entity, epidemiologic studies were also initiated for this condition. This disorder was initially considered to be a form of post-encephalitic parkinsonism, possibly related to prior exposure to Japanese B encephalitis. An outbreak of Japanese B encephalitis had occurred on Guam in 1947–1948, but none of the initially reported cases of parkinsonism–dementia complex had reported a prior history of this form of infection. In 1962, Lessel and colleagues reported a prevalence for parkinsonism–dementia complex of 118/100,000 with a mean age of onset of 50 years and a male/female ratio of 2.5:1. The average annual mortality rate for the condition was 28/100,000 with a median survival of 3.5 years. Based on door-to-door surveys, Reed *et al*. (1966) reported the average annual incidence per 100,000 population for parkinsonism–dementia complex to be 33 for men and 6 for women. Again, in 1985, Garruto *et al*. reported a significant decline in the age-adjusted incidence rates for men and women with parkinsonism–dementia complex. Based on these reports, it was widely reported that the neurodegenerative diseases on Guam were going away and that before long the unique opportunity to identify important etiologic clues, which might represent avenues for understanding comparable diseases elsewhere in the world, would be completely lost (Stone, 1993). In part, based on the concept that the disease had all but disappeared from the at-risk population, the NINCDS research station on Guam was closed.

Nevertheless, in 1990 a small group of committed scientists obtained ongoing funding from the National Institute on Aging to continue studies on Guam. From this began the current Micronesian Health Study Registry, directed by the late Dr. W. C. Wiederholt. Preliminary data from this registry (Wiederholt, 1999) indicate that parkinsonism–dementia complex is not disappearing and during 1980–1989 there was an average annual incidence of 20 cases/100,000 population. Of interest is that the age of onset of this disorder had increased to 59 years in both men and women.

XIV. Overlap between ALS and Parkinsonism–Dementia Complex of Guam: One Disorder or Two?

In the initial clinical and neuropathologic descriptions, ALS and parkinsonism–dementia complex of Guam were considered to be separate and distinct nosologic entities. This concept has been retained by virtually all subsequent epidemiologic studies. Despite this, there has been a substantial amount of clinical and neuropathologic evidence that demonstrates extensive overlap between the two forms of the disease. This evidence has suggested to us (Perl, 1994) that the two forms may simply be extremes of a relatively wide clinicopathologic spectrum within what is actually a single disease entity.

It is rare for cases of ALS of Guam to show evidence of parkinsonian signs on neurologic evaluation. In 1966, Elizan and colleagues reported that only 5 of 104 cases of Guam ALS subsequently developed a definite clinical picture of parkinsonism–dementia complex in the course of their disease. In an additional 5 patients, frank parkinsonian features were identified clinically, but these were seen in the absence of any notable cognitive decline. In an extensive retrospective review, Rogers-Johnson *et al*. (1986) reported that in patients diagnosed with ALS, minimal extrapyramidal features were seen in only 5% and dementia noted in only 4% of cases.

Despite these clinical observations, ^{18}F-6-fluorodopa PET scan studies of four patients with ALS of Guam (without clinical evidence of parkinsonism) showed evidence of decreased striatal fluorodopa uptake (Snow *et al*., 1990). The degree of fluorodopa uptake loss was intermediate between that seen in parkinsonism–dementia complex of Guam cases and normal control levels. Although the number of subjects studied was small, this study suggests that Guam cases of ALS can have a significant degree of substantia nigral cell loss and yet still be free of parkinsonian signs or symptoms.

Neuropathologic studies have provided perhaps the clearest evidence of involvement of the nigrostriatal system in cases of Guamanian ALS. In the initial neuropathologic description of the Guam form of ALS, Malamud *et al*. (1961) reported widespread evidence of NFT formation. In this and subsequent reports (Hirano, and Zimmerman, 1962; Hirano *et al*., 1967b), evidence of NFT formation in the substantia nigra, locus ceruleus, and other brain stem nuclei in cases of Guam ALS were clearly demonstrated. In their review of the neuropathologic features in 209 autopsies carried out on Guam ALS cases, Rogers-Johnson and colleagues (1986) identified depigmentation and neuronal loss in the substantia nigra in 63% of cases.

Despite the striking evidence of significant nigral pathology in a majority of Guam ALS cases, why is there so little clinical evidence of accompanying parkinsonian symptomatology in such patients? One possible explanation is that the patient with ALS has at least a partial degree of denervation of the voluntary musculature. If such a patient then loses the integrity of the nigrastriatal pathways, the parkinsonian features may be clinically masked because the musculature has been effectively denervated by the pathology in the motor system. In addition, it is clear that the striatonigral dopaminergic system has a considerable degree of reserve and that the extent of nigral cell loss may not reach a sufficient level to trigger overt

symptomatology. Nevertheless, it is clear from these neuropathologic studies that in cases of ALS from Guam the substantia nigra is affected in a majority of instances.

However, in many cases of parkinsonism–dementia complex, superimposed amyotrophy is observed clinically later in the course of the illness. This was first pointed out by Elizan *et al*. (1966), who reported that 38% of parkinsonism–dementia complex cases subsequently went on to develop clinical manifestations of ALS. Furthermore, 12 cases of what was thought to be ''pure'' parkinsonism–dementia complex, who were without muscular wasting, showed electromyographic evidence of lower motor neuron damage. In data reviewed by Rogers-Johnson *et al*. (1986) of patients with a diagnosis of parkinsonism–dementia complex, 34% had a positive Babinski sign and 32% showed fasciculations and/or muscular atrophy, again suggesting the presence of superimposed upper and lower motor neuron pathology.

In their first neuropathologic report of parkinsonism–dementia complex, Hirano and colleagues (1961b) described evidence of typical morphologic changes of ALS in the spinal cords of 17 of the 48 cases (38%). Rogers-Johnson's review revealed that of 113 autopsies carried out on parkinsonism–dementia complex cases (on whom spinal cord specimens were available for examination), 35% showed neuropathologic evidence of lateral column myelin loss and anterior horn cell degeneration. Unfortunately, in this retrospective review it is unclear if the presence of motor findings in the clinical presentation influenced whether a spinal cord specimen would be taken at autopsy. Nevertheless, the overall agreement of clinical and neuropathologic data would suggest that motor neuron pathology exists in a substantial proportion of cases of parkinsonism–dementia complex.

Based on these findings, it is clear that relatively few ''pure'' cases of either ALS or parkinsonism–dementia complex are actually encountered. Accordingly, we have argued that ALS/parkinsonism-dementia complex of Guam represents a spectrum of clinical manifestations of a single, broad neurodegenerative disorder. Whether an individual patient on Guam is diagnosed with ALS or parkinsonism–dementia complex will be determined by the nature of the earliest clinical signs and symptoms that presumably correlate with the neuroanatomic site of the initiation of the neurodegenerative process. If that process initiates in the motor system, then weakness and muscular atrophy will be noted and a diagnosis of ALS will be made. Alternatively, if the process begins in the substantia nigra, then rigidity and bradykinesia will be noted, warranting a diagnosis of parkinsonism–dementia complex. Finally, if the initial process involves the hippocampus and aspects of the neocortex, then dementia will be the presenting clinical feature. Presumably, as the disease progresses it goes on to involve these other neuroanatomic sites and introduces much of the overlapping symptomatology discussed earlier.

Of interest, Elizan and colleagues (1996) followed a number of cases presenting with dementia and noted that most evolved to develop extrapyramidal signs and symptoms in the course of their illness. Certainly from the perspective of the neuropathologist it is clear that the various clinical categories encountered on Guam reflect manifestations of widespread progressive neurodegenerative process variably affecting upper and lower motor neurons, nigrostriatal dopaminergic neurons, and neurons of the hippocampus and neocortex. What mediates the relative degree of involvement in the early stages of the disease remains unclear, but eventually all of these neuronal systems become involved to some degree. This, to us, suggests a unifying process and a single disorder leading to a wide spectrum of clinical manifestations. If this concept is correct, then this has important implications for nosologic considerations for the analogous age-related diseases seen elsewhere in the world.

XV. Other Foci of ALS/Parkinsonism–Dementia Complex

In addition to the focus of ALS/parkinsonism–dementia complex identified and studied extensively on Guam, there are two other similar foci of endemic neurodegenerative disease that have been identified. These sites are in the Kii peninsula of Japan and among the Auyu and Jakai people of the Irian Jaya region of southwestern New Guinea. Kinnosuke Miura, a pupil of Charcot, published the first clinical description of a case of ALS in Japan (Miura, 1902). He subsequently claimed that ALS was more common among the Japanese than was seen in Europe and that there was an unusually high incidence of the disease on the Kii peninsula, a remote area on the southern coast of the Japanese island of Honshu (Miura, 1911). Subsequent studies indicated that the high prevalence of ALS was confined to two isolated districts in the Kii peninsula, namely Kozagawa and Hobara (Shiraki and Yase, 1975, 1991). Surveys indicated the annual incidence of ALS was 15 cases per 100,000 population in Kozagawa and 55 cases per 100,000 in Hobara.

Neuropathologic studies of ALS cases from the Kii peninsula have been reported by Shiraki and Yase (1975, 1991) and reveal a pattern of neuropathologic changes that is virtually identical to that seen on Guam. The changes documented in their studies have included widespread NFT formation in a pattern that parallels closely what is seen in the Guam cases. Since the 1980s, the number of cases of ALS encountered in Kozagawa and Hobara has diminished dramatically, similar to what has occurred on Guam. Until recently, documented cases of parkinsonism–dementia complex from the Kii peninsula had not been reported. However, Kuzuhara (1999) has now reported that such cases identified clinically and at autopsy show neuropathologic features that are virtually identical to those encountered on Guam.

The New Guinea focus was first identified by Gajdusek (1963), who briefly described a high incidence of ALS based on brief clinical observations while passing through this extremely remote and primitive region. He subsequently returned to the area and surveyed the population more thoroughly for neurologic disease (Gajdusek and Salazar, 1980). Based on his studies, he reported an average annual incidence of ALS for the period 1974–1980 of 147 per 100,000 (almost 150 times greater than what is seen in the continental United States). In his survey, the incidence of parkinsonism, both with and without dementia, was 79 per 100,000 population. The mean age of onset in the population for ALS and parkinsonism was 33 and 43 years, respectively, both significantly younger than is seen on Guam. Unfortunately, although the clinical features of

these patients appear quite similar to the disorder seen on Guam, no neuropathologic studies have ever been carried out on these cases, which reside in an extremely inaccessible region.

XVI. Etiologic Concepts

Investigation into the epidemic of neurodegenerative diseases among the Chamorro population of Guam has, from the earliest observations of Dr. Zimmerman, offered the promise that a better understanding of the underlying etiologic factors responsible for this high-risk focus would provide important insights regarding the three analogous disorders seen elsewhere in the world: ALS, Parkinson's disease, and Alzheimer's disease. From the earliest days, it was assumed that the high incidence of ALS on Guam reflected a genetic abnormality that had become magnified in an inbred, island-bound population. However, numerous studies have shown repeatedly that if genetic factors are present, and they most certainly are, they are not etiologic in nature and likely represent modifying factors influencing the individual's relative susceptibility to an as yet unidentified environmental etiologic agent (or agents). There are many reasons for this statement.

First, and foremost, is the appearance of cases of ALS and parkinsonism–dementia complex among Filipino migrants to Guam with long-term residence on the island. Since the 1950s to the present time there has been a sizable migration of individuals born and raised in the Philippines who have moved permanently to Guam. These immigrants have been mostly male, in search of stable employment, and they now make up a community of approximately 20,000 stable residents on Guam. Many have married native Chamorro women and have adopted, more or less, a Guamanian way of life. Among this Filipino migrant community, a substantial number of cases of ALS have been documented. Reed and Brody (1975) first noted such cases. Subsequently, Garruto *et al*. (1981) clinically documented nine cases of ALS and two with parkinsonism–dementia complex. Virtually all were Filipino men who moved to Guam in the late 1940s and early 1950s. Except for three cases of ALS that had developed within 3 years of arrival on Guam, the onset of disease was at least 13 years after moving to the island. In a small number of autopsies performed on ALS cases among the Filipino migrants, NFTs were documented in brain specimens in 50% of cases. The extent of NFT involvement was not as dramatically widespread as is typically encountered in the native Chamorro cases, but this finding clearly separates them from sporadic ALS, as seen elsewhere in the world, as NFTs are not a prominent neuropathologic feature of ALS, especially among relatively young patients.

Additional cases of ALS continue to be identified among the Filipino migrant community living on Guam. Neuropathologic examinations of these cases show motor neuron degeneration accompanied by widespread NFTs, ensuring that they are not merely incidental cases of sporadic ALS among this population. Importantly, the appearance of cases of parkinsonism–dementia complex among the Filipino migrant population further reinforces the concept that long-term residence on the island underlies the cause of the outbreak of neurodegenerative disease. ALS is a disorder that has been seen throughout the world, yet parkinsonism–dementia complex remains confined to the three foci of the Western Pacific discussed earlier. As mentioned previously, Garruto *et al*. (1981) reported the occurrence of a single case of parkinsonism–dementia complex in a Filipino migrant to Guam; however, this diagnosis was made on clinical grounds and no autopsy was performed on this individual.

We have had the opportunity to examine the brains of two patients with parkinsonism accompanied by dementia that occurred in Filipino migrants following more than 25 years of residence on Guam (Purohit *et al*., 1992). Both cases showed the characteristic neuropathologic features of parkinsonism–dementia complex and were virtually identical to what we commonly encounter in native Chamorro cases. In both, there was widespread, rather severe NFT formation in the hippocampus, entorhinal cortex, and neocortex in the absence of parenchymal amyloid deposition. NFTs were predominant in the superficial cortical layers. There was a severe loss of pigmented neurons in the substantia nigra and locus coeruleus with globoid forms of NFTs being seen in several remaining neurons in these nuclei. Lewy bodies were not encountered in either the pigmented neurons or the neocortex. An additional autopsy-confirmed case of parkinsonism–dementia complex occurring in a Filipino migrant to Guam was reported by Chen *et al*. (1982). These three autopsy-proven cases represent important evidence that ALS/parkinsonism–dementia complex is not a disorder that is unique to the Chamorro population, but may also be seen in Filipinos who have lived on Guam for many years. Unless there is a hitherto unrecognized focus of ALS/parkinsonism–dementia complex in the Philippine islands, then these findings strongly support the concept that long-term exposure to a putative environmental agent on Guam is responsible for the epidemic.

XVII. Genetic Factors

Over the years, researchers have considered a number of environmentally based agents as the possible etiologic cause of the high-risk foci of neurodegenerative disease in the Western Pacific. Nevertheless, a number of genetic factors have been investigated, not so much as sources of the etiology of the disease, but as potential modifying constituents that may alter relative susceptibility to develop the disease. For example, with the discovery of cases of familial ALS elsewhere in the world related to mutations in the Cu/Zn superoxide dismutase gene, this locus was investigated by Figlewicz *et al*. (1994) in ALS/parkinsonism–dementia complex families on Guam. In this setting, they reported no evidence of gene mutations in the Cu/Zn superoxide dismutase gene. Chen *et al*. (2000) reported a higher frequency of G to C mutations in exon 9 of the CYP2D6 gene in Chamorros and Filipinos living on Guam and in San Diego, California, when compared to Caucasians living in the same areas. However, they found an equivalent mutant allele frequency among both parkinsonism–dementia complex patients and healthy Chamorros. As mentioned previously, studies of the amyloid precursor protein for mutations related to familial AD among ALS/parkinsonism–dementia complex patients revealed negative results (Chartier-Harlin *et al*., 1993), as did surveys of the tau gene (Pérez-Tur *et al*., 1999). In summary, to date, despite searching for clues among the most

reasonable candidate genes, no identifiable genetic abnormality appears to segregate with neurodegenerative disease among the Guamanian Chamorro population.

XVIII. Migration Studies of Chamorros

Studies of potential environmental etiologies must take into consideration the likelihood of a considerable period of latency between initial exposure to such an agent and the eventual onset of neurodegenerative disease. Following the granting of full American citizenship to inhabitants of Guam in 1950, they were allowed to travel freely and resettle anywhere they wished in the continental United States. This newly gained freedom prompted an extensive outmigration of Guamanian Chamorros to selected locations along the West Coast. For example, significant communities of island-born Guamanians developed and continue to thrive in Chula Vista, San Jose, and Oakland, California, as well as Bremerton, Washington. In 1957, a survey of 165 adult Guamanians who had migrated to California revealed two patients with ALS, a rate that was equal to that which was then present on Guam (Torres *et al*., 1957). Those involved in the study also learned of five additional cases of ALS among this migrant population who had died prior to the initiation of the survey. In 1966–1967, a follow-up study by Eldridge *et al*. (1969) found that of 321 Guamanians over age 40 who were then living in California, two patients were suffering from ALS and two had parkinsonism–dementia complex. Finally, Garruto *et al*. (1980) conducted perhaps the broadest survey by attempting to identify all Guamanians who had migrated from the island and eventually developed ALS. They identified a total of 28 cases (some of whom were included in the two prior published surveys). There were 21 cases who had developed ALS while living in the continental United States, 3 cases who initiated in other countries (Japan, Germany, and Korea), and 4 who became ill following a return to Guam but only after long-term residence on the United States mainland. Ten of these cases had undergone autopsy studies with confirmatory neuropathologic findings indicative of the Marianas form of the disease (the finding of superimposed NFT formation). In this study, the mean age at migration from Guam was 29.4 years (range: 18 to 63 years) with an average number of years living off Guam prior to the onset of disease of 13.6 years (range: 1 to 34 years). The mean age of onset was 48.8 years, which, at that time, was 4 years younger than was seen among Chamorros who had never left Guam. Some of these cases developed following more than 20 or more years of migrating from Guam.

Using these data, Garruto and Yanagihara (1991) estimated that ALS mortality rates for Chamorros living on Guam were three times greater than for Chamorros who had migrated to the West Coast, suggesting that outmigration had significantly lowered their subsequent risk of developing the disease. Nevertheless, Chamorros living off Guam retained a 5- to 10-fold increase of developing ALS when compared to the non-Guamanian population of the United States. In addition, Guamanians migrating to the continental United States are at risk of developing parkinsonism–dementia complex, a disease that has no direct counterpart in other populations. A review of all of the migration data suggests that the enhanced risk of developing ALS/parkinsonism–dementia complex is attained within the first 18 years of life and then may be retained for many decades (perhaps even lifelong). Inherent in such considerations is the concept that whatever putative environmental agent is responsible for the development of ALS/parkinsonism–dementia complex is present on Guam but is not available to the Guamanian "diaspora." This assumption may be correct. People tend to bring with them many aspects of their culture, native foods, festivals, and so on, and this may represent opportunities for further exposure to potential environmental agents among an outmigrated community. The Guamanians have a strong attachment to their island culture, and items such as diet have accompanied them in their travels and continue to be enjoyed whenever available. Further, with the ready availability of air travel, return trips to Guam are common among this migrant community.

XIX. Environmental Agents

A. Infectious Organisms

It was the identification of parkinsonism–dementia complex that gave rise to serious consideration that the nature of the outbreak of neurodegenerative disease on Guam might be post infectious in nature. This aspect of the disorder suggested similarities to postencephalitic parkinsonism patients who had survived the epidemic of encephalitis lethargica in the early part of the 20th century. Postencephalitic parkinsonism typically displays a period of latency between the initial episode of encephalitis to the onset of parkinsonian symptoms, which may last as long as a decade or more (6 months to a year is more typical) (Yahr, 1968). Clinically, patients with postencephalitic parkinsonism may have all of the signs and symptoms of idiopathic Parkinson's disease and also demonstrate oculogyric crises. Clinical studies of Guamanian cases of parkinsonism–dementia complex failed to demonstrate oculogyric crises (Elizan *et al*., 1966), although the significance of this negative finding remains unclear. Neuropathologic features of postencephalitic parkinsonism include many encountered in parkinsonism–dementia complex of Guam. These include the finding of NFTs in remaining neurons of the substantia nigra, in the absence of Lewy bodies, and the finding of neocortical NFTs predominating in superficial layers (layer II–III) (Hof *et al*., 1992b).

Despite these similarities, studies of both serum and postmortem brain tissues have failed to show any consistent evidence of a preceding central nervous system infection. Archival death certificates dating from the beginning of the 20 century failed to document any major outbreaks of encephalitis in the community, and interviews with knowledgable elders in the community failed to provide anecdotal accounts of any notable episodes of encephalitis on Guam. Relatively restricted outbreaks of Japanese B encephalitis and mumps did occur on Guam in December 1947 and in April 1948, but subsequent interviews with patients suffering from ALS/parkinsonism–dementia complex and their families have failed to confirm a prior history of encephalitis in such cases.

It should be kept in mind that Guam represents the southernmost island of a small archipelago and one of four

inhabited islands (Guam, Rota, Tinian, and Saipan) that are in very close proximity. Commerce and other traffic among these four islands have always been extensive, as they are within direct visual sighting of each other, which would likely provide a ready means for potential access of any infectious disease-carrying vectors. The two neighboring islands of Guam and Rota have continued to show a high incidence of ALS/parkinsonism–dementia complex among their inhabitants. This is in sharp distinction to the populations living on Tinian and Saipan where repeated clinical surveys have failed to identify any significant number of cases. It is highly unlikely that a classic infectious agent could remain restricted to the two southernmost islands and not extend to the other two islands.

The possibility that Guam ALS/parkinsonism–dementia complex represents an example of a prion-related disease has also been considered. In the laboratories of Gibbs and Gajdusek (1982), numerous attempts were made to transmit neurodegenerative disease through the intracerebral inoculation of Guam-derived brain tissues into nonhuman primates and other susceptible species. All such attempts were negative, including other efforts to recover a transmissible agent through the inoculation of tissue culture. In the face of this extensive amount of negative evidence, it is difficult to consider further a possible infectious etiology for the outbreak.

XX. Cycad

Over the years, an important candidate for the etiology of ALS/parkinsonism–dementia complex of Guam has been exogenous neurotoxins in the seed of the false sago palm, *Cycas circinalis*. In 1964, Marjory Whiting first identified the cycad seed as a potential cause of the outbreak. The cycad tree is an indigenous plant of Guam and its fleshy seed has traditionally served as a food source for the Chamorros. The natives dry the seeds and then grind them into a flour, which is used to prepare tortillas, porridge, and doughnuts and to thicken soups. However, the raw seeds contain a potent hepatotoxin, making them rapidly fatal unless washed thoroughly before eating. The seeds must be soaked in many changes of water over a period of a week or more before they may be eaten safely. Whiting proposed that an additional neurotoxin might be present in the seeds and be responsible for the onset of neurodegenerative disease. This gave rise to a detailed search for putative neurotoxins in the plant. The results of these extensive toxicologic studies were published in a series of six cycad conferences, which were held under the auspices of the NIH (Proceedings of the Third Conference on the Toxicity of Cycads, 1964; Whiting, 1988). The detailed reports of these conferences provide extensive documentation on the numerous studies that were carried out in which a wide range of animals (including nonhuman primates) were exposed to high doses of either raw cycad seeds or their purified constituents.

An important finding of these toxicologic studies was the identification of cycasin (methylmethoxymethanol), a very potent alkylating agent present within the cycad seed. Cycasin comprises 2–4% by weight of the seed and metabolizes to methylmethoxymethanol, a potent carcinogen. Indeed, cycasin represents one of the strongest naturally occurring carcinogens, and many of the cycad-exposed animals eventually developed a wide variety of cancers involving the lungs, kidneys, and liver. Importantly, despite the high doses being used and the wide range of animals that were exposed, little, if any, evidence of neurotoxicity was demonstrated in these experiments. Based on this entirely negative evidence, despite a major research effort, support for the cycad hypothesis as the etiology of the Guam outbreak of neurodegeneration waned.

In 1987, Spencer and colleagues reported producing extrapyramidal dysfunction and motor weakness in cynomologus monkeys fed large oral doses of an ''unusual'' amino acid present in small amounts in the cycad seed, namely β-*N*-methyl-amino-L-alanine (BMAA). BMAA shares chemical similarities with β-*N*-oxalylamino-L-alanine (BOAA), which had been implicated in neural lathyrism, a disease characterized by nonprogressive spastic paraparesis. For up to 13 weeks, each monkey was fed a large daily dose of BMAA via gastric gavage. The BMAA was chemically synthesized by a laboratory chemist and was not derived from the natural source. The animals were reported to develop muscular weakness, masked facies, and a loss of aggressiveness, and the authors suggested their similarity to ALS/parkinsonism–dementia complex in humans. The appropriateness of these conclusions has been questioned on a number of grounds. The doses of BMAA were extremely high (100–300 mg BMAA/kg body weight/day). Duncan *et al*. (1990) reported that BMAA is present in very small concentrations in raw cycad seed (0.1% or less, by weight) and is removed readily by even brief washing. Indeed, they estimated that an adult human would have to ingest approximately 7 kg of unwashed raw seed per day to receive a dose that was comparable to that given to the exposed animals. Because raw cycad seeds are highly toxic to humans and must be washed repeatedly before it may be eaten safely, most of the BMAA would have been removed. Accordingly, a comparable dose of washed cycad seeds would require ingestion of approximately 70 kg of cycad flour per day. Additional studies have questioned whether BMAA crosses the blood–brain barrier (Duncan *et al*., 1990).

Importantly, despite reporting motor weakness, evidence of actual motor neuron loss was not demonstrated in any of the BMAA-exposed monkeys. Furthermore, neither a striatal dopaminergic deficit (indicative of nigral degeneration), nor denervation atrophy (suggesting anterior horn cell pathology) was documented in the exposed animals and it remains unclear why these monkeys displayed any of their neurologic signs. Currently, the role of cycad-containing foods in the outbreak of neurodegeneration on Guam remains unsubstantiated. With regard to BMAA, Spencer and colleagues (1993) have subsequently written that ''the changes [induced in monkeys fed synthetic BMAA] fall short of a model of the human disease.''

XXI. Toxic Metals

In 1972, Yase first suggested the possibility that the neurodegeneration seen on Guam and the Kii peninsula might be related to an abnormal accumulation of potentially neurotoxic metals. He noted that manganese was present in significant amounts in the soils of Guam and the Kii peninsula.

He also observed high levels of aluminum in the soils of both sites. Manganese poisoning is well documented to produce a parkinsonian syndrome; however, it is marked pathologically by degeneration of the striatum with sparing of the substantia nigra, pars compacta (Olanow *et al*., 1994). Aluminum, however, had been experimentally linked to neurofibrillary degeneration through the induction of tangle-like lesions in rabbits following direct exposure to aluminum-containing compounds (Klatzo *et al*., 1965; Terry and Peña, 1965).

In 1980, using the techniques of electron probe microanalysis, Perl and Brody first demonstrated evidence of aluminum accumulation in the neurofibrillary tangle-bearing neurons of AD. Subsequently, using a similar approach, Perl and colleagues showed evidence of dramatic aluminum accumulation in the tangle-bearing neurons of patients with ALS and parkinsonism–dementia complex of Guam. Additional studies indicated that the concentration of aluminum in the tangle-bearing neurons of the Guam cases was approximately 10 times greater than that of AD cases (Good and Perl, 1994). Although the association of aluminum and AD remains controversial, the finding of excess aluminum in the tangles encountered in the Guam cases has now been confirmed using five different physical methods in a number of different laboratories (Garruto *et al*., 1984; Linton *et al*., 1987; Piccardo *et al*., 1988; Good and Perl, 1993).

To date, the environmental source of these dramatic intraneuronal accumulations of aluminum remains unclear. It has been hypothesized that a deficiency in environmental sources of calcium and magnesium, physiologically essential ionic constituents, presumeably related to underlying trace elemental abnormalities of soil and water, could lead to increased aluminum uptake as an alternative dietary source of cations. However, search for evidence of calcium deficiency among Guamanians has not revealed consistent alterations (Steele *et al*., 1990; Ashkog *et al*., 1994), and other alternative explanations must be sought. Guam is partially a volcanic island with an aluminum-rich bauxite soil. The soils of Guam contain soils with 42 times the amount of elutable aluminum when compared to two other bauxite islands, namely Jamaica and Palau (McLachlan *et al*., 1989). This later study suggests that Guam soil may contain a great deal more bioavailable aluminum when compared to other volcanic islands. The extent and nature of these differences and their potential biologic significance remain unclear and largely unstudied to date.

More recent studies using laser microprobe lass analysis demonstrated that the NFTs of Guam cases contained accumulations of iron, as well as aluminum, a finding that parallels that obtained using this technique with cases of AD. Iron and aluminum excess has also been detected in the neuromelanin granules and Lewy bodies of cases of idiopathic Parkinson's disease. Iron, through the Fenton reaction, is a powerful prooxidant and is capable of catalyzing the production of the highly reactive hydroxyl radical from hydrogen peroxide, a by-product of normal dopamine metabolism. We have suggested (Olanow *et al*., 1994) that the combination of iron and aluminum may place a neuron in a state of oxidative stress because of the apparent ability of aluminium to enhance the capacity of iron to induce lipid peroxidation (Gutteridge *et al*., 1985). Findings of mutations in the Cu/Zn superoxide dismutase gene in cases of familial ALS, as well as the production of transgenic models of motor neuron disease through the introduction of such mutations, may have relevance to Guam neurodegeneration. Cu/Zn superoxide dismutase clearly plays a role in the balancing of the production of oxygen radicals through energy utilization and the body's natural defenses against the potentially damaging effects of such radical production. In some way that is still not yet understood, altering this critical balance appears to induce progressive neuronal degeneration in the form of similar neurodegenerative disorders. Whether the striking aluminum and iron accumulations identified in target neurons for neurodegeneration in cases of ALS/parkinsonism–dementia complex of Guam are truly etiologic in nature remains unknown. The source of these deposits and the mechanism by which they occur also remain to be elucidated.

XXII. General Comments

It is a mistake to dismiss ALS/parkinsonism–dementia complex of Guam as a unique and separate disorder without further implications elsewhere in the world. Instead, Guam should be viewed as a rich laboratory in which to explore the interactions between environmental factors and genetic factors in the induction of most of the cardinal features of the age-related neurodegenerative disorders. In this interaction the strongest factor appears to be environmental in nature. In a similar fashion, because important hereditary insights can be gained through the study of genetically based high-risk families in a geographic isolate such as Guam, environmentally based aspects may be addressed more easily. Despite extensive research over several decades, the mystery of the etiology of the remarkable concentration of neurodegeneration on the island of Guam remains unsolved. Nevertheless, Guam represents the richest potential laboratory in which to unravel those critical environmental factors of importance to an understanding of both the disorders seen on Guam but also the analogous disorders encountered throughout the world.

Over the many years the Chamorro community on Guam has patiently awaited for the scientists working on the problem of ALS/parkinsonism–dementia complex to come up with the answers that will lift the staggering burden of suffering represented by this tragic disorder from their shoulders. Despite over 40 years of active research requiring answering innumerable questionnaires, providing gallons of blood samples, and countless autopsy specimens on their loved ones, these answers have yet to emerge. Considering this, the indomitable patience and continuing cooperative spirit of this beleaguered community are noteworthy and remarkable. The author has often noted that if a similar outbreak of ALS/parkinsonism–dementia complex were to be noted in a comparable sized community in the continental United States, the public outcry for immediate solutions to the problem would be deafening and would result in the rapid enlistment of a major research effort to solve the problem as quickly as possible. Unfortunately, despite the fact that the Chamorros of Guam are United States citizens living on American soil, their cries for help are muted by the more than 5000 miles of Pacific Ocean

between them and the mainland. The author can only hope that someday soon the patience of the Guam people will finally be rewarded by the scientific enquiries into their plight. That day will become a cause for rejoicing on Guam and will represent a major milestone in the world's quest for an understanding of the major age-related neurodegenerative diseases for which Guam patients serve as a model.

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References

Anderson, F. H., Richardson, E. P., Okazaki, H., and Brody, J. A. (1979). Neurofibrillary degeneration on Guam. Frequency in Chamorros and non-Chamorros with no known neurological disease. *Brain* **102**, 65–77.

Arnold, A., Edgren, D. C., and Palladino, V. S. (1953). Amyotrophic lateral sclerosis. Fifty cases observed on Guam. *J. Nerv. Ment. Dis*. **117**, 135–139.

Ashkog, J. E., Waring, S. C., Petersen, R. C., Kurland, L. T., Maraganore, D. M., Esteban-Santillan, C., Harmsen, W. S., O'Brien, P. C., and Moyer, T. P. (1994). Guamanian neurodegenerative disease: Failure to find abnormal calcium metabolism. *Neurology* **44**, S2, 193.

Ball, M. J. and Lo, L. (1977). Granulovacuolar degeneration in the aging brain and in dementia. *J. Neuropathol. Exp. Neurol*. **36**, 474–487.

Buée, L., Hof, P. R., Bouras, C., Delacourte, A., Perl, D. P., Morrison, J. H., and Fillit, H. M. (1994). Pathological alterations of the cerebral microvasculature in Alzheimer's disease and related dementing disorders. *Acta Neuropathol*. **87**, 469–480.

Buée, L., Pérez-Tur, J., Leveugle, B., Buée-Scherrer, V., Mufson, E. J., Loerzel, A. J., Chartier-Harlin, M. C., Perl, D. P., Delacourte, A., and Hof, P. R. (1996). Apolipoprotein E in Guamanian amyotrophic lateral sclerosis/parkinsonism-dementia complex: Genotype analysis and relationships to neuropathologic changes. *Acta Neuropathol*. **91**, 247–253.

Buée-Scherrer, V., Buée, L., Hof, P. R., Leveugle, B., Gilles, C., Loerzel, A. J., Perl, D. P., and Delacourte, A. (1995a). Neurofibrillary degeneration in amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam: Immunochemical characterization of tau proteins. *Am. J. Pathol*. **68**, 924–932.

Buée-Scherrer, V., Buée, L., Hof, P. R., Vermersch, P., Leveugle, B., Wattez, A., Perl, D. P., and Delacourte, A. (1995b). Tau variants in aging and neurodegenerative disease. *In* "Alzheimer's Disease: Lessons from Cell Biology" (K. S. Kosik, Y. Christen, and D. J. Selkoe, eds.), pp. 132–149. Springer-Verlag, Berlin.

Bunina, T. L. (1962). On intracellular inclusions in familial amyotrophic lateral sclerosis. *Korsakov J. Neurol. Psychiatry* **62**, 1293–1299.

Chartier-Harlin, M. C., Crawford, F., Perl, D. P., Steele, J., and Hardy, J. (1993). Sequencing of exon 16 and 17 of the beta-amyloid precursor protein gene reveals the beta-amyloid sequence to be normal in cases of the parkinsonism dementia complex of Guam. *J. Neural Transm*. **5**, 63–65.

Chen, K. M., Makifuchi, T., Garruto, R. M., and Gajdusek, D. C. (1982). Parkinsonism-dementia in a Filipino migrant: A clinicopathologic case report. *Neurology* **32**, 1221–1226.

Chen, L. (1981). Neurofibrillary change on Guam. *Arch. Neurol. (Chicago)* **38**, 16–18.

Chen, X., Xia, Y., Gresham, L. S., Molgaard, C. A., Thomas, R. G., Galasko, D., Wiederholt, W. C., and Saitoh, T. (2000). APOE and CYP2D6 polymorphism in Chamorro with and without parkinsonism dementia complex of Guam. *Neurology* (in press).

Duncan, M. W., Steele, J. C., Kopin, I. J., and Markey, S. P. (1990). 2-Amino-3-(methylamino)-propanoic acid (BMAA) in cycad flour: An unlikely cause of amyotrophic lateral sclerosis and parkinsonism–dementia of Guam. *Neurology* **40**, 767–772.

Eldridge, R., Ryan, E., Rosario, J., and Brody, J. A. (1969). Amyotrophic lateral sclerosis and parkinsonism-dementia in a migrant population from Guam. *Neurology* **19**, 1029–1037.

Elizan, T. S., Hirano, A., Abrams, B. M., Need, R. L., Van Nuis, C., and Kurland, L. T. (1966). Amyotrophic lateral sclerosis and parkinsonism-dementia complex of Guam. Neurological reevaluation. *Arch. Neurol. (Chicago)* **14**, 356–368.

Figlewicz, D. A., Garruto, R. M., Krizus, A., Yanagihara, R., and Rouleau, G. A. (1994). The Cu/Zn superoxide dismutase gene in ALS and parkinsonism-dementia of Guam. *NeuroReport* **5**, 557–560.

Gajdusek, D. C. (1963). Motor neuron disease in natives of New Guinea. *N. Engl. J. Med*. **268**, 474–476.

Gajdusek, D. C., and Salazar, A. M. (1980). Amyotrophic lateral sclerosis and parkinsonian syndromes in high incidence among the Auyu and Jakai people in West New Guinea. *Neurology* **32**, 107–126.

Galloway, P. G., Perry, G., and Gambetti, P. (1987a). Hirano bodies contain actin and actin-associated proteins. *J. Neuropathol. Exp. Neurol*. **46**, 185–199.

Galloway, P. G., Perry, G., Kosik, K., and Gambetti, P. (1987b). Hirano bodies contain tau protein. *Brain Res*. **403**, 337–340.

Garruto, R. M., and Yanagihara, R. (1991). Amyotrophic lateral scle rosis in the Marianas Islands. *In* "Handbook of Clinical Neurology" (J.M.B.V. DeJong, ed.), Vol. 15, pp. 253–277. Elsevier, Amsterdam.

Garruto, R. M., Gajdusek, D. C., and Chen, K. M. (1980). Amyotrophic lateral sclerosis among Chamorro migrants from Guam. *Neurology* **8**, 612–619.

Garruto, R. M., Gajdusek, D. C., and Chen, K. M. (1981). Amyotrophic lateral sclerosis and parkinsonism-dementia among Filipino migrants to Guam. *Ann. Neurol*. **10**, 341–350.

Garruto, R. M., Fukatsu, R., Yanagihara, R., Gajdusek, D. C., Hook, G., and Fiori, C. (1984). Imaging of calcium and aluminum in neurofibrillary tangle-bearing neurons in parkinsonism-dementia of Guam. *Proc. Natl. Acad. Sci. U.S.A*. **81**, 1875–1879.

Garruto, R. M., Yanagihara, R., and Gajdusek, D. C. (1985). Disappearance of high-incidence amyotrophic lateral sclerosis and parkinsonism-dementia on Guam. *Neurology* **35**, 193–198.

Gentleman, S. M., Perl, D., Allsop, D., Clinton, J., Royston, M. C., and Roberts, G. W. (1991). Beta (A4)-amyloid protein and parkinsonian dementia complex of Guam. *Lancet* **337**, 55–56.

Gibbs, C. J., Jr., and Gajdusek, D. C. (1982). An update on long-term in vivo and in vitro studies designed to identify a virus as the cause of amyotrophic lateral sclerosis, parkinsonism dementia and Parkinson's disease. *Adv. Neurol*. **36**, 343–353.

Gibson, P. H., and Tomlinson, B. E. (1977). Numbers of Hirano bodies in the hippocampus of normal and demented people with Alzheimer's disease. *J. Neurol. Sci*. **33**, 199–206.

Goldman, J. E. (1983). The association of actin with Hirano bodies. *J. Neuropathol. Exp. Neurol*. **42**, 146–152.

Good, P. F., and Perl, D. P. (1993). Aluminium in Alzheimer's? *Nature (London)* **362**, 418.

Good, P. F., and Perl, D. P. (1994). A quantitative comparison of aluminum concentration in neurofibrillary tangles of Alzheimer's disease and

parkinsonian dementia complex of Guam by laser microprobe mass analysis. *Neurobiol. Aging* **15**, S28.

Gutteridge, J. M., Quinlan, G. J., Clark, I., and Halliwell, B. (1985). Aluminum salts accelerate peroxidation of membrane lipids stimulated by iron salts. *Biochim. Biophys. Acta* **835**, 441–447.

Hirano, A. (1973). Progress in the pathology of motor neuron diseases. *Prog. Neuropathol*. **2**, 181–215.

Hirano, A. (1994). Hirano bodies and related neuronal inclusions. *Neuropathol. Appl. Neurobiol*. **20**, 3–11.

Hirano, A., and Zimmerman, H. M. (1962). Alzheimer's neurofibrillary changes. A topographic study. *Neurology* **7**, 227–242.

Hirano, A., Kurland, L. T., Krooth, R. S., and Lessell, S. (1961a). Parkinsonism-dementia complex, an endemic disease on the island of Guam. I. Clinical features. *Brain* **84**, 642–661.

Hirano, A., Malamud, N., and Kurland, L. T. (1961b). Parkinsonism-dementia complex, an endemic disease on the island of Guam. II. Pathological features. *Brain* **84**, 662–679.

Hirano, A., Kurland, L. T., and Sayre, G. P. (1967a). Familial amyotrophic lateral sclerosis: A subgroup characterized by posterior and spinocerebellar tract involvement and hyaline inclusions in the anterior horns. *Arch. Neurol. (Chicago)* **16**, 232–243.

Hirano, A., Arumugasamy, N., and Zimmerman, H. M. (1967b). Amyotrophic lateral sclerosis. A comparison of Guam and classical cases. *Arch. Neurol. (Chicago)* **16**, 357–363.

Hirano, A., Dembitzer, H. M., Kurland, L. T., and Zimmerman, H. M. (1968). The fine structure of some intraganglionic alterations. Neurofibrillary tangles, granulovacuolar bodies and "rod-like" structures as seen in Guam amyotrophic lateral sclerosis and parkinsonism-dementia complex. *J. Neuropathol. Exp. Neurol*. **27**, 167–182.

Hof, P. R., Perl, D. P., Loerzel, A. J., and Morrison, J. H. (1991). Neurofibrillary tangle distribution in the cerebral cortex of parkinsonism-dementia cases from Guam: Differences with Alzheimer's disease. *Brain Res*. **564**, 306–313.

Hof, P. R., Delacourte, A., and Bouras, C. (1992a). Distribution of cortical neurofibrillary tangles in progressive supranuclear palsy: A quantitative analysis of six cases. *Acta Neuropathol*. **84**, 45–51.

Hof, P. R., Charpiot, A., Delacourte, A., Buée, L., Purohit, D., Perl, D. P., and Bouras, C. (1992b). Distribution of neurofibrillary tangles and senile plaques in the cerebral cortex in postencephalitic parkinsonism. *Neurosci. Lett*. **139**, 10–14.

Hof, P. R., Bouras, C., Buée, L., Delacourte, A., Perl, D. P., and Morrison, J. H. (1992c). Differential distribution of neurofibrillary tangles in the cerebral cortex of dementia pugilistica and Alzheimer's disease cases. *Acta Neuropathol*. **85**, 23–30.

Hof, P. R., Nimchinsky, E. A., Buée-Scherrer, V., Buée, L., Nasrallah, J., Hottinger, A. F., Purohit, D. P., Loerzel, A. J., Steele, J. C., Delacourte, A., Bouras, C., Morrison, J. H., and Perl, D. P. (1994a). Amyotrophic lateral sclerosis/parkinsonism–dementia complex of Guam: Quantitative neuropathology, immunohistochemical analysis of neuronal vulnerability, and comparison with related neurodegenerative disorders. *Acta Neuropathol.* **88**, 397–404.

Hof, P. R., Perl, D. P., Loerzel, A. J., Steele, J. C., and Morrison, J. H. (1994b). Amyotrophic lateral sclerosis and parkinsonism-dementia from Guam: Differences in neurofibrillary tangle distribution and density in the hippocampal formation and neocortex. *Brain Res.* **650**, 107–116.

Ito, H., Goto, S., Hirano, A., and Yen, S. H. (1991). Immunohistochemical study of the hippocampus in parkinsonism-dementia complex on Guam. *J. Geriatr. Psychiatry Neurol*. **4**, 134–142.

Klatzo, I., Wisniewski, H., and Streicher, E. (1965). Experimental production of neurofibrillary pathology: I. Light microscopic observations. *J. Neuropathol. Exp. Neurol*. **24**, 187–199.

Kurland, L. T., and Mulder, D. W. (1954). Epidemiologic investigations of amyotrophic lateral sclerosis. 1. Preliminary report on geographic distribution, with special reference to the Mariana Islands, including clinical and pathologic observations. *Neurology* **4**, 355–378, 438–448.

Kurland, L. T., Mulder, D. W., Sayre, G. P., Lambert, E., Hutson, W., Iriarte, L. G., and Imus, H. A. (1956). Amyotrophic lateral sclerosis in the Marianas Islands. *Arch. Neurol. Psychiatry* **75**, 435–441.

Kuzuhara, S. (1999). ALS and parkinsonism-dementia complex of Kii peninsula. *Ryoik. Shokogun Shirizu* **27**, 335–338.

Lepore, F. E., Steele, J. C., Cox, T. A., Tillson, G., Calne, D. B., Duvoisin, R. C., Lavine, L., and McDarby, J. V. (1988). Supranuclear disturbances of ocular motility in Lytico-Bodig. *Neurology* **38**, 1849–1853.

Lessel, S., Hirano, A., Torres, J., and Kurland, L. T. (1962). Parkinsonism-dementia complex, epidemiologic considerations in the Chamorros of the Marianas islands, including clinical and pathologic observations. *Arch. Neurol. (Chicago)* **7**, 377–385.

Leveugle, B., Spik, G., Perl, D. P., Bouras, C., Fillit, H. M. and Hof, P. R. (1994). The iron-binding protein lactoferrin is present in pathologic lesions in a variety of neurodegenerative disorders: A comparative immunohistochemical analysis. *Brain Res*. **650**, 20–31.

Linton, R. W., Bryan, S. R., Cox, X. B., Griffis, D. P., Shelburne, J. D., Fiori, C. E., and Garruto, R. M. (1987). Digital imaging studies of aluminum and calcium in neurofibrillary tangle-bearing neurons using SIMS (secondary ion mass spectrometry). *Trace Elem. Med*. **4**, 99–104.

Malamud, N., Hirano, A., and Kurland, L. T. (1961). Pathoanatomic changes in amyotrophic lateral sclerosis on Guam. *Neurology* **5**, 401–414.

Mawal-Dewan, M., Schmidt, M. L., Balin, B., Perl, D. P., Lee, V. M., and Trojanowski, J. Q. (1996). Identification of phosphorylation sites in PHF-TAU from patients with Guam amyotrophic lateral sclerosis/parkinsonism-dementia complex. *J. Neuropathol. Exp. Neurol*. **55**, 1051–1059.

McLachlan, D. R., McLachlan, C. D., Krishnan, B., Krishnan, S. S., Dalton, A. J., and Steele, J. C. (1989). Aluminum and calcium in soil and food from Guam, Palau and Jamaica: Implications for amyotrophic lateral sclerosis and parkinsonism-dementia syndromes of Guam. *Environ. Geochem. Health* **11**, 45–53.

Miura, K. (1902). Über die amyotrophische Lateralsklerose. *Neurol. Jpn*. **1**, 1–15.

Miura, K. (1911). Amyotrophische Lateralsklerose unter dem Bilde von sog. Bulbarparalyse. *Neurol. Jpn*. **10**, 366–369.

Mourelatos, Z., Adler, H., Hirano, A., Donnenfeld, H., Gonatas, J. O., and Gonatas, N. K. (1990). Fragmentation of the Golgi apparatus of motor neurons in amyotrophic lateral sclerosis revealed by organelle-specific antibodies. *Proc. Natl. Acad. Sci. U.S.A*. **87**, 4393–4395.

Mourelatos, Z., Hirano, A., Rosenquist, A., and Gonatas, N. (1994). Fragmentation of the Golgi apparatus of motor neurons in amyotrophic lateral sclerosis (ALS). Clinical studies in ALS of Guam and experimental studies in deafferented neurons and in beta,beta′-iminodipropionitrile axonopathy. *Am. J. Pathol*. **144**, 1288–1300.

Olanow, C. W., and Perl, D. P. (1994). Free radicals and neurodegeneration. *Trends Neurosci*. **17**, 193–194.

Olanow, C. W., Calne, D. B., Chu, N. S., and Perl, D. P. (1994). Manganese-induced neurotoxicity. *In* "Advances in Research on Neurodegeneration Etiopathogenesis" (Y. Mizuno, D. B. Calne, and R. Horowski, eds.), Vol. II, pp. 53–62. Birkhauser, Boston.

Pérez-Tur, J., Buée, L., Morris, H. R., Waring, S. C., Onstead, L., Wavrant-De Vrièze, F., Crook, R., Buée-Scherrer, V., Hof, P. R., Petersen, R. C., McGeer, P. L., Delacourte, A., Hutton, M., Siddique, T., Ashkog, J. E., Hardy, J., and Steele, J. C. (1999). Neurodegenerative diseases of Guam: Analysis of tau. *Neurology* **53**, 411–413.

Perl, D. P. (1994). Amyotrophic lateral sclerosis and parkinsonism-dementia complex of Guam: Two distinct entities or a single disease process with a spectrum of neuropathologic and clinical expressions? *In* "Advances in Research on Neurodegeneration" (Y. Mizuno, D. B. Calne, and R. Horowski, eds.), Vol. II, pp. 209–218. Birkhauser, Boston.

Perl, D. P., Hof, P. R., Steele, J. C., Purohit, D. P., Esteban-Santillan, C., Peterson, R., and Kurland, L. T. (1994). Marianas dementia: A purely

dementing form of ALS/parkinsonism-dementia complex of Guam. *Soc. Neurosci. Abstr.* **20**, 1649–1650.

Piccardo, P., Yanagihara, R., Garruto, R. M., Gibbs, C. J., Jr., and Gajdusek, D. C. (1998). Histochemical and X-ray microanalytical localization of aluminum in amyotrophic lateral sclerosis and parkinsonism-dementia of Guam. *Acta Neuropathol*. **77**, 1–4.

Proceedings of the Third Conference on the Toxicity of Cycads. (1964). *Fed. Proc*. **23**.

Purohit, D. P., Perl, D. P., and Steele, J. C. (1992). ALS/parkinsonism dementia-complex among Filipino migrants to Guam: Report of two cases with parkinsonian features. *J. Neuropathol. Exp. Neurol*. **51**, 323.

Reed, D., Plato, C., Elizan, T., and Kurland, L. T. (1966). The amyotrophic lateral sclerosis/parkinsonism-dementia complex: A ten-year follow-up on Guam. Part I. Epidemiologic studies. *Am. J. Epidemiol*. **83**, 54–73.

Reed, D. M., and Brody, J. A. (1975). Amyotrophic lateral sclerosis and parkinsonism-dementia of Guam, 1945–1972. I. Descriptive epidemiology. *Am. J. Epidemiol*. **101**, 287–301.

Rogers-Johnson, P., Garruto, R. M., Yanagihara, R., Chen, K., Gajdusek, D. C., and Gibbs, C. J., Jr. (1986). Amyotrophic lateral sclerosis and parkinsonism-dementia on Guam: A 30-year evaluation of clinical and neuropathologic trends. *Neurology* **36**, 7–13.

Schmidt, M. L., Lee, V. M. Y., and Trojanowski, J. Q. (1989). Analysis of epitopes shared by Hirano bodies and neurofilament proteins in normal and Alzheimer's disease hippocampus. *Lab. Invest*. **60**, 513–522.

Schnur, J. A., Chase, T. N., and Brody, J. A. (1971). Parkinsonism-dementia of Guam: Treatment with L-dopa. *Neurology* **21**, 1236–1242.

Shankar, S. K., Yanagihara, R., Garruto, R. M., Grundke-Iqbal, I., Kosik, K. S., and Gajdusek, D. C. (1989). Immunocytochemical characterization of neurofibrillary tangles in amyotrophic lateral sclerosis and parkinsonism-dementia of Guam. *Ann. Neurol*. **25**, 146–151.

Shiraki, H., and Yase, Y. (1975). Amyotrophic lateral sclerosis in Japan. *In* ''Handbook of Clinical Neurology'' (P. J. Vinken and G. W. Bruyn, eds.), Vol. 22, pp. 353–419. Elsevier, New York.

Shiraki, H., and Yase, Y. (1991). Amyotrophic lateral sclerosis and parkinsonism-dementia in the Kii Peninsula: Comparison with the same disorders in Guam and with Alzheimer's disease. *In* ''Handbook of Clinical Neurology'' (J. M. B. V. DeJong, ed.), Vol. 15, pp. 273–300. Elsevier, Amsterdam.

Snow, B. J., Peppard, R. F., Guttman, M., Okada, J., Martin, W. R., Steele, J., Eisen, A., Carr, G., Schoenberg, B., and Calne, D. (1990). Positron emission tomographic scanning demonstrates a presynaptic dopaminergic lesion in Lytico-Bodig. The amyotrophic lateral sclerosis-parkinsonism-dementia complex of Guam. *Arch. Neurol. (Chicago)* **47**, 870–874.

Spencer, P. S., Kisby, G. E., Ross, S. M., Roy, D. N., Hugon, J., Ludolph, A. C., and Nunn, P. B. (1993). Guam ALS-PDC: Possible causes. *Science* **262**, 825–826.

Steele, J. C., Guzman, T. Q., and Driver, M. G. (1990). Nutritional factors in amyotrophic lateral sclerois on Guam: Observations from Umatac. *In* ''Amyotrophic Lateral Sclerosis: Concepts in Pathogenesis and Etiology'' (A. J. Hudson, ed.), pp. 193–223. University of Toronto Press, Toronto.

Stone, R. (1993). Guam: Deadly disease dying out. *Science* **261**, 424–426.

Terry, R. D., and Peña, C. (1965). Experimental production of neurofibrillary pathology: Electron microscopy, phosphate histochemistry and electron probe analysis. *J. Neuropathol. Exp. Neurol*. **24**, 200–210.

Tomlinson, B. E., and Kitchener, D. (1972). Granulovacuolar degeneration of hippocampal cells. *J. Pathol*. **106**, 165–185.

Torres, J., Iriarte, L. L., and Kurland, L. T. (1957). Amyotrophic lateral sclerosis among Guamanians in California. *Calif. Med*. **4**, 385–388.

Uebayashi, Y. (1984). Long survival cases of motor neuron disease in the Kii peninsula and Guam. *In* ''Amyotrophic Lateral Sclerosis in Asia and Oceania'' (K. M. Chen and Y. Yase, eds.), pp. 43–64. National Taiwan University, Taiwan.

Umahara, T., Hirano, A., Kato, S., Shibata, N., and Yen, S. H. (1994). demonstration of neurofibrillary tangles and neuropil thread-like structures in spinal cord white matter in parkinsonism-dementia complex on Guam and in Guamanian amyotrophic lateral sclerosis. *Acta Neuropathol*. **88**, 180–184.

Wakayama, I., Kihira, T., Yoshida, S., and Garruto, R. M. (1993). Rare neuropil threads in amyotrophic lateral sclerosis and parkinsonism-dementia on Guam and in the Kii Peninsula of Japan. *Dementia* **4**, 75–80.

Waring, S. C. (1994). Amyotrophic lateral sclerosis and parkinsonism-dementia complex of Guam: Descriptive epidemiology, secular trends, and birth cohort effects on incidence, 1950–1989. Dissertation, University of Texas, Houston.

Waring, S. C., O'Brien, P. C., Kurland, L. T., Thibodeau, S. N., Tsai, M. S., Petersen, R. C., and Esteban-Santillan, C. E. (1994). Apolipoprotein E allele in Chamorros with amyotrophic lateral sclerosis/parkinsonism-dementia complex. *Lancet* **343**, 611.

Whiting, M. (1988). ''Toxicity of Cycads: Implications for Neurodegenerative Diseases and Cancer. Transcripts of Four Cycad Conferences'' Third World Medical Research Foundation, New York.

Whiting, M. G. (1964). Toxicity of cycads. *Econ. Bot*. **17**, 64–71.

Wiederholt, W. C. (1999). Neuroepidemiologic research initiative on Guam: Past and present. *Neuroepidemiology* **18**, 279–291.

Yahr, M. D. (1968). Encephalitis lethargica (Von Economo's disease, epidemic encephalitis). *In* ''Handbook of Clinical Neurology'' (P. J. Vinken and G. W. Bruyn, eds.), Vol. 34, pp. 451–457. Elsevier/North-Holland, Amsterdam.

Yase, Y., Chen, K. M., Brody, J. A., and Toyokura, Y. (1978). An historical note on the parkinsonism-dementia complex of Guam. *Neurol. Med*. **8**, 583–589.

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Pierre J. Magistretti, Sabine Joray, and Luc Pellerin

16

Brain Energy Metabolism: Cellular Aspects and Relevance to Functional Brain Imaging

Brain energy metabolism and blood flow are tightly coupled to neuronal activity. Functional brain imaging techniques, such as positron emission tomography and functional magnetic resonance imaging, detect metabolic and vascular signals that are associated with neural activity. Results have clarified the mechanisms that underlie the coupling between neuronal activity and glucose metabolism. These results indicate a role of astrocytes in this coupling. During healthy aging, marked changes in brain energy metabolism are revealed by functional imaging. The patterns of changes associated with aging are distinct from those seen in Alzheimer's disease.

I. Energy Metabolism and Blood Flow

A. Glucose Is the Main Energy Substrate for the Brain

The brain represents only 2% of the body weight in humans, yet the energy-consuming processes that ensure proper brain function account for approximately 25% of total body glucose utilization. With a few exceptions that will be reviewed later, glucose is the obligatory energy substrate of the brain. (Kety, 1957; Sokoloff, 1960; Edvinsson *et al*., 1993).

In any tissue, glucose can follow various metabolic pathways; in the brain, glucose is almost entirely oxidized to CO_2 and water through its sequential processing via glycolysis, the tricarboxylic acid (TCA) cycle, and the associated oxidative phosphorylation, which yields 38 ATP per glucose on a molar basis. The oxygen consumption of the brain, which accounts for almost 20% of the oxygen consumption of the whole organism, is 160 µmol/100 g of brain weight/min and roughly corresponds to the value determined for CO_2 production. This O_2/CO_2 relation corresponds to what is known in metabolic physiology as a respiratory quotient of nearly 1 and provides the demonstration that carbohydrates, and glucose in particular, are the exclusive substrates for oxidative metabolism. In normal adults, the calculated glucose utilization by the brain is 31 µmol/100 g of brain weight/min (Kety and Schmidt, 1948; Sokoloff, 1960) and cerebral blood flow (CBF) is approximately 57 ml/100 g of brain weight/min.

Exactly like in other tissues, the metabolism of glucose, the main energy substrate of the brain, produces two forms of energy: ATP and NADPH. Glycolysis and the TCA cycle produce ATP, whereas energy under the form of reducing equivalents stored in the NADPH molecule is produced predominantly through the pentose phosphate pathway. Maintenance of the electrochemical gradients, particularly for Na^+ and K^+, needed for electrical signaling, via the action potential, and for chemical signaling, through synaptic transmission, is the main energy-consuming process of neural cells.

B. Blood Flow Regulation

Since the seminal article of Roy and Sherrington (1890), the search has been intense for the identification of chemical mediators that could couple neuronal activity with local increases in blood flow. These signals can be broadly grouped into two categories: (i) molecules or ions that transiently accumulate in the extracellular space following neuronal activity and (ii) specific neurotransmitters that mediate the coupling in anticipation, or at least in parallel, with local activation (neurogenic mechanisms).

Several products of activity-dependent neuronal and glial metabolism, such as lactate, H^+, adenosine, and K^+, have vasoactive effects and are therefore putative mediators of coupling, although the kinetics and spatial resolution of this mode do not account for all the observed phenomena. As attractive as it is, an exclusively neurogenic mechanism of coupling neuronal activity to blood flow is unlikely and moreover it still awaits firm functional confirmation *in vivo*. Nitric oxide (NO) is certainly a key element in coupling, although evidence indicates that it can only be considered as one of the mechanisms contributing to the coupling between activity and local increases in blood flow (Villringer and Dirnagl, 1995).

The consequence of the activity-linked increase in blood flow is that more substrates, namely glucose and oxygen, necessary to meet the additional energy demands are delivered to the activated area per unit time.

II. Coupling and Functional Imaging

An important principle in brain physiology is indeed the tight coupling of neuronal activity with both energy metabolism and blood flow. As noted earlier, this principle was already formulated over a hundred years ago by Roy and Sherrington (1890) : "... we conclude then that the chemical products of cerebral metabolism ... of the brain can cause variations of the caliber of the cerebral vessels ... that in this reaction the brain possesses an intrinsic mechanism by which the vascular supply can be varied locally in correspondence with local variations of functional activity." This physiological principle, which has also been validated for the coupling between activity and energy metabolism with the determination of local rates of glucose utilization with the 2-deoxyglucose technique (Sokoloff *et al.*, 1977), has provided the basis for all functional imaging techniques, as local increases in brain activity produce blood flow and metabolism signals that can be detected with various imaging techniques. Positron emission tomography (PET) can monitor the increase in cerebral blood flow (CBF) in glucose utilization and oxygen consumption (Phelps *et al.*, 1979; Frackowiak *et al.*, 1980); the degree of blood oxygenation yields the signals that are detected with functional magnetic resonance imaging (fMRI) (Ogawa *et al.*, 1992). However, PET and fMRI do not detect synaptic activity directly but measure signals that reflect energy consumption by activity-dependent neural processes. Despite the remarkable advances in imaging techniques and their extensive applications to neurology, neuropsychology, and psychiatry, the precise understanding of the cellular and molecular mechanisms that underlie the signals detected with these imaging techniques, which rely on the coupling of synaptic activity with energy metabolism and blood flow, is still quite fragmentary. Evidence indicates that astrocytes play a key role in coupling synaptic activity to glucose utilization and metabolism (Magistretti *et al.*, 1999).

III. Cellular Mechanism of Brain Energy Metabolism

A. Neuronal Activity Is Tightly Coupled to Glucose Utilization

The 2-deoxyglucose (2-DG) technique developed by Louis Sokoloff for laboratory animals (Sokoloff *et al.*, 1977) and its adaptation to PET for humans has provided a direct demonstration of the coupling between neuronal activation and glucose (Magistretti *et al.*, 1999). Despite this compelling evidence, the cellular and molecular mechanisms that underlie such a coupling are still under scrutiny. A current assumption is that neuronal signals produced by synaptic activity act directly on brain capillaries to increase locally the delivery of energy substrates. However, this view does not take into account an important cellular component of the brain: the astrocytes. Indeed, particular astrocytic profiles, the end-feet, surround intraparenchymal capillaries, whereas other astrocytic processes ensheath synaptic contacts (Peters *et al.*, 1991) and possess receptors and reuptake sites for neurotransmitters. (Magistretti and Pellerin, 1996). These features imply that astrocytes are ideally positioned to sense increases in synaptic activity and to couple them with energy metabolism.

B. Astrocytes Couple the Activity of Glutamatergic Synapses with Glucose Utilization

From the foregoing, it follows that astrocytes may play a prominent role in coupling neuronal activity to local increases in glucose utilization during activation as observed in animals and humans. To explore this hypothesis, we have studied glucose utilization by mouse cerebral cortex astrocytes in culture using the [^{3}H]-2-deoxyglucose as a marker of glucose utilization. We have shown that glutamate stimulates 2-DG uptake and phosphorylation in astrocytes in a concentration-dependent manner with an EC_{50} of approximately 80 μM. (Pellerin and Magistretti, 1994). This effect is not receptor mediated as it cannot be prevented or mimicked by glutamate receptor antagonists and agonists, respectively (Pellerin and Magistretti, 1994). Rather, it is mediated by a Na^+-dependent glutamate transporter, as supported by pharmacological evidence (Pellerin and Magistretti, 1994, 1996; Takahashi *et al.*, 1995) (Fig. 16.1).

The intracellular molecular mechanisms of this coupling indicate a critical involvement of the Na^+/K^+-ATPase, as ouabain completely inhibits the glutamate-evoked 2-DG uptake by astrocytes (Pellerin and Magistretti, 1994, 1996). The astrocytic Na^+/K^+-ATPase responds predominantly to increases in intracellular Na^+ for which it shows a K_m of about 10 mM (Kimelberg *et al.*, 1993). Because the $(Na^+)_i$ concentration ranges between 10 and 20 mM (12) in cultured astrocytes, Na^+/K^+-ATPase is set to be readily activated when $(Na^+)_i$ raises concomitantly with glutamate uptake (Bowman and Kimelberg, 1984). The effect of glutamate is likely due to the mobilization of a subunit of the pump that is highly sensitive to ouabain, probably the α_2 subunit (Pellerin and Magistretti, 1997).

There is ample evidence from studies in a variety of cellular systems, including the brain, kidney, vascular smooth muscle, and erythrocytes, that increases in the activity of the Na^+/K^+ ATPase stimulate glucose uptake and glycolysis (Parker and Hoffman, 1967; Lipton and Robacker, 1983). Consistent with this view, glutamate stimulates the glycolytic processing of glucose in astrocytes, as indicated by the increase in lactate release (Fig. 16.1). The proposed stoichoimetry of the molecular steps involved in the coupling between glutamate uptake and glucose utilization is the following: the uptake of one glutamate with three sodium ions triggers the entry of one glucose, which, through glycolysis, produces two ATPs, one of which is consumed by the pump, resulting in the extrusion of three sodium ions, whereas the other fuels the enzymatic conversion of glutamate to glutamine, an ATP-requiring, astrocyte-specific reaction catalyzed by glutamine synthase (Fig. 16.1); the glycolytic processing of glucose results in approximately two lactate molecules produced per one glucose

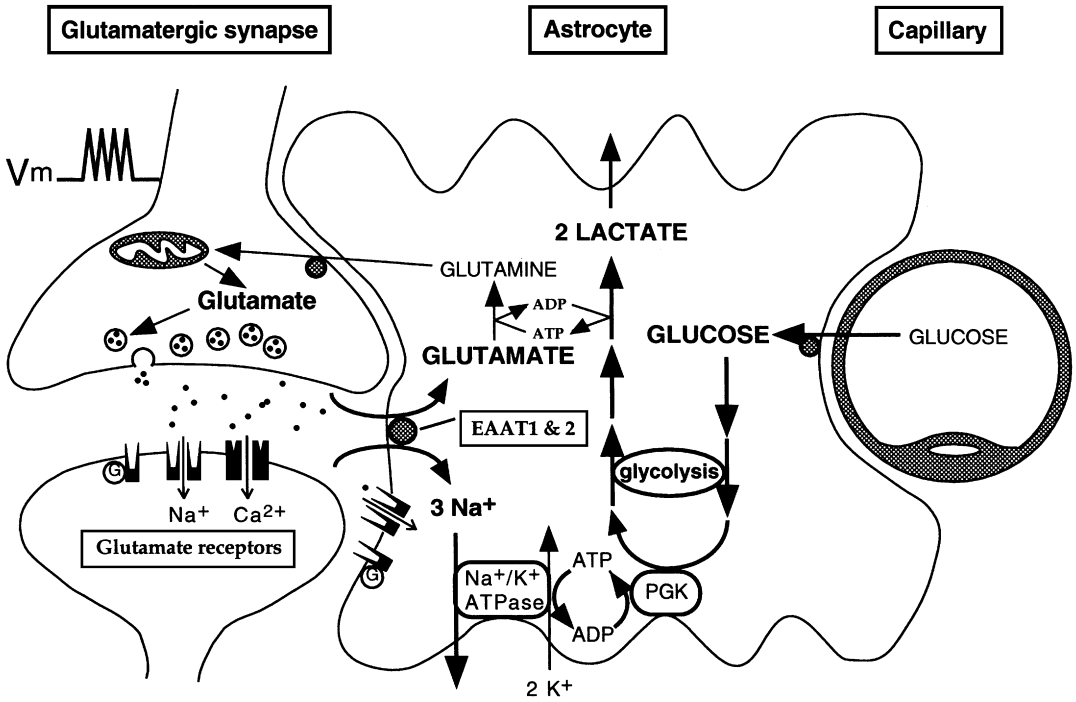

FIG. 16.1. Schematic representation of the mechanism for glutamate-induced glycolysis in astrocytes during physiological activation. At glutamatergic synapses, presynaptically released glutamate depolarizes postsynaptic neurons by acting at specific receptor subtypes. The action of glutamate is terminated by an efficient glutamate uptake system located primarily in astrocytes. Glutamate is cotransported with Na^+, resulting in an increase in the intraastrocytic concentration of Na^+, leading to an activation of the astrocyte Na^+/K^+-ATPase. Activation of Na^+/K^+-ATPase stimulates glycolysis, i.e., glucose use and lactate production. Once released by astrocytes, lactate can be taken up by neurons and serves them as an adequate energy substrate. (For graphic clarity, only lactate uptake into presynaptic terminals is indicated. However, this process could also occur at the postsynaptic neuron.) This model, which summarizes *in vitro* experimental evidence indicating glutamate-induced glycolysis, is taken to reflect cellular and molecular events occurring during activation of a given cortical area.

molecule, i.e., a stoichoimetrical relationship between glucose and lactate, as expected (Fig. 16.1).

These data indicate that glutamate stimulates aerobic glycolysis (i.e., the transformation of glucose into lactate in the presence of sufficient oxygen) in astrocytes by a mechanism involving an activation of the Na^+/K^+ ATPase. In this context, it is important to note that *in vivo*, the main mechanism that accounts for the activation-induced 2-DG uptake is represented by the activity of the Na^+/K^+-ATPase (Sokoloff, 1991).

This view raises the question of the usefulness of lactate as an energy substrate for neurons. A vast array of experimental data has accumulated over the years, indicating that *in vitro*, lactate can adequately maintain synaptic activity in the absence of glucose (Schurr *et al*., 1988; Tsacopoulos and Magistretti, 1996). *In vivo*, lactate is not an adequate substrate, as it crosses the blood–brain barrier only marginally (Pardridge and Oldendorf, 1977); however, if formed within the brain parenchyma through the mechanism described earlier (Fig. 16.1) or if applied to *in vitro* preparations, lactate may in fact be consumed preferentially to glucose, particularly during periods of intense activity (Larrabee, 1995).

Immunohistochemical data indicate a cellular distribution of lactate dehydrogenase (LDH) that is consistent with the model. LDH is the enzyme that catalyzes the interconversion of lactate and pyruvate. In the human hippocampus and visual cortex, immunoreactivity against LDH_5 (the form enriched in lactate-producing tissues) is restricted to a population of astrocytes, whereas neurons are stained only by an antibody directed against LDH_1 (the form enriched in lactate-consuming tissues) (Bittar *et al*., 1996). These data thus support the idea that some astrocytes would preferentially process glucose glycolytically into lactate, which, once released, could be transformed by neurons into pyruvate and enter the TCA cycle to serve as an energy fuel. It should be stressed that one molecule of lactate entering the TCA cycle through the LDH-catalyzed reaction can yield, in normoxic conditions, 17 ATPs.

In summary, because glutamate release occurs following the modality-specific activation of a brain region, these data and the proposed model are consistent with the view that during activation, glutamate uptake into astrocytes leads to increased glucose utilization and lactate production, which can be subsequently used by neurons to meet their energy needs. Further support for this notion of an ''astrocyte–neuron lactate shuttle'' in the brain (Fig. 16.2) has been provided by the identification of two lactate transporters, MCT-1 and MCT-2, selectively expressed in astrocytes or neurons. Thus, MCT-1 is enriched in astrocytes in culture, whereas MCT-2 is expressed predominantly in neurons (Bröer *et al*., 1997; Pellerin *et al*., 1998).

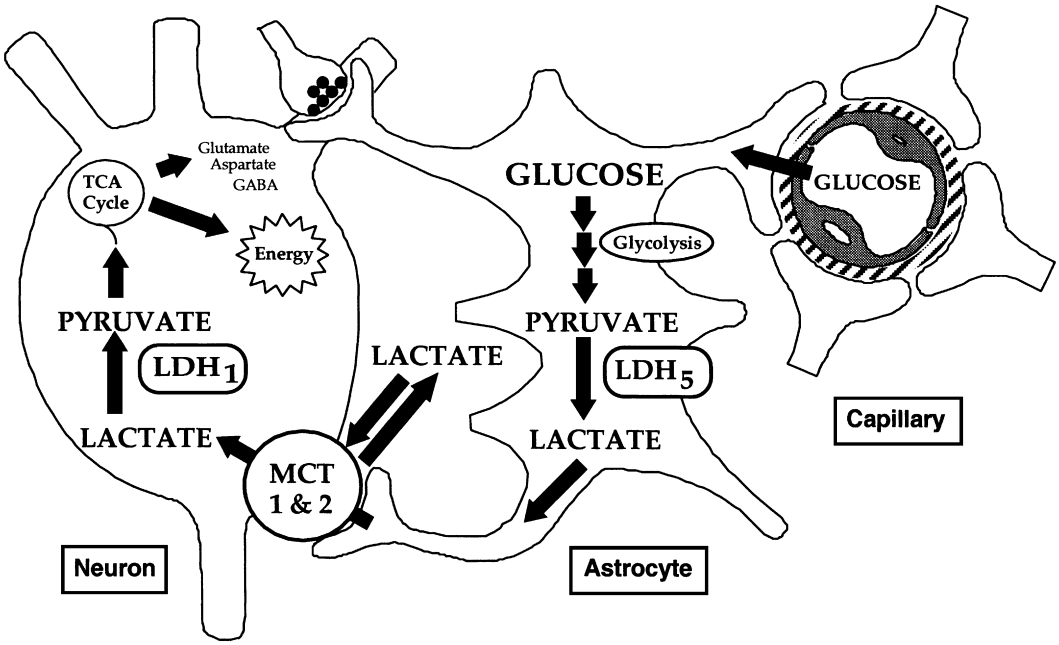

FIG. 16.2. Schematic representation of the proposed astrocyte–neuron lactate shuttle. Following neuronal activation and synaptic glutamate release, glutamate reuptake into astrocytes triggers increased glucose uptake from capillaries via activation of an isoform of the Na^+/K^+-ATPase, which is highly sensitive to ouabain, possibly the $\alpha 2$ isoform (Pellerin and Magistretti, 1994, 1997). Glucose is then processed glycolytically to lactate by astrocytes, which are enriched in the muscle form of LDH (LDH_5). The exchange of lactate between astrocytes and neurons is operated by monocarboxylate transporters (MCTs). Lactate is then converted to pyruvate, as neurons contain the heart form of LDH (LDH_1). Pyruvate, via the formation of acetyl-CoA by pyruvate dehydrogenase (PDH), enters the TCA cycle, thus generating 17 ATP molecules per lactate molecule.

IV. Relevance to Functional Brain Imaging

Results obtained in a variety of *in vivo* paradigms both in laboratory animals and in humans support the existence of such a transient lactate production during activation. Thus, marked increases in the concentration of extracellular lactate have been monitored by microdialysis studies in rat striatum and hippocampus during physiological sensory stimulation (Fellows *et al*., 1993). This activity-linked increase in lactate is completely inhibited by the glutamate uptake inhibitor THA, thus providing further support to the existence of glutamate-stimulated glycolysis during activation (Fray *et al*., 1996). Magnetic resonance spectroscopy (MRS) in humans has also revealed that during physiological activation of the visual system, a transient lactate peak is observed in the primary visual cortex (Prichard *et al*., 1991; Frahm *et al*., 1996). These microdialysis and MRS data *in vivo* would support the notion of a transient glycolytic processing of glucose during activation. PET analyses pioneered by Raichle and Fox have suggested that oxygen consumption does not increase commensurately with blood flow and glucose utilization in activated brain areas (Fox *et al*., 1988), raising the much debated possibility that an activity-dependent glycolytic processing of glucose may occur. Other investigators have found that the degree of uncoupling between glucose utilization and oxygen consumption during activation may actually vary, and even may not occur, depending on the stimulations used (Marrett *et al*., 1995). In addition, using [^{13}C]-glucose MRS, Shulman and colleagues have reported data consistent with a significant increase in oxygen utilization during activation (Hyder *et al*., 1996). Finally, results by Sibson *et al*. (1998) indicate that glutamatergic transmission and glucose oxidation monitored *in vivo* by MRS show a linear relationship with a stoichiometry of 1:1.

On the basis of studies at the cellular level, the model proposed for the coupling between neuronal activity and glucose utilization (Fig. 16.1) would be consistent with an initial glycolytic processing of glucose occurring in astrocytes during activation, resulting in a transient lactate overproduction, followed by a recoupling phase during which lactate would be oxidized by neurons.

Finally, the model proposed in Figs. 16.1 and 16.2 is consistent with the notion that signals detected during physiological activation in humans with ^{18}F-2-DG PET may reflect predominantly uptake of the tracer into astrocytes (Pellerin and Magistretti, 1994; Magistretti *et al*., 1999). This conclusion does not question the validity of the 2-DG-based techniques, rather it provides a cellular and molecular basis for these functional brain-imaging techniques.

V. Brain Energy Metabolism and Aging

A. Brain Glucose Metabolism in Healthy Aging

Studies on age-related changes in resting CMRglc have been discrepant, probably because of methodological difficulties and differences in criteria selection. For example, use of the region-of-interest approach does not provide sufficient anato-

mical references and reliability across reports. Measurements should indeed be corrected for cortical atrophy. In some studies the spatial resolution of PET-used cameras is low. A variable among studies is the selection criteria for subjects. Criteria should ideally allow the evaluation of the effect of age unconfounded by diseases associated with advancing age.

During normal aging, the brain shows a reduction in whole brain glucose metabolism (De Santi *et al*., 1995; Loessner *et al*., 1995; Moeller *et al*., 1996; Murphy *et al*., 1996; Petit-Taboue *et al*., 1998). This decline in global glucose metabolic rate ranges from 2% per decade (Moeller *et al*., 1996) to 6% (Petit-Taboue *et al*., 1998). Most studies indicate that this age-related decrease in glucose metabolism is prominent in the frontal and temporal lobes (De Santi *et al*., 1995; Loessner *et al*., 1995; Murphy *et al*., 1996). De Santi *et al*. (1995) reported a stronger metabolic change with age for the frontal lobe than for the temporal lobe. This metabolic decline in frontal lobe is more important in the premotor, orbitofrontal (Petit-Taboue *et al*., 1998), dorsolateral (De Santi *et al*., 1995), medial frontal, and frontal operculum areas (Moeller *et al*., 1996). Regional cerebral metabolic differences appear to exist within the temporal lobe (Eberling *et al*., 1995), with largest decreases in the anterior temporal cortex. Certain studies have also reported age-related decrements in glucose metabolism in the parietal cortex (Moeller *et al*., 1996; Murphy *et al*., 1996). A rCMRglc decline was also shown in the association neocortex, particularly in anterior temporal and perisylvian temporoparietal areas, the anterior cingulate cortex, and the insula, as well as in the caudate nucleus and the anterior thalamus (Petit-Taboue *et al*., 1998). In a study of 40 young and 31 old male individuals, an age-related glucose metabolism change in the hippocampus was also reported (De Santi *et al*., 1995). Posterior brain areas, such as the occipital cortex and cerebellum, may in contrast show an increased metabolic activity with age (De Santi *et al*., 1995; Loessner *et al*., 1995; Moeller *et al*., 1996; Petit-Taboue *et al*., 1998). Similar observations have been made in brain stem and basal ganglia (Moeller *et al*., 1996). In summary, there appears to be an age-related shift of regional metabolism that decreases in the anterior and lateral areas, accompanied by an increase in more posterior regions. This decreased anterior–posterior gradient may be associated with a decreased subcortical–cortical gradient.

B. Brain Glucose Metabolism in At-Risk Individuals for Alzheimer's Disease

Several factors appear to be associated with an individual at risk for AD. Familial Alzheimer disease is a form of AD with an onset before 60 years of age. At least three fully penetrant genes have been associated with the development of this early-onset AD: the amyloid precursor protein gene on chromosome 21, the presenilin-1 on chromosome 14, and presenilin-2 on chromosome 1 (see Chapter 22). Late-onset AD (after the age of 60) is familial or sporadic and is associated with a gene on chromosome 19, the apolipoprotein E (APOE). This gene has three allelic variants, types 2, 3, and 4, and five common genotypes: 2/3, 3/3, 2/4, 3/4, and 4/4; the presence of APOE4 has been validated as a risk factor for AD (Saunders *et al*., 1993). Individuals with age-associated memory impairment, having severe memory loss and no other type of cognitive impairment, show a progression to AD. Over 45% of them developed AD in a 48-month follow-up (Bowen *et al*., 1997) and up to 55% in a 54-month follow-up (Petersen *et al*., 1995). APOE4 is a strong predictor of the outcome of these patients. Two other conditions put an individual at risk for AD: twins, monozygotic or dizygotic, when discordant for AD, and Down syndrome.

Studies of cognitive normal individuals at risk for AD reveal a pattern of reduced cerebral metabolic rate for glucose in the parietal and temporal neocortex (Azari *et al*., 1994; Newman *et al*., 1994; Kennedy *et al*., 1995; Small *et al*., 1995; Reiman *et al*., 1996; Minoshima *et al*., 1997; Pietrini *et al*., 1997; see Chapter 18). Some of these studies also reveal global (Newman *et al*., 1994; Kennedy *et al*., 1995; Pietrini *et al*., 1997) and prefrontal (Wang *et al*., 1994; Reiman *et al*., 1996) hypometabolism. Two studies also reported a significant metabolic reduction in the posterior cingulate cortex (Reiman *et al*., 1996; Minoshima *et al*., 1997).

In summary, studies of individuals at risk for AD confirm that metabolic dysfunction precedes the onset of the clinical symptoms of dementia. An important consideration in evaluating the brain glucose metabolism pattern in AD and in the preclinical stages of AD is the characterization of the changes in CMRglc occurring during normal aging. In healthy aging, a global reduced metabolic rate for glucose is observed, which is most prominent in frontal, temporal, and parietal lobes. Studies of cognitively unaffected individuals at risk for AD reveal temporal and parietal hypometabolism. Some of these studies also reveal a global and a prefrontal deficit. Importantly, pattern of glucose metabolism described in the preclinical stages of AD appear to differ from that observed in normal aging, as the earliest changes in the preclinical stages of AD appear to affect mostly the posterior cingulate cortex.

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References

Azari, N. P., Pettigrew, K. D., Pietrini, P., Horwitz, B., and Schapiro, M. B. (1994). Detection of Alzheimer's disease pattern of cerebral metabolism in Down syndrome. *Dementia* **5**, 69–78.

Bittar, P., Charnay, Y., Pellerin, L., Bouras, C., and Magistretti, P. J. (1996). Selective distribution of lactate dehydrogenase isoenzymes in neurons and astrocytes of the human brain. *J. Cereb. Blood Flow Metab.* **16**, 1079–1089.

Bowen, J., Teri, L., Kukull, W., McCormick, W., McCurry, S., and Larson, E. (1997). Progression to dementia in patients with isolated memory loss. *Lancet* **349**, 763–765.

Bowman, C. L., and Kimelberg, H. K. (1984). Excitatory amino acids directly depolarize rat brain astrocytes in primary culture. *Nature (London)* **311**, 656–659.

Bröer, S., Rahman, B., Pellegri, G., Pellerin, L., Martin, J.-L., Verleysdonk, S., Hamprecht, B., and Magistretti, P. J. (1997). Comparison of lactate transport in astroglial cells and monocarbosylate transporter 1 (MCT1) expressing xenopus laevis oocytes. *J. Biol. Chem.* **272**, 30096–30102.

De Santi, S., de Leon, M. J., Convit, A., Tarshish, C., Rusinek, H., Tsui, W. H., Sinaiko, E., Wang, G. J., Bartlet, E., and Volkow, N. (1995). Age-related changes in brain: II. Positron emission tomography of frontal and temporal lobe glucose metabolism in normal subjects. *Psychiatr. Q.* **66**, 357–370.

Eberling, J. L., Nordahl, T. E., Kusubov, N., Reed, B. R., Budinger, T. F., and Jagust, W. J. (1995). Reduced temporal lobe glucose metabolism in aging. *J. Neuroimaging* **5**, 178–182.

Edvinsson, L., MacKenzie, E. T., and McCulloch, J. (1993). Cerebral blood flow and metabolism. *In* "Cerebral Blood Flow and Metabolism" pp. 161–168. Raven Press, New York.

Fellows, L. K., Boutelle, M. G., and Fillenz, M. (1993). Physiological stimulation increases nonoxidative glucose metabolism in the brain of the freely moving rat. *J. Neurochem.* **60**, 1258–1263.

Fox, P. T., Raichle, M. E., Mintun, M. A., and Dence, C. (1988). Non-oxidative glucose consumption during focal physiologic neural activity. *Science* **241**, 462–464.

Frackowiak, R. S. J., Lenzi, G. L., Jones, T., and Heather, J. D. (1980). Quantitative measurement of regional cerebral blood flow and oxygen metabolism in man using ^{15}O and positron emission tomography: theory, procedure and normal values. *J. Comput. Assist. Tomogr.* **4**, 727–736.

Frahm, J., Krüger, G., Merboldt, K.-D., and Kleinschmidt, A. (1996). Dynamic uncoupling and recoupling of perfusion and oxidative metabolism during focal brain activation in man. *Magn. Reson. Med.* **35**, 143–148.

Fray, A. E., Forsyth, R. J., Boutelle, M. G., and Fillenz, M. (1996). The mechanisms controlling physiologically stimulated changes in rat brain glucose and lactate — a microdialysis study. *J. Physiol. (London)* **496**, 49–57.

Hyder, F., Chase, J. R., Behar, K. L., Mason, G. F., Siddeek, M., Rothman, D. L., and Shulman, R. G. (1996). Increased tricarboxylic acid cycle flux in rat brain during forepaw stimulation detected with ^{1}H [^{13}C] NMR. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 7612–7617.

Kennedy, A. M., Frackowiak, R. S., Newman, S. K., Bloomfield, P. M., Seaward, J., Roques, P., Lewington, G., Cunningham, V. J., and Rossor, M. N. (1995). Deficits in cerebral glucose metabolism demonstrated by positron emission tomography in individuals at risk of familial Alzheimer's disease. *Neurosci. Lett.* **186**, 17–20.

Kety, S. S. (1957). The general metabolism of the brain in vivo. *In* "The Metabolism of the Nervous System" (D. Richter, ed.), pp. 221–237. Pergamon, London.

Kety, S. S., and Schmidt, C. F. (1948). The nitrous oxide method for the quantitative determination of cerebral blood flow in man: Theory, procedure and normal values. *J. Clin. Invest.* **27**, 476–483.

Kimelberg, H. K., Jalonen, T., and Walz, W. (1993). Regulation of brain microenvironment: transmitters and ions. *In* "Astrocytes: Pharmacology and Function" (S. Murphy, ed.), p. 193. Academic Press, San Diego, CA.

Larrabee, M. G. (1995). Lactate metabolism and its effects on glucose metabolism in an excised neural tissue. *J. Neurochem.* **64**, 1734–1741.

Lipton, P., and Robacker, K. (1983). Glycolysis and brain function: $[K^+]0$ stimulation of protein synthesis and K^+ uptake require glycolysis. *FASEB J.* **42**, 2875–2880.

Loessner, A., Alavi, A., Lewandrowski, K. U., Mozley, D., Souder, E., and Gur, R. E. (1995). Regional cerebral function determined by FDG-PET in healthy volunteers: Normal patterns and changes with age. *J. Nucl. Med.* **36**, 1141–1149.

Magistretti, P. J., and Pellerin, L. (1996). Cellular bases of brain energy metabolism and their relevance to functional brain imaging: Evidence for a prominent role of astrocytes. *Cereb. Cortex* **6**, 50–61.

Magistretti, P. J., Pellerin, L., Rothman, D. L., and Shulman, R. G. (1999). Energy on demand. *Science* **283**, 496–497.

Marrett, S., Meyer, E., Kuwabara, H., Evans, A., and Gjedde, A. (1995). Differential increases of oxygen metabolism in visual cortex. *J. Cereb. Blood Flow Metab.* **15**, S80.

Minoshima, S., Giordani, B., Berent, S., Frey, K. A., Foster, N. L., and Kuhl, D. E. (1997). Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease. *Ann. Neurol.* **42**, 85–94.

Moeller, J. R., Ishikawa, T., Dhawan, V., Spetsieris, P., Mandel, F., Alexander, G. E., Grady, C., Pietrini, P., and Eidelberg, D. (1996). The metabolic topography of normal aging. *J. Cereb. Blood Flow Metab.* **16**, 385–398.

Murphy, D. C., DeCarli, C., McIntosh, A. R., Daly, E., Mentis, M. J., Pietrini, P., Szczepanik, J., Schapiro, M. B., Grady, C. L., Horwitz, B., and Rapoport, S. I. (1996). Sex differences in human brain morphometry and metabolism: An in vivo quantitative magnetic resonance imaging and positron emission tomography study on the effect of aging. *Arch. Gen. Psychiatry* **53**, 585–594.

Newman, S., Kennedy, A., and Frackowiak, R. (1994). Presymptomatic cognitive changes in an individual with familial Alzheimer's disease: A neuropsychological and PET study. *J. Neurol.* **241**(Suppl.), 127.

Ogawa, S., Tank, D. W., Menon, R., Ellermann, J. M., Kim, S. G., Merkle, H., and Ugurbil, K. (1992). Intrinsic signal changes accompanying sensory stimulation: Functional brain mapping with magnetic resonance imaging. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 5951–5955.

Pardridge, W. M., and Oldendorf, W. H. (1977). Transport of metabolic substrates through the blood-brain barrier. *J. Neurochem.* **28**, 5–12.

Parker, J. C., and Hoffman, J. F. (1967). The role of membrane phosphoglycerate in the control of glycolytic rate by active cation transport in human red blood cells. *J. Gen. Physiol.* **60**, 893–916.

Pellerin, L., and Magistretti, P. J. (1994). Glutamate uptake into astrocytes stimulates aerobic glycolysis: A mechanism coupling neuronal activity to glucose utilization. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 10625–10629.

Pellerin, L., and Magistretti, P. J. (1996). Excitatory amino acids stimulate aerobic glycolysis in astrocytes via an activation of the Na^+/K^+-ATPase. *Dev. Neurosci.* **18**, 336–342.

Pellerin, L., and Magistretti, P. J. (1997). Glutamate uptake stimulates Na^+/K^+ ATPase activity in astrocytes via activation of a distinct subunit highly sensitive to ouabain. *J. Neurochem.* **69**, 2132–2137.

Pellerin, L., Pellegri, G., Bittar, P. G., Bouras, C., Martin, J.-L., Stella, N., and Magistretti, P. J. (1998). Evidence supporting the existence of an activity-dependent astrocyte-neuron lactate shuttle. *Dev. Neurosci.* **20**, 291–299.

Peters, A., Palay, S. L., and Webster, H. deF. (1991). "The Fine Structure of the Nervous System: Neurons and their Supporting Cells." Saunders, Philadelphia.

Petersen, R. C., Smith, G. E., Ivnik, R. J., Tangalos, E. G., Schaid, D. J., Thibodeau, S. N., Kokmen, E., Waring, S. C., and Kurland, L. T. (1995). Apolipoprotein E status as a predictor of the development of Alzheimer's disease in memory-impaired individuals. *JAMA, J. Am. Med. Assoc.* **273**, 1274–1278.

Petit-Taboue, M. C., Landeau, B., Desson, J. F., Desgranges, B., and Baron, J. C. (1998). Effects of healthy aging on the regional cerebral metabolic rate of glucose assessed with statistical parametric mapping. *Neuroimage* **7**, 176–184.

Phelps, M. E., Huang, S. C., Hoffman, E. J., Selin, C., Sokoloff, L., and Kuhl, D. E. (1979). Tomographic measurement of local cerebral glucose metabolic rate in humans with (F-18)2-fluoro-2-deoxy-d-glucose: Validation of method. *Ann. Neurol.* **6**, 371–388.

Pietrini, P., Dani, A., Furey, M. L., Alexander, G. E., Freo, U., Grady, C. L., Mentis, M. J., Mangot, D., Simon, E. W., Horwitz, B., Haxby, J. V., and Shapiro, M. B. (1997). Low glucose metabolism during brain stimulation in older Down's syndrome subjects at risk for Alzheimer's disease prior to dementia. *Am. J. Psychiatry* **154**, 1063–1069.

Prichard, D., Rothman, D., Novotny, E., Petroff, O., Kuwabara, T., Avison, M., Howseman, A., Hanstock, C., and Shulman, R. (1991). Lactate rise detected by ^{1}H NMR in human visual cortex during physiologic stimulation. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 5829–5831.

Reiman, E. M., Caselli, R. J., Yun, L. S., Chen, K., Bandy, D., Minoshima, S., Thibodeau, S. N., and Osborne, D. (1996). Preclinical evidence of

Alzheimer's disease in persons homozygous for the epsilon 4 allele for apolipoprotein E. *N. Engl. J. Med.* **334**, 752–758.

Roy, C. S., and Sherrington, C. S. (1890). On the regulation of the blood supply of the brain. *J. Physiol. (London)* **11**, 85–108.

Saunders, A. M., Strittmatter, W. J., and Schmechel, D. E. (1993). Association of apolipoprotein E allelee 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* **43**, 1467–1472.

Schurr, A., West, C. A., and Rigor, B. M. (1988). Lactate-supported synaptic function in the rat hippocampal slice preparation. *Science* **240**, 1326–1328.

Sibson, N. R., Dhankhar, A., Mason, G. F., Rothman, D. L., Behar, K. L., and Shulman, R. G. (1998). Stoichiometric coupling of brain glucose metabolism and glutamatergic neuronal activity. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 316–321.

Small, G. W., Mazziotta, J. C., Collins, M. T., Baxter, L. R., Phelps, M. E., Mandelkern, M. A., Kaplan, A., La Rue, A., Adamson, C. F., and Chang, L. (1995). Apolipoprotein E type 4 allele and cerebral glucose metabolism in relatives at risk for familial Alzheimer disease. *JAMA, J. Am. Med. Assoc.* **273**, 942–947.

Sokoloff, L. (1960). The metabolism of the central nervous system in vivo. *In* ''Handbook of Physiology-Neurophysiology'' (J. Field, H. W. Magoun, and V. E. Hall, eds.), pp. 1843–1864. American Physiological Society, Washington, DC.

Sokoloff, L. (1991). Relationship between functional activity and energy metabolism in the nervous system: Whether, where and why? *In* ''Brain Work and Mental Activity'' (N. A. Lassen, D. H. Ingvar, M. E. Raichle, and L. Friberg, eds.), p. 52. Munksgaard, Copenhagen.

Sokoloff, L., Reivich, M., Kennedy, C., Des Rosiers, M. H., Patlak, C. S., Pettigrew, K. D., Sakurada, O., and Shinohara, M. (1977). The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anesthetized albino rat. *J. Neurochem.* **28**, 897–916.

Takahashi, S., Driscoll, B. F., Law, M. J., and Sokoloff, L. (1995). Role of sodium and potassium ions in regulation of glucose metabolism in cultured astroglia. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 4616–4620.

Tsacopoulos, M., and Magistretti, P. J. (1996). Metabolic coupling between glia and neurons. *J. Neurosci.* **16**, 877–885.

Villringer, A., and Dirnagl, U. (1995). Coupling of brain activity and cerebral blood flow: Basis of functional neuroimaging. *Cerebrovasc. Brain Metab. Rev.* **7**, 240–276.

Wang, G., Volkow, N. D., Wolf, A. P., Brodie, J. D., and Hitzemann, R. J. (1994). Intersubject variability of brain glucose metabolic measurements in young normal males. *J. Nucl. Med.* **35**, 1457–1466.

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Functional Imaging in Cognitively Intact Aged People

This chapter reviews the functional correlates of perception, attention, and memory deficits that occur over the course of normal aging. Two general patterns of findings emerge from functional neuroimaging studies of cognitive aging. One is characterized by age-related *declines* in brain activation in functional networks important for cognition, and the other by age-related *increases* in brain activation in other brain regions that are not typically associated with the cognitive process under study. Age-related reductions in functional brain activity are more pronounced during demanding tasks that require self-initiated processes, but are abated during supportive tasks that guide appropriate information processing. Age-related increases in functional brain networks occur during both relatively simple perceptual tasks and during more complex, demanding memory tasks and may reflect inefficient processing and/or compensatory changes in the elderly. The most direct way to distinguish these alternative interpretations is via direct investigations of the relationship between brain activity and behavioral performance. Such investigations have been carried out by only a few investigators and should be a major focus of future research, as their outcome will help shape future rehabilitation efforts.

I. Introduction

The fact that the majority of older adults live their later years free of neurodegenerative disease is reassuring, but even normal aging is associated with perceptual and cognitive changes that impinge on older adults' quality of life. Section II describes functions that decline or are spared by aging. Due to space limitations, we have limited our review to those functions that have been tested in neuroimaging studies, but interested readers are encouraged to start with the two editions of the *"Handbook of Aging and Cognition"* (Craik and Salthouse, 1992, 2000) for more thorough reviews of cognitive aging. Section III reviews results from nonhuman animal studies, neuropsychological studies of patients with brain damage, and neuroimaging studies with younger adults that help elucidate the brain regions that are important for various cognitive functions. Sections IV and V review functional neuroimaging studies that have been conducted with healthy older adults and describe how brain activation during nonmemory and memory tasks differs between younger and older adults. All of the cognitive aging studies described used positron emission tomography (PET), as studies using functional magnetic resonance imaging (fMRI) have not yet been published; however, readers interested in the effects of aging on sensorimotor functioning are referred to fMRI studies by Ross *et al*. (1997), Taoka *et al*. (1998), and D'Esposito *et al*. (1999). Finally, Section VI combines the available aging PET data and attempts to identify brain regions in which activation in the face of a cognitive challenge is consistently altered by aging.

II. Cognitive Changes and Spared Functions in Healthy Elderly

In most cognitive aging studies, particularly in neuroimaging studies of aging, strict inclusion criteria are applied in order to rule out extracognitive influences on performance. Typically, to be included in a study of cognitive aging, participants must live independently (i.e., not in a nursing home), be relatively well educated, and have normal or corrected-to-normal vision and audition. In addition, participants are screened for disease that might compromise brain function, such as cardiovascular disease, history of stroke or neurological disorders, history of psychiatric illness, or drug abuse, as well as for use of medications that might affect brain function. One result of these criteria is that the older adults tested in most studies are archetypes of ''successful'' aging, and thus may not represent the average older individual; however, one can infer that any deficits in cognition or in brain function identified in these older adults would be even greater in their less fortunate peers. With this in mind, we review below perceptual and cognitive functions that are changed or spared by normal (i.e., successful) aging.

A. Perception and Attention

The amount of stimuli present externally (e.g., visual and auditory information) and internally (i.e., thoughts) at any given moment far exceeds our attentional capacity, and thus we must selectively attend to particular aspects of the environment while ignoring irrelevant information. Older adults have been shown to be impaired in many aspects of selective attention, primarily because of distraction caused by irrelevant information in the environment. For example, relative to their younger counterparts, older adults are disproportionately slower to read words that have been perceptually degraded, such as h*o*r*s*e, than intact words (Allen *et al*., 1993), and to locate objects or faces when they are presented among distractors versus in isolation (Plude and Hoyer, 1986; Sekuler and Ball, 1986; Ball *et al*., 1990).

The distinction between ''bottom-up'' and ''top-down'' influences on attention helps explain these findings. Bottom-up influences on selective attention occur when a stimulus or stimulus feature is particularly salient, because it is the only thing in the environment, because features such as its color or abrupt onset make it stand out from the background, or because it activates a highly practiced, automated process such as word reading. In contrast, top-down influences are intentional, or goal directed, and act to prevent interference from irrelevant stimuli or stimulus features. In general, top-down processes require attentional control and are compromised by aging, whereas bottom-up processes run off more automatically and are spared by aging (Hasher and Zacks, 1977; Jennings and Jacoby, 1993).

B. Semantic Memory

The distinction between bottom-up and top-down processes also helps explain the presence and absence of age-related declines in different types of memory. Semantic memory refers to general knowledge of facts and words that has been built up over a lifetime of experience (Tulving, 1983). Numerous studies have found that the content and organization of older adults' semantic memory are comparable or even richer than that of younger adults in terms of vocabulary, general world knowledge, and the tendency to provide similar semantic associates to words (for reviews, see Light and Burke, 1988; Light, 1991). The fact that semantic memory ability does not differ between young and older adults is consistent with the notion that well-learned, highly automated abilities initiate bottom-up processes, which are spared by aging.

C. Perceptual Priming

Perceptual priming is a type of implicit memory that refers to the facilitated identification or completion of a stimulus by recent exposure to that item. Following the presentation of a list of words, participants are typically presented with incomplete stimuli and are asked to complete each stimulus with the first thing that comes to mind. Although no reference is made to the previous study episode and participants do not intentionally retrieve information from memory, there is a greater tendency to complete the stimuli with items that had been presented previously than with new items. For example, if ''string'' had been presented, participants would be more likely to complete the word stem ''str___'' with ''string'' than with ''strain'' or ''strong.'' Numerous studies have shown that perceptual priming is comparable in younger and older adults (Light and Burke, 1988; Light, 1991), which is in line with the idea that bottom-up processes guided by the stimulus environment remain stable across the life span.

D. Working Memory

An example of top-down processes in memory functioning is working memory, which refers to the active maintenance and manipulation of information held in mind (Baddeley, 1986). Examples of working memory include everyday activities, such as mental arithmetic, and laboratory tests, such as backwards digit span or tests in which participants must hold a picture or face in mind over a delay and then determine which of two perceptually similar stimuli it matches. Working memory is to be contrasted with primary memory (Waugh and Norman, 1965), which is the short-term maintenance of information with no requirement to manipulate the information (e.g., remembering a telephone number until the number is dialed). In general, age-related differences in primary memory are slight or nonexistent, whereas age-related deficits in working memory are more robust (e.g., Craik, 1986; Dobbs and Rule, 1989).

E. Episodic Memory

Semantic memory is distinguished from episodic memory, which refers to conscious memory for a particular event or episode in the past, including contextual information such as when and where the episode occurred (Tulving, 1983). For example, semantic memory allows one to know the meaning of the words ''sweater,'' ''bean,'' and ''wrench,'' but episodic memory allows one to remember that those words were presented in a memory list a few minutes ago. Numerous studies have demonstrated that episodic memory is quite vulnerable to aging in that older adults tend to remember less information and less contextual detail regarding particular events that have been presented (for reviews, see Craik *et al*., 1995; Balota *et al*., 2000). Episodic memory involves several stages of processing, including an encoding stage in which information is perceived, analyzed, and related to previously stored information and a retrieval stage in which information is searched for (via an external or self-generated retrieval cue) and brought back into consciousness. Previous research has found that older adults suffer both encoding and retrieval deficits. Evidence in favor of an age-related encoding deficit includes findings that older adults are less likely to engage in the types of operations that facilitate proper learning, such as mental imagery (Treat and Reese, 1976), elaborative encoding to make the information semantically rich and distinctive (Rankin and Collins, 1986), and active organization of information (Smith, 1980), but when older adults are instructed to use these strategies, their memory performance improves. Evidence in favor of a retrieval deficit includes the fact that episodic memory impairments are typically larger on tests of recall (free and cued recall) than on tests of recognition (Schonfield and Robertson, 1966). These findings apply to a variety of stimulus types. For example, both younger and older adults remember pictures better than words, but age-related

decrements in memory for pictures are larger when tested using free or cued recall (Puglisi and Park, 1987) than when tested using recognition (Till *et al*., 1982; Park *et al*., 1986). Similarly, Bartlett and Leslie (1986) found no age-related decline in the recognition of faces when studied faces were discriminated from new faces, but found that older adults did perform more poorly when a studied face had to be discriminated from the same face pictured from different angles or displaying different expressions. Together, these results indicate that age-related episodic memory decrements, although ubiquitous, vary considerably in magnitude depending on the encoding and retrieval demands at hand.

F. Theoretical Explanations for Age-Related Cognitive Changes

Variants of the top-down/bottom-up distinction have been formulated to explain age-related deficits in episodic and working memory. For example, Jennings and Jacoby (1993) argued that conscious recollection of a past event is impaired by aging, but automatic familiarity-driven processes remain intact. More broadly, Craik (1983, 1986) proposed that the greater the demands are for attention-demanding, self-initiated operations during encoding, maintenance, or retrieval, the worse older adults perform because the amount of attentional resources available for such operations declines with age. Conversely, the more the task stimuli or task environment guides appropriate encoding and retrieval operations (i.e., a bottom-up influence), the smaller the age-related deficit. As will be seen later in this chapter, results from neuroimaging studies indicate that these results hold at the level of brain activity; specifically, age-related decrements in brain activation appear to be greater during memory tasks that require attention-demanding encoding and retrieval processes than during memory tasks that facilitate these processes.

The neural mechanisms of cognitive aging undoubtedly are numerous and complex, but one hypothesis is particularly relevant to this discussion. A number of investigators have postulated that older adults' cognitive impairments stem primarily from frontal lobe dysfunction. This hypothesis is based both on anatomical and imaging evidence that age-related brain atrophy and hypometabolism may be exaggerated in anterior brain regions (see Madden and Hoffman, 1997; Raz *et al*., 1997; Raz, 2000; Chapter 18) and on evidence for a similarity between the types of attention and episodic memory deficits experienced by older adults and patients with frontal lobe damage (Moscovitch and Winocur, 1992; West, 1996). The major appeal of this hypothesis is that it proposes a neural basis for older adults' difficulty with ''top-down'' or controlled, attention resource-demanding cognitive processes. Indeed, many of the neuroimaging studies of younger and older adults described in Sections IV and V have found age-related differences in prefrontal lobe activity that presumably reflects age-related changes in ''top-down'' processing.

III. Brain Areas Involved in Cognition in Young Adults

Until recently, our knowledge of the functional neuroanatomy of perception and cognition was gained exclusively from work with nonhuman animals and from patients with brain damage; since the early 1990s, however, advances in functional neuroimaging techniques such as PET and fMRI have made it possible to study perception and cognition *in vivo* in healthy humans. This section reviews studies that help elucidate the neural mechanisms of perception and attention, semantic memory, working memory, and episodic memory.

A. Perception and Attention

Studies with nonhuman primates (e.g., Ungerleider and Mishkin, 1982; Felleman and Van Essen, 1991) and with patients with brain damage (e.g., Damasio *et al*., 1989; Goodale and Milner, 1992), as well as neuroimaging studies of healthy younger adults (Haxby *et al*., 1991; Sergent *et al*., 1992; Köhler *et al*., 1995), have revealed two distinct pathways in visual perception: a ventral occipitotemporal pathway involved in object perception and a dorsal occipitoparietal pathway involved in the perception of spatial relationships among objects. Neuroimaging studies also show that how an object is processed, and thus the pathway that is activated, depend on the stimulus feature to which attention is paid. For example, in a PET study conducted by Corbetta *et al*. (1991), stimulus arrays were presented that changed in shape and speed of motion, and during each scan subjects were instructed to attend selectively to one of these features. The results were consistent with the dual-pathway model; specifically, attention to shape activated ventral occipitotemporal regions, whereas attention to speed activated dorsal occipitoparietal regions; i.e., selective attention helps determine the neural mechanisms involved in processing particular features of a stimulus.

Other cognitive tasks require more complex attention. For example, in some circumstances, attention must be divided between two or more aspects of the environment, attention must be paid to one stimulus feature while other highly salient features are ignored, attention must be switched between different features of a stimulus or between parts of the environment, or directed searches must be made for additional stimulus properties. Each of these conditions has been linked to activation of the anterior cingulate, inferior parietal lobule, and dorsolateral prefrontal lobe (e.g., see Corbetta *et al*., 1990, 1991; Pardo *et al*., 1991; Kosslyn *et al*., 1994, 1995; Shallice *et al*., 1994; Fletcher *et al*., 1995; Nobre *et al*., 1997), among other brain regions depending on the particular sensory and cognitive demands of the task. These are examples of tasks that require top-down perceptual processing, and given that top-down processes are characterized as being controlled, attention demanding, and vulnerable to aging, we should find age-related declines in activation of this attentional network.

B. Semantic Memory

The use of language is one instantiation of retrieval from semantic memory. The fact that word meaning is automatically triggered by the perception of a word is perhaps best exemplified by the Stroop task (Stroop, 1935), in which reading color words printed in incompatible colors (e.g., ''red'' written in blue ink) is much more difficult than reading color words printed either in black ink or a compatible ink color (e.g., ''red'' in red ink). Early evidence from brain-damaged

patients and newer evidence using neuroimaging techniques have linked a large network of brain regions to language functioning, including Broca's area for speech production and Wernicke's area for language comprehension (for a review, see Damasio, 1981), as well as visual or auditory association cortices (depending on the mode of presentation), and left inferior frontal cortex (for a review, see Price, 1998). In particular, the left inferior frontal cortex is associated with the internally driven search through semantic memory, as it is active during verb generation tasks (e.g., Wise *et al*., 1991; Warburton *et al*., 1996), and is more active during encoding tasks that require semantic processing than tasks that require perceptual processing (Grady *et al*., 1998b; Kapur *et al*., 1994).

C. Perceptual Priming

The main result from neuroimaging studies of perceptual priming is a significant *deactivation* in posterior sensory regions. For example, in the case of visual word stem completion (described in Section II,C), reliable deactivation of extrastriate cortex (Brodmann's area 19) was shown by Buckner *et al*. (1995), Blaxton *et al*. (1996), and Schacter *et al*. (1996a). These results are similar to findings obtained from single-cell recordings in monkey cortex, in which firing rates are suppressed upon the repetition of a stimulus (see Desimone, 1996). Thus, these findings suggest that prior stimulus exposure reduces the amount of perceptual processing required by that stimulus in the future, which thereby facilitates its identification or completion.

D. Working Memory

Working memory is presumed to be more demanding of attention than maintenance rehearsal because one must simultaneously hold *and* manipulate the information held in mind; thus, it should come as no surprise that working memory and complex attention tasks activate overlapping functional brain networks. Earlier research with nonhuman animals and with brain-damaged patients has linked the prefrontal cortex to spatial working memory tasks, such as delayed alternation (Oscar-Berman and Bardenhagen, 1998), and neuroimaging studies of working memory in healthy humans have found activation of the inferior parietal lobule and dorsolateral prefrontal regions (Jonides *et al*., 1993; Paulescu *et al*., 1993; Petrides *et al*., 1993a,b; Berman *et al*., 1995; Smith *et al*., 1995; Awh *et al*., 1996; Courtney *et al*., 1996; D'Esposito *et al*., 1998). Moreover, neuroimaging studies have revealed that the left hemisphere is preferentially involved in verbal working memory, whereas the right hemisphere is preferentially involved in spatial working memory (Smith and Jonides, 1997), although activation of parietal and prefrontal areas in both hemispheres increases with the memory load (Braver *et al*., 1997; Jonides *et al*., 1997).

E. Episodic Memory

For nearly half a century now, investigators have been studying the brain mechanisms involved in episodic memory processing. Lesion studies in nonhuman animals and studies with brain-damaged patients identified medial temporal areas, namely the hippocampus and adjacent cortical areas, as crucial for episodic memory (Scoville and Milner, 1957; Zola-Morgan and Squire, 1985; Zola-Morgan *et al*., 1986, 1989; Eichenbaum *et al*., 1992; Squire, 1992). In addition, patients with frontal lobe lesions suffer moderate, but significant disabilities recalling information from memory (Wheeler *et al*., 1995). Although these approaches have been an essential tool in advancing our knowledge of the neural mechanisms of cognition, one problem with lesion approaches is determining whether memory deficits are due to encoding or retrieval impairments. This distinction is particularly relevant in the study of normal aging because one long-standing issue in the cognitive aging literature is whether encoding or retrieval plays a greater role in older adults' memory impairments (Burke and Light, 1981).

A major advantage of functional neuroimaging technology is that separate images of brain activity during encoding and retrieval can be obtained. Although early neuroimaging studies failed to find consistent activation of medial temporal lobe regions during episodic memory tasks (for reviews, see Cabeza and Nyberg, 1997; Fletcher *et al*., 1997), more recent studies have reported significant medial temporal activation during encoding and retrieval in both verbal and nonverbal domains (Desgranges *et al*., 1998). Imaging studies also identify prefrontal regions in memory, although activation of the prefrontal cortex tends to be asymmetric, with more left prefrontal activation during episodic encoding and more right prefrontal activation during retrieval. This pattern, which was named HERA (hemispheric encoding-retrieval asymmetry) by Tulving *et al*. (1994b), describes a very general functional pattern of brain activity during episodic memory and, as such, is susceptible to exceptions. For example, the left prefrontal cortex is often activated by retrieval, particularly during more difficult or demanding retrieval tasks (Nolde *et al*., 1998).

Posterior regions are also activated during episodic memory tasks. Some of these activations can be linked to the perception of the items being encoded or of the retrieval cue (see Section III,A). In addition, retrieval is frequently associated with parietal activation, both in the inferior parietal lobule (Brodmann's area 40) and in medial regions such as the precuneus (Cabeza and Nyberg, 1997; Desgranges *et al*., 1998). Finally, the nature of encoding or retrieval affects the pattern of brain activation. ''Deeper'' or more elaborative semantic encoding, relative to more ''shallow'' or perceptually based encoding, is associated with greater activation in left prefrontal (Kapur *et al*., 1994; Grady *et al*., 1998b) and medial temporal (Nyberg *et al*., 1998) regions. There is also considerable controversy over the significance of right prefrontal activation during retrieval. Some investigators have found that the right prefrontal cortex is active independently of the type of memory task or memory performance levels and have thus inferred that it reflects a general retrieval mental set, or ''retrieval mode'' (Kapur *et al*., 1995; Nyberg *et al*., 1995). Other investigators have found greater right prefrontal activation during more difficult retrieval conditions, suggesting that the activity reflects the degree of ''retrieval effort'' (Schacter *et al*., 1996a). Others have found greater right prefrontal activation during recognition tasks that consist of higher proportions of ''old'' than ''new'' items, suggesting that it is a marker of ''retrieval success'' (Tulving *et al*., 1994a;

Rugg *et al*., 1996). Still other investigators suggest that retrieval-related right prefrontal activity reflects postretrieval monitoring processes (Rugg *et al*., 1998). Such debates are the fodder on which good science feeds, and indeed neuroimaging studies of memory and aging (in which older adults often but not always perform more poorly on memory tests, and often have monitoring problems) may play an important role in the resolution of this debate.

IV. Age-Related Differences in Brain Activation during Nonmemory Tasks

We remind the readers that in neuroimaging studies of cognitive aging, care is taken to ensure that participants have normal or corrected-to-normal vision and hearing. However, even if the younger and older adults in a cognitive aging study have comparable sensory function as measured by standard acuity tests, it is possible for the functional neuroanatomy of perceptual processing to change with age.

The first PET studies demonstrating age-related differences in brain activity during cognition involved face and location perception in order to compare the effects of aging on the activation of the ventral and dorsal processing streams described in Section III,A (Grady *et al*., 1992, 1994). These studies involved a match-to-sample task for faces or locations in which the sample and two choices were present simultaneously on the computer screen, thus eliminating any demands on memory. Response accuracy did not differ between the two age groups, but the older adults were slower to respond on both tasks. Blood flow data revealed that in both age groups, face matching activated primarily the occipitotemporal cortex while location matching activated primarily the occipitoparietal cortex, a finding that is consistent with the dual-path model of visual processing. In addition, however, the young subjects showed greater activation in early visual processing regions (in prestriate cortex), and the older adults showed greater activation either along the ventral or dorsal pathways or outside of them (e.g., in frontal cortex). Grady *et al*. concluded that younger adults make more efficient use of occipital visual areas, whereas older adults rely on additional cortical networks, which results in slower performance.

This conclusion was supported by a path analysis of blood flow data from the face-matching task (McIntosh *et al*., 1994; Horwitz *et al*., 1995). Path analysis is a variant of structural equation modeling that can determine the strength and direction of influence between brain regions in a functional model and assess how these differ between tasks or subject groups (McIntosh and Gonzalez-Lima, 1994). Analysis showed that in both age groups there were positive influences from the ventral occipitotemporal cortex to anterior temporal regions, and from there to the inferior frontal cortex. However, older adults' data also showed a strong feedback influence from frontal cortex to occipital cortex. These analyses suggest that although the network of ventral regions involved in face processing may not change with age, the functional interaction between regions in this network is altered by aging, such that there is an age-related increase in the reliance on frontally mediated strategic monitoring of low-level processes.

More recently, Grady *et al*. (2000) investigated age-related differences in brain activity during the perception of degraded and nondegraded faces. In this study, response accuracy was higher for the younger adults (particularly with the degraded faces), but the two age groups did not differ in terms of response time. Brain activity during nondegraded face matching essentially replicated the earlier findings: activity was seen in ventral occipital temporal areas in both age groups, but the younger adults had greater activity in prestriate and bilateral parietal regions, whereas the older adults had greater activity in left ventral prefrontal cortex, left medial temporal, and bilateral fusiform areas. Degraded face matching should reduce the ability to rely on simple perceptual matching and should increase the need for strategic monitoring processes. Indeed, data from both younger and older adults showed that increasing levels of face degradation led to decreased activity in occipital regions and to increased activity in bilateral prefrontal regions.

Madden and colleagues (1996) conducted a PET study focusing on age-related differences in brain activity during word processing. The passive viewing of words and nonwords relative to a fixation control activated striate and inferior temporal cortices in the left hemisphere for both age groups, although the level of temporal lobe activation was greater in the younger than in the older adults. In another condition, participants were required to press a button in response to words but not nonwords, an activity that is presumed to involve semantic retrieval. In this case, relative to passive viewing of words and nonwords, widespread activation in ventral occipital, lingual, and fusiform regions was bilateral, but stronger in the left hemisphere. These activations were comparable in younger and older adults, except for an age-related decrease in activity in a small region situated between the left lingual and fusiform gyri. In another condition the words and nonwords were perceptually degraded by the inclusion of asterisks between each letter (e.g., H*O*R*S*E), and again participants were required to respond to words but not to nonwords. In this condition, relative to the processing of nondegraded words, additional activity was found in the left lingual gyrus, which did not differ between the two age groups. The older adults were slowed disproportionately by the distracting asterisks, a finding that is consistent with the behavioral results mentioned in Section II,A, yet there was no neural correlate of this effect. Madden *et al*. (1996) did not find activation of the left inferior prefrontal cortex during word processing probably because in no case was a strategic search through semantic memory required (see Section III,B). Nevertheless, results from this study demonstrated that in very simple tasks that activated well-learned, highly automated processes, such as word reading, age-related differences in brain activity are minimal.

Investigations of age-related differences in brain activity during attention tasks have also been conducted. Only middle-aged to older adults (ages 51–73) were tested in a study conducted by Johannsen *et al*. (1997). They presented visual and vibrotactile stimuli, and participants were required to detect changes in one stimulus (simple attention) or both (divided attention). Both simple and divided attention activated the prefrontal cortex (Brodmann's area 46) and the inferior parietal lobule (Brodmann's area 40) in the right hemisphere.

As mentioned in Section III,A, these are the same brain regions that younger adults have activated in previous studies of attention (e.g., Corbetta *et al*., 1990, 1991; Pardo *et al*., 1991). Thus, although it appears from this study that the brain networks involved in attention do not change as a function as age, we do not know whether younger adults would have activated these brain regions under these particular conditions, whether they would be activated to a greater or lesser extent, or whether the strength and direction of influence among brain regions (as assessed by path analysis) would differ with age.

Madden *et al*. (1997) also investigated simple and divided attention, but tested both younger and older adults. Two letters were preassigned as target letters, and on each trial a 3×3 grid of letters was presented and participants pressed one of two buttons depending on which target was present. In a ''central condition,'' a target letter appeared in the central grid position on every trial, and in the ''divided attention'' condition, one target was present on every trial, but its location in the grid varied from trial to trial. One could question whether this latter condition represents true divided attention (attending to two sources or types of information simultaneously), but it seems clear that the divided attention condition placed greater attentional demands on the participants than the other two conditions. Indeed, errors and reaction times increased with the task demands, particularly for the older adults.

Blood flow data revealed that the divided condition, relative to the central condition, was associated with increased activity along the occipitotemporal (i.e., ventral) processing stream for the younger adults, but in medial prefrontal regions for the older adults. These findings are broadly consistent with those of Grady *et al*. (1992, 1994), although the younger adults' activity was more lateral and the older adults' activity was more medial in the study by Madden *et al*. (1997), and suggest that while younger adults relied more on object processing to perform the divided attention task, the older adults relied more on strategic control and response monitoring.

Esposito *et al*. (1999) examined cerebral blood flow while participants performed Raven's progressive matrices, a task that taps problem solving and abstract reasoning. In this task, subjects are shown a 3×3 matrix with one empty cell and must select the missing element from a number of alternatives displayed beneath the matrix. Relative to a control task matched for sensorimotor demands, activation during the Raven's task was greater for the younger than the older adults in the left inferior parietal lobule, inferior and middle temporal lobe, left parahippocampal gyrus, and cerebellum. In addition, the younger adults showed more *suppression* of brain activity during the Raven's test relative to the control task in the posterior cingulate, bilateral frontal pole, and in superior temporal lobe regions. Thus, there was less difference in brain activity between the Raven's test and the control task in the older adults, which suggests that older adults were less sensitive to the demands of the task at hand.

In summary, every study of the effects of aging on brain activity during nonmemory tasks has found an age-related reduction in the recruitment of functional networks important for attention, and many of these studies have found an age-related increase in the recruitment of areas that are not activated by younger adults. We will defer our explanations for these findings until later because the same pattern of findings appears in the memory literature (see Section V), and thus any account of age-related decreases and increases of neural activity will need to encompass both nonmemory and memory tasks.

V. Age-Related Differences in Brain Activation during Memory Tasks

As noted in Section II, the magnitude of age-related memory decrements is sensitive to encoding and retrieval task demands; specifically, age-related memory impairments are greater when the encoding or retrieval task requires complex, self-initiated processes, but smaller when the task facilitates encoding or retrieval (Craik, 1983, 1986). Studies of working memory and episodic memory reveal that tasks requiring greater degrees of self-initiated mnemonic activity recruit more extensive frontal lobe involvement (e.g., Petrides *et al*., 1993a,b; Cabeza *et al*., 1997a). Thus, a reasonable prediction is that the behavioral findings will extend to measures of cerebral blood flow in that age-related decrements in brain activity during memory tasks will be greater when the task requires self-initiated encoding or retrieval activities and smaller when the memory task provides encoding or retrieval support. A small but growing number of neuroimaging studies of memory and aging are available from which this hypothesis can be tested. This section discusses age-related differences in brain activity during implicit memory, working memory, and episodic memory.

A. Perceptual Priming

Bäckman *et al*. (1997) investigated brain activity during a word stem perceptual priming task in younger and older adults. Behavioral data were consistent with previous studies (see Section II,A) in that there was no significant age-related difference in the amount of perceptual priming. Cerebral blood flow data showed a reduction in extrastriate cortex (Brodmann's area 19) activity during the word stem completion test relative to a control task, which in fact was greater in the older than younger adults. Although clearly more evidence is needed, results from this study are promising in that they demonstrate how bottom-up processing driven by external stimuli is maintained with age, as revealed by both behavior and blood flow.

B. Working Memory

One example of a task that requires working memory abilities is the Wisconsin card sorting task (WCST), in which participants sort cards differing in the number, shape, and color of stimuli onto one of four reference piles. Participants must discover the sorting rule (e.g., color), and after they have successfully sorted a series of cards according to this rule, the rule is switched without notice (e.g., to number). Thus, this task assesses a number of higher-order attention, problem-solving, and working memory abilities (Goldman-Rakic, 1987; Berman *et al*., 1995). Both Nagahama *et al*. (1997) and Esposito *et al*. (1999) examined age-related differences in cerebral blood flow during the WCST relative to a control task that matched the sensorimotor demands of the WCST. However, it should be noted that Nagahama *et al*. (1997) used

a modified WCST in which each stimulus card shared only one attribute with a reference card, and thus their task was less demanding than the original WCST. The other difference between these two studies is that Esposito *et al*. (1999) tested 41 adults between the ages of 18 and 80, and thus were able to examine linear relationships between age and behavioral performance or cerebral blood flow.

The results of these two studies were rather compatible, despite their procedural differences. Nagahama *et al*. (1997) found that the older adults were slower and made more errors (both perseverative and nonperseverative) than the younger adults, and Esposito *et al*. (1999) reported a significant negative correlation between age and WCST performance (percentage correct). Both studies found age-related decreases in activation during the WCST in the left dorsolateral prefrontal cortex and in the left inferior parietal lobule. These brain regions have been associated with other complex attention and working memory tasks (see Sections III,A and III,D), and thus these results suggest that there is an age-related decrement in the ability to activate brain networks involved in strategic processes such as problem-solving and response monitoring during complex or difficult tasks. In addition, Nagahama *et al*. (1997) reported age-related decreases in the activation of a number of posterior brain regions associated with visual processing, but they did not find any brain regions in which activation was greater in the older than in the younger adults. In contrast, Esposito *et al*. (1999) reported age-related decreases in activation of the anterior cingulate and cerebellum, as well as age-related increases in activation of the cuneus, bilateral frontal poles, and right parahippocampal gyrus; indeed, in the younger adults these latter three regions were suppressed during the WCST relative to its control. Discrepancies between these two studies are probably due to the different versions of the WCST used and to differences in their statistical power ($n = 6$ per age group in Nagahama *et al*.). Nevertheless, the finding from Esposito *et al*. that older adults activate a more extensive neural network is reminiscent of the findings from complex attentional tasks (Grady *et al*., 1992, 1994; Madden *et al*., 1997).

One study investigated age-related differences in cerebral blood flow during a more traditional working memory task (Grady *et al*., 1998a). On each trial, a stimulus face was presented and was followed by a 1, 6, 11, 16, or 21 sec delay, after which the stimulus face and a new face were presented, and participants identified the stimulus face. Thus, this task is similar to the face matching task used previously by these investigators (Grady *et al*., 1992, 1994, 2000), but with the added requirement to hold the face in mind over the delay period and then match it from memory. The younger adults were faster and slightly more accurate than their older counterparts, but the two age groups' response times increased similarly with increasing delay. Furthermore, the delay manipulation had similar effects for the two age groups on brain activation in a number of brain regions. For example, activity in the ventral extrastriate cortex and in an inferior part of the right prefrontal cortex decreased, whereas activity in left prefrontal regions increased with longer memory delays. These effects suggest that as the delay between study and test increased, there was a decrease in the participation of early visual processing and a decrease in memory retrieval, along with an increase in elaboration of the studied faces. Furthermore, these aspects of working memory for faces were invariant with age. Despite these similarities, during the working memory task as a whole, compared to the control task, the older adults had greater activity in left prefrontal regions. Grady *et al*. suggested that this result may reflect an age-related increase in the reliance on elaborative strategies, such as relating the faces to someone familiar, or an age-related increase in general working memory demands (such as response monitoring and attention). In contrast, the younger adults had greater activity in the right ventrolateral prefrontal cortex, which has been associated with the maintenance of information online in order to facilitate discrimination (Petrides *et al*., 1993a). In addition, there were age-related differences in brain activity in other regions that showed the greatest change in activity from the 1 to 6 sec delay. One of these regions was the left hippocampus, in which activity increased from the 1 to 6 sec delay in the younger adults, but decreased over these delays in the older adults, suggesting that older adults are less able to engage medial temporal lobe structures that are important for remembering (see Section III,E). In summary, this study indicated that although younger and older adults' neural response to a working memory task is similar in a number of ways, the older adults seem to approach the task less efficiently.

C. Episodic Memory

The remaining neuroimaging studies of memory and aging focused on episodic memory and scanned participants separately during encoding and/or retrieval. We discuss age-related differences in brain activity during encoding and retrieval separately because these two memory stages recruit different functional networks (see Section III,E).

Four studies investigated age-related differences in cerebral blood flow during encoding. It is important to consider the similarities and differences among their findings in terms of the study conditions used in each study because conditions that guide elaborative encoding lead to better memory performance (Craik and Tulving, 1975). As foreshadowed in Section II,F, we might expect that such conditions will ''guide'' effective functional networks, and thus lead to fewer age-related reductions in encoding-related cerebral blood flow.

First of all, Madden *et al*. (1999) presented concrete nouns and required participants to make a living/nonliving judgment on each item, a task that guides attention to the meaning of the words and leads to better memory performance (Craik and Tulving, 1975). The younger adults remembered more of the words on a later recognition memory test, but performance was above 75% (hits minus false alarms) in both age groups. The older adults' cerebral blood flow during encoding, relative to a baseline task, showed encoding-related activity in bilateral prefrontal regions, the left parahippocampal gyrus, left fusiform gyrus, and the thalamus. Curiously, there were no regions of significant encoding-related activity in the younger adults, but only in the thalamus did encoding-related activity interact with the age group, which suggests that younger and older adults activate similar encoding-related brain networks (albeit at subthreshold levels in the younger adults) when the study task guides elaborative encoding.

Cabeza *et al*. (1997b) presented moderately related word pairs (e.g., parent–piano), and participants were instructed to consider a meaningful relationship between the two words during the encoding task. Thus, this task did require some degree of self-initiated encoding, as the words were not highly related, and it was up to participants to refine the semantic association between the words. The younger and older adults' memory for these words (assessed by cued recall and recognition) did not differ, but there were significant age-related differences in encoding-related cerebral blood flow. Relative to brain activity during retrieval, there were age-related declines in encoding-related brain activity in left prefrontal cortex and in bilateral occipitotemporal regions, together with age-related increases in activity in bilateral insular regions, indicating that aging is associated with altered self-initiated encoding operations.

This same paired associate task was used by Anderson *et al*. (2000), but in this case the word pairs were presented under conditions of full or divided attention. This study was motivated by findings from the cognitive aging literature that younger adults learning under conditions of divided attention have memory impairments that closely resemble those associated with aging (Craik, 1982; Rabinowitz *et al*., 1982; Jennings and Jacoby, 1993; Anderson *et al*., 1998). One goal of the PET study was to see whether aging and divided attention were associated with similar reductions in encoding-related brain activity, and indeed very similar reductions in left inferior prefrontal cortex were found. These results suggest that the behavioral effects of aging and divided attention resemble each other because both reduce the ability to engage in elaborative encoding.

Finally, one could argue that even less encoding support was provided in the study conducted by Grady *et al*. (1995), in which unfamiliar faces were presented and participants were instructed simply to remember the faces. The younger adults recognized more of the faces on a later recognition memory task. These behavioral differences were accompanied by greater encoding-related activation (relative to baseline tasks) in the younger than in the older adults in the left inferior prefrontal cortex, the anterior cingulate, left temporal and fusiform regions, and in the right hippocampus and parahippocampal gyrus, but no regions in which encoding-related activity was greater in the older than in the younger adults. These results suggest that when elaborative encoding processes are not guided by the task, age-related declines in memory performance are associated with reductions in encoding-related brain activity.

Obviously, it is difficult to draw conclusions about the relationship between elaborative encoding and age-related differences in cerebral blood flow from only four studies. Nevertheless, these studies seem to demonstrate that older adults benefit from supportive study conditions because such conditions guide brain activity in the functional network associated with successful encoding.

Retrieval support comes most typically in the form of retrieval cues, which lessen the need for top-down or self-initiated memory search. Episodic memory tasks thus differ in the amount of retrieval support they offer, ranging from free recall in which participants are told to recall everything they can remember from the study episode to item recognition, in which the studied items are represented along with new items, and participants indicate whether each item was on the study list. Retrieval support reduces age-related memory decrements (Craik, 1986), and we might expect that it would also diminish age-related reductions in retrieval-related cerebral blood flow.

Two studies used item recognition memory paradigms, paradigms that are thought to provide the greatest amount of retrieval support of all episodic memory tasks. First of all, the study conducted by Grady *et al*. (1995) included a two-choice face recognition test for the studied faces described earlier. The younger adults showed retrieval-related brain activity (relative to control tasks) in right prefrontal regions, right parietal cortex, extrastriate regions, and the cerebellum; the older adults showed a similar pattern of retrieval-related brain activity, but with less activation of the right parietal and right extrastriate regions. Importantly, however, there were no age-related differences in right prefrontal activity during retrieval. Similarly, Madden *et al*. (1999) scanned during a yes/no word recognition test and found that younger and older adults activated right prefrontal regions comparably during recognition, relative to a baseline task. However, older adults also had prefrontal activation in the left hemisphere during recognition, whereas in the younger adults it was right lateralized. The results of these two studies indicate that in the context of a very supportive retrieval task, areas important for episodic retrieval are recruited similarly by younger and older adults, although older adults may recruit additional neural systems to enhance task performance.

One additional recognition task was used by Cabeza *et al*. (1997b), but in this case it was a semantic recognition task. After studying moderately related word pairs, participants were presented with some of the original word pairs (e.g., ''parent–piano'') and with some altered pairs (e.g., ''parent–guitar''), and participants said ''yes'' to the pairs they thought they had studied. This task requires participants to remember the specific semantic connection between the originally presented words, and in that sense resembles a recall test rather than a recognition test. Indeed, this study also included a cued recall test in which the first word of each pair was presented, and participants had to recall the second word of the pair or say ''pass.'' For the most part, brain activity during semantic recognition and cued recall was similar, and thus we will consider them together as retrieval-related brain activity. Right prefrontal retrieval-related brain activity was comparable between the younger and the older adults (Brodmann's areas 9, 10, and 47), although the younger adults also activated medial prefrontal regions. There were, however, age-related reductions in right parietal activity, and age-related increases were found in the left prefrontal cortex. These results are similar to those of Madden *et al*. (1999) in demonstrating a more extensive retrieval-related brain network in older adults.

A cued recall task was used in the study conducted by Anderson *et al*. (2000), and retrieval was conducted under conditions of full or divided attention. The younger adults recalled more words than the older adults overall, and the effect of the attention manipulation replicated previous reports of slight or no effects of divided attention during retrieval (Baddeley *et al*., 1984; Craik *et al*., 1996; Anderson *et al*., 1998). These behavioral results were reflected in blood flow data; specifically, there were age-related decreases in right

prefrontal activity during retrieval, but divided attention had no effect on retrieval-related right prefrontal activity in either age group. In addition, the older adults activated the left inferior frontal gyrus, and the younger adults activated the left middle frontal gyrus. The bilaterality of retrieval-related activity was surprising, but it should be noted that the area activated by the older adults was closer to regions associated more typically with encoding. Two conclusions can be drawn from these findings: (1) at least in the presence of a moderately helpful retrieval cue, divided attention during retrieval leaves memory performance intact because it does not interfere with functioning in right prefrontal regions that are important for retrieval from episodic memory and (2) the older adults' lower activation of retrieval-related networks may partly contribute to their lower memory performance.

Two studies used word stem cued recall tests in which the first few letters of studied words were presented during retrieval, and participants tried to complete the stems with words from the studied list (e.g., "string" for "str___"). Bäckman *et al*. (1997) found that both younger and older adults activated right prefrontal regions during this task, relative to a control task, but only the older adults showed additional activation of left prefrontal regions, a finding that is consistent with the results of Cabeza *et al*. (1997b) and Madden *et al*. (1999). In addition, the older adults showed greater activation of bilateral medial temporal lobe regions, whereas this pattern was not seen in the younger adults, which Bäckman and colleagues interpreted as reflecting an age-related increase in the use of cue-dependent—or data-driven—retrieval (see Moscovitch and Umilta, 1991). Schacter *et al*. (1996b) tested word stem cued recall following perceptual or semantic study conditions that led to low and high memory performance, respectively. In the low recall minus baseline comparison, Schacter *et al*. reported an age-related decrease in the activation of the left and right anterior prefrontal cortex. However, in the high recall minus baseline comparison, there was no significant frontal lobe activation, but significant bilateral hippocampal activation that did not differ between the two age groups. Our interpretation of these results rests on a consideration of the interaction between encoding and retrieval support. That is, in low recall conditions, relatively degraded information had been encoded into memory, and thus, even when presented with a moderately supportive retrieval cue (the word stem), participants must engage in strategic memory searches. The age-related reduction in right prefrontal activity indicates that the older adults are less able to engage the retrieval networks necessary to guide strategic retrieval. In contrast, in the high recall condition, relatively meaningful information was encoded into memory, and thus the moderately supportive retrieval cue is sufficient to retrieve information from memory in a cue-dependent or data-driven manner. In this case, medial temporal and not prefrontal regions are activated (cf, Moscovitch and Umilta, 1991), and these bottom-up processes are resilient to aging.

Finally, Cabeza *et al*. (2000) compared item recognition with temporal order memory. In the item recognition condition, participants were shown one word from a study list and one new word, and they indicated which word had been on the study list. In the temporal order condition, participants were shown two words from the study list, and they indicated which word had been shown more recently in the list. The two main regions that were activated during the item retrieval task were medial temporal lobe regions and prefrontal regions: whereas activity in temporal lobe regions was relatively comparable in the two age groups, the older adults showed less right prefrontal but more left prefrontal activity than their younger counterparts, which is reminiscent of the more extensive item retrieval-related networks in older adults reported by Cabeza *et al*. (1997b), Bäckman *et al*. (1997), and Madden *et al*. (1999). In contrast, cerebral blood flow during the temporal order task activated right prefrontal regions significantly more for the younger than older adults. Thus, this study also showed that when the memory task requires the intentional retrieval of contextual details from a previous episode, there are age-related decrements in activity in the brain networks required to perform such searches.

The conclusion that can be drawn from these studies of brain activity during retrieval is that age-related decrements in brain activity are greatest during tasks that require controlled, self-initiated retrieval searches, but are minimized during tasks that offer more retrieval support. In particular, older adults show less right prefrontal activation during difficult recall tasks, and as the study by Cabeza *et al*. (1997b) showed, even during recognition tasks that require retrieval of associative information. The other interesting finding from these studies is that these age-related decreases in right prefrontal activity during difficult memory tasks are often accompanied by age-related increases in left prefrontal activation. That is, in the face of retrieval tasks that require self-initiated memory search, the network of brain activity is more extensive in older than in younger adults.

VI. Common Age-Related Differences in Brain Activation across Studies

Two broad patterns emerge from the studies of age-related differences in cerebral blood flow during cognitive tasks. First, there are age-related decreases in brain activity in the functional networks associated with cognitive tasks, and these decrements are most pronounced during conditions that require more self-initiated processing, but are reduced in more supportive conditions. Second, under some circumstances the older adults activate more extensive networks of brain activity than do their younger counterparts. In an effort to visualize these two patterns, we selected all of the voxels in which significant age X task interactions were reported in the studies described in Sections IV and V, classified them according to the cognitive process thought to be isolated by the task in question, and visually inspected these results as a function of brain region.

Brain regions that showed clear patterns of age-related differences were the occipital cortex, the ventral and medial portions of the temporal lobes, and the frontal lobes. Figure 17.1 (see color insert) shows significant interactions with age in occipital and temporal lobe regions. The "warmer" colors (reds, oranges, and yellows) represent regions that the older adults activated more than the younger adults, and the "cooler" colors (blues and greens) represent regions that the

younger adults activated more than the older adults. The first striking finding is the age difference noted during perception. Specifically, in perception tasks, as well as in attention tasks, the younger adults were more likely to activate visual processing regions in extreme posterior regions of the occipital lobes, which suggests that younger adults perceive and attend to objects at very early stages of processing. In contrast, during perception tasks the older adults were more likely to activate regions further along the ventral processing stream in both occipital and temporal regions, a finding that indicates that perceptual analysis requires more extensive visual processing in older than younger individuals. Figure 17.2 (see color insert) shows regions in the prefrontal cortex that showed significant age X task interactions. Here we find that many sites in lateral and medial prefrontal cortex showed age-related increases in activation during perception tasks, suggesting that perceptual stimuli undergo much more elaborate processing in older adults.

It may be instructive to consider these findings in terms of Salthouse's (e.g., 1996) theory of cognitive slowing. According to this theory, the speed of perceptual-cognitive processing declines with age, such that the information available from earlier stages of information processing is incompletely analyzed when it is needed by higher stages of processing. Slowing has a cascading effect, such that at each stage information is more degraded and sometimes arrives too late to be useful for higher stages of processing. While it is impossible to ascertain from PET data whether speed is the root of the age-related differences in perceptual networks (because of the temporal constraints of this scanning technique), this theory could explain why older adults activate more extensive brain regions than their younger counterparts. That is, areas may be activated in older but not younger adults because additional neural systems may be brought on line to help decipher information delivered by earlier processing stages. The path analyses of brain activity during face matching reported by McIntosh *et al*. (1994) and Horwitz *et al*. (1995) are consistent with this view, as the older adults showed greater feedback influences from frontal to posterior brain regions, suggesting more elaborative processing was required by older adults in order to resolve the perceptual information. Indeed, in tasks that are thought to isolate other cognitive functions (e.g., memory), some of the brain regions found to be activated by older but not younger adults may be related to the resolution of perceptual information.

Turning now to memory tasks, a particular pattern seems to emerge from the age-related differences in working memory and episodic encoding activity in occipitotemporal regions (see Fig. 17.1). During working memory tasks, younger adults showed greater activity in posterior occipital regions, whereas older adults showed very few regions of greater activity. During episodic encoding tasks, the age-related increases tended to be more posterior to the age-related decreases. These results suggest that there is an age-related decrease in the ability to utilize posterior occipital lobe regions to maintain visual information in working memory, as well as an age-related decrease in the ability to process that information more ''deeply'' in anterior regions along the ventral processing stream during encoding. A more striking comparison can be seen in age-related differences in the frontal lobes, however (see Fig. 17.2). While there were few frontal sites of greater encoding-related brain activity in the older adults, there were many instances of greater encoding activity in the younger adults, and the majority of these occurred in lateral aspects of left hemisphere. The younger adults' left prefrontal activity during encoding is consistent with HERA (Tulving *et al*., 1994b) and supports the conclusion that elaborative episodic encoding declines with age.

While there is no clear pattern in the few age-related differences in retrieval-related brain activity in posterior brain regions, a pattern is much more apparent in the frontal lobes. In particular, age-related decreases in retrieval-related brain activity were found mainly in the right prefrontal cortex, whereas age-related increases in retrieval-related brain activity were found mainly in the left prefrontal cortex. That is, there is an age-related reduction in the activation of brain regions important for episodic retrieval (right prefrontal cortex), together with an age-related increase in the activation of brain regions more typically associated with encoding and other cognitive functions (left prefrontal cortex). If we return to the explanations that have been brought forth in order to account for right prefrontal activity during retrieval (see Section III,E), the ''retrieval effort'' and ''retrieval success'' hypotheses are not consistent with the data, as Cabeza *et al*. (1997b) found no age-related differences in memory performance together with age-related declines in right prefrontal activitation, whereas Grady *et al*. (1995) and Madden *et al*. (1999) found worse memory performance in the older adults together with no age-related differences in right prefrontal activation. The other two hypotheses fare better, namely that older adults may have more difficulty maintaining a general retrieval mental set or may be less efficient at monitoring retrieval. Maintaining mental set and monitoring cognitive operations have both been linked to frontal lobe functioning, and older adults are more likely to lose set on other cognitive tasks (e.g., the WCST) and to show evidence of monitoring failures (e.g., poorer inhibition of prepotent responses and higher intrusion rates) (for a review, see West, 1996). Finally, these results could also be viewed as consistent with the cortical asymmetry of reflective activity pattern described by Nolde *et al*. (1998), in which retrieval tasks that require more complex, evaluative processes (i.e., self-initiated processes) are more likely to activate the left prefrontal cortex. This view would suggest that the retrieval cue is less likely to activate the desired information in older adults, and thus there is an age-related increase in the need to engage in strategic retrieval processes mediated by the left prefrontal cortex.

Perhaps the best way to arrive at a theoretical explanation for age-related differences in cerebral blood flow during perceptual and cognitive tasks is to analyze the relationship between blood flow and behavior. Relatively few analyses of this sort have been performed, but those that have are instructive. For example, in the divided attention study conducted by Madden *et al*. (1997), response times were correlated with activity in the visual cortex in the young adults and with activity in the prefrontal cortex in the older adults. These findings highlight the conclusion that younger adults relied more on object processing to perform the attention task, whereas older adults relied more on strategic control and response monitoring. In the working memory task for faces

conducted by Grady *et al*. (1998a), right prefrontal (Brodmann's area 45/46) and left medial temporal regions were more active and were associated with *faster* responses in the younger adults, and the left prefrontal cortex was more active and was associated with *slower* responses in the older adults. These results suggest that the efficiency of the functional networks activated declined with age. In the word recognition study reported by Madden *et al*. (1999), the activation of Brodmann's area 10 in the right prefrontal cortex during retrieval did not differ between younger and older adults and was associated with longer recognition latencies for both age groups, which suggests that this location may be involved in retrieval effort or retrieval monitoring processes. Finally, in the WCST study by Nagahama *et al*. (1997), decrements in left dorsolateral prefrontal activation (which were greater in the older adults) were related to an increased number of perseverative errors on that task.

While these findings suggest that the age-related diffusion of brain networks is generally associated with poorer cognitive performance, there are other cases in which it is associated with better performance. For example, in the recognition study of Madden *et al*. (1999), increased blood flow in posterior brain regions was associated with faster responding on the part of the older adults. In the study by Grady *et al*. (2000), nondegraded face-matching activity in the left hippocampus and left thalamus was greater for older than younger adults and was associated with slower, but more accurate performance in the older adults on the degraded tasks. These findings are consistent with the suggestion that in some cases, the older adults' more extensive brain networks may reflect "functional compensation," or the use of different strategies in order to improve performance (Grady *et al*., 1994; Cabeza *et al*., 1997b).

Finally, we would like to point out one additional pattern of results that emerges from these studies of perception and memory in healthy adults. During some perceptual tasks (e.g., face matching in Grady *et al*., 1992, 1994, 2000; divided attention in Madden *et al*., 1997), the older adults showed more extensive networks of brain activity that extended into frontal cortex, and these results indicated an age-related increase in the need for more elaborative processes in order to resolve perceptual information. However, during memory tasks that required more elaborative encoding or retrieval mediated by frontal lobe regions (Grady *et al*., 1995; Schacter *et al*., 1996a; Cabeza *et al*., 1997b, 2000; Madden *et al*., 1999; Anderson *et al*., 2000), the older adults failed to activate prefrontal regions as much as their younger counterparts. How can these two seemingly contradictory patterns be integrated?

We suggest that these results are consistent with the attentional resource account (Craik, 1983, 1986) that was introduced in Section II,F. This account proposes that cognitive tasks consume attentional resources to varying degrees and that there is an age-related reduction in the amount of attentional resources available to fuel complex cognitive tasks. Neuroimaging studies are beginning to elucidate the neural correlates of attentional resources. Specifically, Goldberg *et al*. (1998) reported that performance on the WCST and on a fast-paced auditory shadowing task declined when these two tasks were performed simultaneously, a finding that demonstrates that attentional capacity was exceeded by the dual-task requirement. Furthermore, relative to the full attention conditions, activity in prefrontal cortex declined during the dual-task condition. D'Esposito (2000) considered these results in conjunction with other findings from his laboratory and hypothesized that prefrontal activity may increase to meet the attentional demands of the task, but only to the point at which attentional capacity is reached, beyond which prefrontal activity may decline. A merger of this idea of an inverted U-shaped function of prefrontal activity with Craik's (1983, 1986) reduced attentional resources hypothesis leads to the suggestion that older adults reach their asymptote more quickly.

Figure 17.3 shows a hypothetical pattern of prefrontal brain activation (relative to a control task) during four types of task of the sort reviewed in this chapter. Perception and attention tasks that engage bottom-up processes would fall in the leftmost column. During these tasks, younger and older adults show comparably little activation of prefrontal regions, as reported in the study by Madden *et al*. (1996). More difficult attention or perceptual tasks that require top-down processes would fall in the second column to the left. In this case, older adults activate prefrontal regions *more* than younger adults, as shown by Grady *et al*. (1992, 1994, 2000) and Madden *et al*. (1997), perhaps because older adults need to engage more elaborative processes in order to manage the task. At this point in Fig. 17.3, the older adults have already reached their attentional capacity, and any increases in attentional demands beyond this point will result in decreases in prefrontal activity. The decrease in older adults' level of frontal activation is evident in the third column, which represents memory tasks that guide appropriate encoding or retrieval processing. In this case, younger and older adults' degree of frontal lobe activity is comparable, as was shown in the left prefrontal cortex during encoding by Madden *et al*. (1999) and during retrieval in the right prefrontal cortex by Grady *et al*. (1995), Cabeza *et al*. (1997b), and Bäckman *et al*. (1997). Finally, memory tasks that require self-initiated encoding or retrieval operations are represented in the rightmost column, where age-related decrements in frontal lobe activity are found. This finding was reported for encoding by Grady *et al*. (1995), Cabeza *et al*. (1997b), and Anderson *et al*. (2000) and during retrieval by Schacter *et al*. (1996b), Anderson *et al*. (2000), and Cabeza *et al*. (2000). According to this hypothesis, if even more

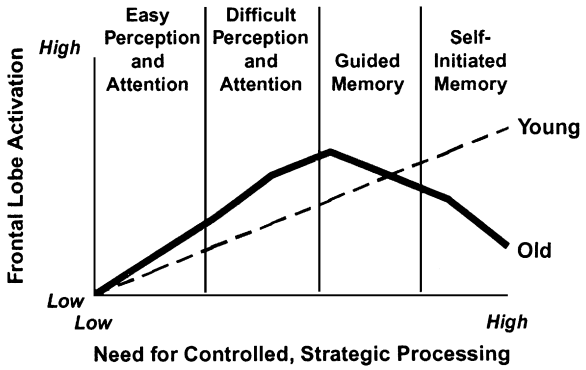

FIG. 17.3 Theoretical model of age-related differences in prefrontal brain activation as a function of the need for controlled processing (e.g., attentional demand) in the cognitive task.

attention-demanding tasks were employed, younger adults' prefrontal activity would decline and approach that of older adults. Although data available at the present time cannot confirm this model, it is nevertheless appealing because it is consistent with the reduced attentional resource hypothesis put forth by Craik (1983, 1986) and it provides an explanation for the seemingly contradictory findings of age-related increases in prefrontal activity during relatively simple attentional or perceptual tasks and age-related decreases in prefrontal activity during difficult memory tasks.

VII. Conclusions and Future Directions

The existing neuroimaging studies of aging show two broad patterns of findings. One pattern is characterized by age-related decreases in the activation of functional brain networks important for cognitive performance, particularly during complex tasks that require self-initiated operations. The other pattern is characterized by age-related increases in brain activation in regions that are not typically activated by younger adults. This more extensive functional network is in some cases associated with poorer cognitive performance and in some cases with better performance. Some brain regions are involved in both of these two patterns of age differences, depending on the task assessed. For example, older adults appear to activate frontal lobe regions to a greater extent during some perceptual tasks, but less during some memory tasks. We suggested that these results are consistent with age-related reductions in attentional resources controlled by frontal lobe systems and presented a hypothetical model (based on Goldberg *et al*., 1998; D'Esposito, 2000) of age-related differences in prefrontal involvement as a function of the attentional demands of the task.

Indeed, in recent years there has been increasing interest in what is referred to as the ''frontal lobe hypothesis'' of cognitive aging, and our review of the neuroimaging studies of aging indicates that age-related changes in prefrontal activity play an important role in distinguishing the functional networks of younger and older adults. However, we would like to emphasize that the frontal lobes never work in isolation, but rather as part of a functional network. That is, if one role of the frontal lobes is to orchestrate the participation of other brain regions, then frontal lobe deficits will result in changes in the involvement of more posterior brain regions, and functional deficits in posterior brain regions may change functioning in the frontal lobes. Thus, to understand the neural underpinnings of cognitive aging, we must consider age-related changes in the *pattern* of activity throughout the brain resulting from a cognitive challenge.

Furthermore, we need to gain a better understanding of how age-related differences in brain activation relate to differences in behavioral performance. This is a tall order, indeed, because we are only beginning to understand how brain activation is related to behavioral performance in younger adults, let alone how these relationships change with aging. The brain–behavior data available thus far provide evidence that older adults' functional networks are associated with worse performance in some cases and better performance in others. Only one study (Grady *et al*., 2000) has correlated blood flow with response accuracy in addition to response latency. It is instructive that Grady and co-workers found that increasing blood flow was generally associated with slower, but more accurate performance because it reminds us that faster performance does not always lead to better performance. Future studies should endeavor to include more than one measure of behavioral performance, as a comprehensive analysis of brain–behavior relationships will likely be our best avenue for understanding cognitive aging and for developing appropriate rehabilitation strategies.

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References

Allen, P. A., Madden, D. J., Weber, T. A., and Groth, K. E. (1993). Influence of age and processing stage on visual word recognition. *Psychol. Aging* **8**, 274–282.

Anderson, N. D., Craik, F. I. M., and Naveh-Benjamin, M. (1998). The attentional demands of encoding and retrieval in younger and older adults: I. Evidence from divided attention costs. *Psychol. Aging* **13**, 405–423.

Anderson, N. D., Iidaka, T., Cabeza, R., Kapur, S., McIntosh, A. R., and Craik, F. I. M. (2000). The effects of divided attention on encoding- and retrieval-related brain activity: A PET study of younger and older adults. *J. Cogn. Neurosci.* (in press).

Awh, E., Jonides, J., Smith, E. E., Schumacher, E. H., Koeppe, R. A., and Katz, S. (1996). Dissociation of storage and rehearsal in verbal working memory: Evidence from PET. *Psychol. Sci.* **7**, 25–31.

Bäckman, L., Almkvist, O., Andersson, J., Nordberg, A., Winblad, B., Reineck, R., and Långström, B. (1997). Brain activation in young and older adults during implicit and explicit retrieval. *J. Cogn. Neurosci.* **9**, 378–391.

Baddeley, A. D. (1986). ''Working Memory.'' Oxford University Press, Oxford, UK.

Baddeley, A. D., Lewis, V., Eldridge, M., and Thomson, N. (1984). Attention and retrieval from long-term memory. *J. Exp. Psychol.*: *Gen.* **113**, 518–540.

Ball, K. K., Roenker, D. L., and Bruni, J. R. (1990). Developmental changes in attention and visual search throughout adulthood. *In* ''The Development of Attention: Research and Theory'' (J. T. Enns, ed.), pp. 489–508. Elsevier, Amsterdam.

Balota, D., Dolan, P. O., and Duchek, J. (2000). *In* ''The Oxford Handbook on Memory'' (E. Tulving and F. I. M. Craik, eds.), Oxford University Press, Cambridge, England, pp 395–409.

Bartlett, J. C., and Leslie, J. E. (1986). Aging and memory for faces versus single views of faces. *Mem. Cogn.* **14**, 271–281.

Berman, K. F., Ostrem, J. L., Randolph, C., Gold, J., Goldberg, T. E., Coppola, R., Carson, R. E., Herscovitch, P., and Weinberger, D. R. (1995). Physiological activation of a cortical network during performance on the Wisconsin Card Sorting Test: A positron emission tomography study. *Neuropsychologia* **33**, 1027–1046.

Blaxton, T. A., Bookheimer, S. Y., Zeffiro, T. A., Figlozzi, C. M., Gaillard, W. D., and Theodore, W. H. (1996). Functional mapping of human memory using PET: Comparisons of conceptual and perceptual tasks. *Can. J. Exp. Psychol.* **50**, 42–56.

Braver, T. S., Cohen, J. D., Jonides, J., Smith, E. E., and Noll, D. C. (1997). A parametric study of prefrontal cortex involvement in human working memory. *NeuroImage* **5**, 49–62.

Buckner, R., Peterson, S. E., Ojemann, J. G., Meizin, F. M., Squire, L. R., and Raichle, M. E. (1995). Functional anatomical studies of explicit and implicit memory retrieval tasks. *J. Neurosci.* **15**, 12–29.

Burke, D. M., and Light, L. L. (1981). Memory and aging: The role of retrieval processes. *Psychol. Bull.* **90**, 513–546.

Cabeza, R., and Nyberg, L. (1997). Imaging cognition: An empirical review of PET studies with normal subjects. *J. Cogn. Neurosci.* **9**, 1–26.

Cabeza, R., Kapur, S., Craik, F. I. M., McIntosh, A. R., Houle, S., and Tulving, E. (1997a). Functional neuroanatomy of recall and recognition: A PET study of episodic memory. *J. Cogn. Neurosci.* **9**, 254–265.

Cabeza, R. Grady, C. L., Nyberg, L., McIntosh, A. R., Tulving, E., Kapur, S., Jennings, J. M., Houle, S., and Craik, F. I. M. (1997b). Age-related differences in neural activity during memory encoding and retrieval: A positron emission tomography study. *J. Neurosci.* **17**, 391–400.

Cabeza, R., Anderson, N. D., Mangels, J., Nyberg, L., and Houle, S. (2000). Age-related differences in neural activity during item and temporal-order memory retrieval: A positron emission tomography study. *J. Cogn. Neurosci.*, **12**, 197–206.

Corbetta, M., Miezin, F. M., Dobmeyer, S. M., and Petersen, S. E. (1990). Attentional modulation of neural processing of shape, color, and velocity in humans. *Science* **248**, 1556–1559.

Corbetta, M., Miezin, F. M., Dobmeyer, S., Shulman, G. L., and Petersen, S. E. (1991). Selective and divided attention during visual discriminations of shape, color, and speed: Functional anatomy by positron emission tomography. *J. Neurosci.* **11**, 2383–2402.

Courtney, S. M., Ungerleider, L. G., Keil, K., and Haxby, J. V. (1996). Object and spatial visual working memory activate separate neural systems in human cortex. *Cereb. Cortex* **6**, 39–49.

Craik, F. I. M. (1982). Selective changes in encoding as a function of reduced processing capacity. *In* ''Cognitive Research in Psychology'' (F. Klix, J. Hoffmann, and E. van der Meer, eds.), pp. 152–161. Deutscher Verlag der Wissenschaffen, Berlin.

Craik, F. I. M. (1983). On the transfer of information from temporary to permanent memory. *Philos. Trans. R. Soc. London, Ser. B* **302**, 341–359.

Craik, F. I. M. (1986). A functional account of age differences in memory. *In* ''Human Memory and Cognitive Capabilities, Mechanisms, and Performances'' (F. Klix and H. Hagendorf, eds.) pp. 409–422. Elsevier, Amsterdam.

Craik, F. I. M., and Salthouse, T. A., eds. (1992). ''Handbook of Aging and Cognition.'' Lawrence Erlbaum, Hillsdale, NJ.

Craik, F. I. M., and Salthouse, T. A., eds. (2000). ''Handbook of Aging and Cognition,'' 2nd ed. Lawrence Erlbaum, Mahwah, NJ.

Craik, F. I. M., and Tulving, E. (1975). Depth of processing and the retention of words in episodic memory. *J. Exp. Psychol.* **104**, 268–294.

Craik, F. I. M., Anderson, N. D., Kerr, S. A., and Li, K. Z. H. (1995). Memory changes in normal ageing. *In* ''Handbook of Memory Disorders'' (A. D. Baddeley, B. A. Wilson, and F. N. Watts, eds.), pp. 211–241. Wiley, Chichester, England.

Craik, F. I. M., Govoni, R., Naveh-Benjamin, M., and Anderson, N. D. (1996). The effects of divided attention on encoding and retrieval processes in human memory. *J. Exp. Psychol.* **125**, 159–180.

Damasio, A. R., Tranel, D., and Damasio, H. (1989). Disorders of visual recognition. *In* ''Handbook of Neuropsychology'' (H. Goodglass and A. R. Damasio, eds.), Vol. 2, pp. 317–332. Elsevier, Amsterdam.

Damasio, H. (1981). Cerebral localization of the aphasias. *In* ''Acquired Aphasia'' (M. T. Sarno, ed.), pp. 27–50. Academic Press, New York.

Desgranges, B., Baron, J.-C., and Eustache, F. (1998). The functional neuroanatomy of episodic memory: The role of the frontal lobes, the hippocampal formation, and other areas. *NeuroImage* **8**, 198–213.

Desimone, R. (1996). Neural mechanisms for visual memory and their role in attention. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 13494–13499.

D'Esposito, M. (2000). Working memory. *In* ''Handbook of Functional Neuroimaging of Cognition'' (R. Cabeza and A. Kingstone, eds.). MIT Press, Cambridge, MA (in press).

D'Esposito, M., Aguirre, G. K., Zarahn, E., Ballard, D., Shin, R. K., and Lease, J. (1998). Functional MRI studies of spatial and nonspatial working memory. *Cogn. Brain Res.* **7**, 1–13.

D'Esposito, M., Zarahn, E., Aguirre, G. K., and Rypma, B. (1999). The effect of normal aging on the coupling of neural activity to the bold hemodynamic response. *NeuroImage* **10**, 6–14.

Dobbs, A. R., and Rule, B. G. (1989). Adult age differences in working memory. *Psychol. Aging* **4**, 500–503.

Eichenbaum, H., Otto, T., and Cohen, N. J. (1992). The hippocampus: What does it do? *Behav. Neural Biol.* **57**, 2–36.

Esposito, G., Kirkby, B. S., Van Horn, J. D., Ellmore, T. M., and Berman, K. F. (1999). Context-dependent, neural system-specific neurophysiological concomitants of ageing: Mapping PET correlates during cognitive activation. *Brain* **112**, 963–979.

Felleman, D. J., and Van Essen, D. C. (1991). Distributed hierarchical processing in primate cerebral cortex. *Cereb. Cortex* **1**, 1–47.

Fletcher, P. C., Frith, C. D., Grasby, P. M., Shallice, T., Frackowiak, R. S. J., and Dolan, R. J. (1995). Brain systems for encoding and retrieval of auditory-verbal memory: An *in vivo* study in humans. *Brain* **118**, 401–416.

Fletcher, P. C., Frith, C. D., and Rugg, M. D. (1997). The functional neuroanatomy of episodic memory. *Trends Neurosci.* **20**, 213–218.

Goldberg, T. E., Berman, K. F., Fleming, K., Ostrem, J., Van Horn, J. D., Esposito, G., Mattay, V. S., Gold, J. M., and Weinberger, D. R. (1998). Uncoupling cognitive workload and prefrontal cortical physiology: A PET rCBF study. *NeuroImage* **7**, 296–303.

Goldman-Rakic, P. S. (1987). Circuitry of primate prefrontal cortex and regulation of behavior by representational memory. *In* ''Handbook of Physiology'' (V. Mountcastle and F. Plum, eds.), Vol. 5, pp. 373–417. American Physiological Society, Bethesda, MD.

Goodale, M. A., and Milner, A. D. (1992). Separate visual pathways for perception and action. *Trends Neurosci.* **15**, 20–25.

Grady, C. L., Haxby, J. V., Horwitz, B., Ungerleider, L. G., Schapiro, M. B., Carson, R. E., Herscovitch, P., Mishkin, M., and Rapoport, S. I. (1992). Dissociation of object and spatial vision in human extrastriate cortex: Age-related changes in activation of regional cerebral blood flow measured with [^{15}O]water and positron emission tomography. *J. Cogn. Neurosci.* **4**, 23–34.

Grady, C. L., Maisog, J. M., Horwitz, B., Ungerleider, L. G., Mentis, M. J., Salerno, J. A., Pietrini, P., Wagner, E., and Haxby, J. V. (1994). Age-related changes in cortical blood flow activation during visual processing of faces and location. *J. Neurosci.* **14**, 1450–1462.

Grady, C. L., McIntosh, A. R., Horwitz, B., Maisog, J. M., Ungerleider, L. G., Mentis, M. J., Pietrini, P., Schapiro, M. B., and Haxby, J. V. (1995). Age-related reductions in human recognition memory due to impaired encoding. *Science* **269**, 218–221.

Grady, C. L., McIntosh, A. R., Bookstein, F., Horwitz, B., Rapoport, S. I., and Haxby, J. V. (1998a). Age-related changes in regional cerebral blood flow during working memory for faces. *NeuroImage* **8**, 409–425.

Grady, C. L., McIntosh, A. R., Rajah, M. N., and Craik, F. I. M. (1998b). Neural correlates of the episodic encoding of pictures and words. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 2703–2708.

Grady, C. L., McIntosh, A. R., Horwitz, B., and Rapoport, S. I. (2000). Age-related changes in the neural correlates of degraded and nondegraded face processing. *Cogn. Neuropsychol.* **17**, 165–186.

Hasher, L., and Zacks, R. T. (1977). Automatic and effortful processes in memory. *J. Exp. Psychol.* **108**, 356–388.

Haxby, J. V., Grady, C. L., Horwitz, B., Ungerleider, L. G., Mishkin, M., Carson, R. E., Herscovitch, P., Schapiro, M. B., and Rapoport, S. I. (1991). Dissociation of object and spatial visual processing pathways in human extrastriate cortex. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 1621–1625.

Horwitz, B., McIntosh, A. R., Haxby, J. V., Furey, M., Salerno, J., Schapiro, M. B., Rapoport, S. I., and Grady, C. L. (1995). Network analysis of PET-mapped visual pathways in Alzheimer type demential. *NeuroReport* **6**, 2287–2292.

Jennings, J. M., and Jacoby, L. L. (1993). Automatic versus intentional uses of memory: Aging, attention, and control. *Psychol. Aging* **8**, 283–293.

Johannsen, P., Jakobsen, J., Bruhn, P., Hansen, S. B., Gee, A., Stødkilde-Jørgensen, H., and Gjedde, A. (1997). Cortical sites of sustained and divided attention in normal elderly humans. *NeuroImage* **6**, 145–155.

Jonides, J., Smith, E. E., Koeppe, R. A., Awh, E., Minoshima, S., and Mintun, M. A. (1993). Spatial working memory in humans as revealed by PET. *Nature, (London)* **363**, 623–625.

Jonides, J., Schumacher, E. H., Smith, E. E., Lauber, E. J., Awh, E., Minoshima, S., and Koeppe, R. T. (1997). Verbal working memory load affects regional brain activation as measured by PET. *J. Cogn. Neurosci.* **9**, 462–475.

Kapur, S., Craik, F. I. M., Tulving, E., Wilson, A. A., Houle, S., and Brown, G. M. (1994). Neuroanatomical correlates of encoding in episodic memory: Levels of processing effect. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 2008–2011.

Kapur, S., Craik, F. I. M., Jones, C., Brown, G. M., Houle, S., and Tulving, E. (1995). Functional role of the prefrontal cortex in retrieval of memories: A PET study. *NeuroReport* **6**, 1880–1884.

Köhler, S., Kapur S., Moscovitch M., Winocur G., and Houle S. (1995). Dissociation of pathways for object and spatial vision: A PET study in humans. *NeuroReport* **6**, 1865–1868.

Kosslyn, S. M., Alpert, N. M., Thompson, W. L., Chabris, C. E., Rauch, S. L., and Anderson, A. K. (1994). Identifying objects seen from different viewpoints: A PET investigation. *Brain* **117**, 1055–1071.

Kosslyn, S. M., Alpert, N. M., and Thompson, W. L. (1995). Identifying objects at different levels of hierarchy: A positron emission tomography study. *Hum. Brain Mapp.* **3**, 107–132.

Light, L. L. (1991). Memory and aging: Four hypotheses in search of data. *Annu. Rev. Psychol.* **41**, 333–376.

Light, L. L., and Burke, D. M. (1988). Patterns of language and memory in old age. *In* ''Language, Memory, and Aging'' (L. L. Light and D. M. Burke, eds.), pp. 244–277. Cambridge University Press, New York.

Madden, D. J., and Hoffman, J. M. (1997). Application of positron emission tomography to age-related cognitive changes. *In* ''Brain Imaging in Clinical Psychiatry'' (K. R. R. Krishnan and P. M. Doraiswamy, eds.), pp. 575–613. Dekker, New York.

Madden, D. J., Turkington, T. G., Coleman, R. E., Provenzale, J. M., DeGrado, T. R., and Hoffman, J. M. (1996). Adult age differences in regional cerebral blood flow during visual word identification: Evidence from $H_2{}^{15}O$ PET. *NeuroImage* **3**, 127–142.

Madden, D. J., Turkington, T. G., Provenzale, J. M., Hawk, T. C., Hoffman, J. M., and Coleman, R. E. (1997). Selective and divided attention: Age-related changes in regional cerebral blood flow measured by $H_2{}^{15}O$ PET. *Hum. Brain Mapp.* **5**, 389–409.

Madden, D. J., Turkington, T. G., Provenzale, J. M., Denny, L. L., Hawk, T. C., Gottlob, L. R., and Coleman, R. E. (1999). Adult age differences in the functional neuroanatomy of verbal recognition memory. *Hum. Brain Mapp.* **7**, 115–135.

McIntosh, A. R., and Gonzalez-Lima, F. (1994). Structural equation modeling and its application to network analysis in functional brain imaging. *Hum. Brain Mapp.* **2**, 2–22.

McIntosh, A. R., Grady, C. L., Ungerleider, L. G., Haxby, J. V., Rapoport, S. I., and Horwitz, B. (1994). Network analysis of cortical visual pathways mapped with PET. *J. Neurosci.* **14**, 655–666.

McIntosh, A. R., Bookstein, F. L., Haxby, J. V., and Grady, C. L. (1996). Spatial pattern analysis of functional brain images using partial least squares. *NeuroImage* **3**, 143–157.

Moscovitch, M., and Umilta, C. (1991). Concious and nonconscious aspects of memory: A neuropsychological framework of modules and central systems. *In* ''Perspectives in Cognitive Neuroscience'' (R. Lister and H. Weingartner, eds.), pp. 229–266. Oxford University Press, New York.

Moscovitch, M., and Winocur, G. (1992). The neuropsychology of memory and aging. *In* ''Handbook of Aging and Cognition'' (F. I. M. Craik and T. A. Salthouse, eds.), pp. 315–372. Lawrence Erlbaum, Hillsdale, NJ.

Nagahama, Y., Fukuyama, H., Yamauchi, H., Katsumi, Y., Magata, Y., Shibasaki, H., and Kimura, J. (1997). Age-related changes in cerebral blood flow activation during a card sorting test. *Exp. Brain Res.* **114**, 571–577.

Nobre, A. C., Sebestyen, G. N., Gitelman, D. R., Mesulam, M. M., Frackowiak, R. S., and Frith, C. D. (1997). Functional localization of the system for visuospatial attention using positron emission tomography. *Brain* **120**, 515–533.

Nolde, S. F., Johnson, M. K., and Raye, C. L. (1998). The role of the prefrontal cortex during tests of episodic memory. *Trends Cogn. Sci.* **2**, 399–406.

Nyberg, L., Tulving, E., Habib, R., Nilsson, L.-G., Kapur, S., Houle, S., Cabeza, R., and McIntosh, A. R. (1995). Functional brain maps of retrieval mode and recovery of episodic information. *NeuroReport* **7**, 249–252.

Nyberg, L., McIntosh, A. R., Houle, S., Nilsson, L.-G., and Tulving, E. (1998). Activation of medial temporal structures during episodic memory retrieval. *Nature, (London)* **380**, 715–717.

Oscar-Berman, M., and Bardenhagen, F. (1998). Nonhuman primate models of memory dysfunction in neurodegenerative disease: Contributions from comparative neuropsychology. *In* ''Memory in Neurodegenerative Disease: Biological, Cognitive, and Clinical Perspectives'' (A. I. Tröster, ed.), pp. 3–20. Cambridge University Press, Cambridge, England.

Pardo, J. V., Fox, P. T., and Raichle, M. E. (1991). Localization of a human system for sustained attention by positron emission tomography. *Nature, (London)* **349**, 61–64.

Park, D., Puglisi, J. T., and Smith, A. D. (1986). Memory for pictures: Does an age-related decline exist? *Psychol. Aging* **1**, 11–17.

Paulescu, E., Frith, C. D., and Frackowiak, R. S. J. (1993). The neural correlates of the verbal component of working memory. *Nature, (London)* **362**, 342–344.

Petrides, M., Alivisatos, B., Evans, A. C., and Meyer, E. (1993a). Dissociation of human middorsolateral from posterior dorsolateral prefrontal cortex in memory processing. *Proc. Nati. Acad. Sci. U.S.A.* **90**, 873–877.

Petrides, M., Alivisatos, B., Meyer, E., and Evans, A. C. (1993b). Functional activation of the human frontal cortex during performance of verbal working memory tasks. *Proc. Nati. Acad. Sci. U.S.A.* **90**, 878–882.

Plude, D. J., and Hoyer, W. J. (1986). Age and the selectivity of visual information processing. *Psychol. Aging* **1**, 4–10.

Price, C. J. (1998). The functional anatomy of word comprehension and production. *Trends Cogn. Sci.* **2**, 281–288.

Puglisi, J. T., and Park, D. C. (1987). Perceptual elaboration and memory in older adults. *J. Gerontol.* **42**, 160–162.

Rabinowitz, J. C., Craik, F. I. M., and Ackerman, B. P. (1982). A processing resource account of age differences in recall. *Can. J. Psychol.* **36**, 325–344.

Rankin, J. L., and Collins, M. (1986). The effects of memory elaboration on adult age differences in incidental recall. The effects of memory elaboration on adult age differences in incidental recall. *Exp. Aging Res.* **12**, 231–234.

Raz, N. (2000). Aging of the brain and its impact on cognitive performance: Integration of structural and functional findings. In ''Handbook of Aging and Cognition'' (F. I. M. Craik and T. A. Salthouse, eds.), 2nd ed., pp. 1–90. Lawrence Erlbaum, Mahwah, NJ.

Raz, N., Gunning, F. M., Head, D., Dupuis, J. H., McQuain, J., Briggs, S. D., Loken, W. J., Thornton, A. E., and Acker, J. D. (1997). Selective aging of the human cerebral cortex observed *in vivo*: Differential vulnerability of the prefrontal gray matter. *Cereb. Cortex* **7**, 268–282.

Ross, M. H., Yurgelun-Todd, D. A., Renshaw, P. F., Maas, L. C., Mendelson, J. H., Mello, N. K., Cohen, B. M., and Levin, J. M. (1997).

Age-related reduction in functional MRI response to photic stimulation. *Neurology* **48**, 173–176.

Rugg, M. D., Fletcher, P. C., Frith, C. D., Frackowiak, R. S. J., and Dolan, R. J. (1996). Differential activation of the prefrontal cortex in successful and unsuccessful memory retrieval. *Brain* **119**, 2073–2082.

Rugg, M. D., Fletcher, P. C., Allan, K., Frith, C. D., Frackowiak, R. S. J., and Dolan, R. J. (1998). Neural correlates of memory retrieval during recognition memory and cued recall. *NeuroImage* **8**, 262–273.

Salthouse, T. A. (1996). The processing-speed theory of adult age differences in cognition. *Psychol. Rev.* **103**, 403–428.

Schacter, D. L., Alpert, N. M., Savage, C. R., Rauch, S. L., and Albert, M. S. (1996a). Conscious recollection and the human hippocampal formation: Evidence from positron emission tomography. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 321–325.

Schacter, D. L., Savage, C. R., Alpert, N. M., Rauch, S. L., and Albert, M. S. (1996b). The role of the hippocampus and frontal cortex in age-related memory changes: A PET study. *NeuroReport* **7**, 1165–1169.

Schonfield, D., and Robertson, B. (1966). Memory storage and aging. *Can. J. Psychol.* **20**, 228–236.

Scoville, W. B., and Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *J. Neurol. Neurosurg. Psychiatry* **20**, 11–19.

Sekuler, R., and Ball, K. (1986). Visual localization: Age and practice. *J. Opt. Soc. Am. A* **3**, 864–867.

Sergent, J., Ohta, S., and MacDonald, B. (1992). Functional neuroanatomy of face and object processing: A positron emission tomography study. *Brain* **115**, 15–36.

Shallice, T., Fletcher, P., Frith, C. D., Grasby, P., Frackowiak, R. S. J., and Dolan, R. J. (1994). Brain regions associated with acquisition and retrieval of verbal episodic memory. *Nature, (London)* **368**, 633–635.

Smith, A. D. (1980). Age differences in encoding, storage, and retrieval. *In* ''New Directions in Memory and Aging'' (L. W. Poon, J. L. Fozard, L. S. Cermak, D. Arenberg, and L. W. Thompson, eds.), pp. 23–45. Lawrence Erlbaum, Hillsdale, NJ.

Smith, E. E., and Jonides, J. (1997). Working memory: A view from neuroimaging. *Cogn. Psychol.* **33**, 5–42.

Smith, E. E., Jonides, J., Koeppe, R. A., Awh, E., Schumacher, E. H., and Minoshima, S. (1995). Spatial vs. object working memory: PET investigations. *J. Cogn. Neurosci.* **7**, 337–356.

Squire, L. R. (1992). Memory and the hippocampus: A synthesis from findings with rats, monkeys, and humans. *Psychol. Rev.* **99**, 195–231.

Stroop, J. R. (1935). Studies of interference in serial verbal reactions. *J. Exp. Psychol.* **18**, 643–662.

Talairach, J., and Tournoux, P. (1988). ''A Co-planar Stereotaxic Atlas of the Human Brain.'' Thieme, Stuttgart.

Taoka, T., Iwasaki, S., Uchida, H., Fukusumi, A., Nakagawa, H., Kichikawa, K. Takayama, K. Yoshioka, T., Takewa, M., and Ohishi, H. (1998). Age correlation in the time lag in signal change on EPI-fMRI. *J. Comput. Assist. Tomogr.* **22**, 514–517.

Till, R. E., Bartlett, J. C., and Doyle, A. H. (1982). Age differences in picture memory with resemblance and discrimination tasks. *Exp. Aging Res.* **8**, 179–184.

Treat, N. J., and Reese, H. W. (1976). Age, pacing, and imagery in paired-associate learning. *Dev. Psychol.* **12**, 119–124.

Tulving, E. (1983). ''Elements of Episodic Memory.'' Oxford University Press, Cambridge, England.

Tulving, E., Kapur, S., Markowitsch, H. J., Craik, F. I. M., Habib, R., and Houle, S. (1994a). Neuroanatomical correlates of retrieval in episodic memory: Auditory sentence recognition. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 2012–2015.

Tulving, E., Kapur, S., Craik, F. I. M., Moscovitch, M., and Houle, S. (1994b). Hemispheric encoding/retrieval asymmetry in episodic memory: Positron emission tomography findings. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 2016–2020.

Ungerleider, L. G., and Mishkin, M. (1982). Two cortical visual systems. *In* ''Analysis of Visual Behavior'' (D. J. Ingle, M. A. Goodale, and R. J. W. Mansfield, eds.), pp. 549–586. MIT Press, Cambridge, MA.

Warburton, E., Wise, R. J., Price, C. J., Weiller, C., Hadar, U., Ramsey, S., and Frackowiak, R. S. J. (1996). Studies with positron emission tomography of noun and verb retrieval in normal subjects. *Brain* **119**, 159–180.

Waugh, N. C., and Norman, D. A. (1965). Primary memory. *Psychol. Rev.* **72**, 89–104.

West, R. L. (1996). An application of prefrontal cortex function theory to cognitive aging. *Psychol. Bull.* **120**, 272–292.

Wheeler, M. A., Stuss, D. T., and Tulving, E. (1995). Frontal lobe damage produces episodic memory impairment. *J. Int. Neuropsychol. Soc.* **1**, 525–536.

Wise, R., Chollet, R., Hadar, U., Friston, K., Hoffner, E., and Frackowiak, R. S. J. (1991). Distribution of cortical neural networks involved in word comprehension and word retrieval. *Brain* **114**, 1803–1817.

Zola-Morgan, S., and Squire, L. R. (1985). Medial temporal lesions in monkeys impair memory on a variety of tasks sensitive to human amnesia. *Behav. Neurosci.* **99**, 22–34.

Zola-Morgan, S., Squire, L. R., and Amaral, D. G. (1986). Human amnesia and the medial temporal regions: Enduring memory impairment following in a bilateral lesion limited to field CA1 of the hippocampus. *J. Neurosci.* **6**, 2950–2967.

Zola-Morgan, S., Squire, L. R., Amaral, D. G., and Suzuki, W. A. (1989). Lesions of perirhinal and parahippocampal cortex that spare the amygdala and hippocampal formation produce severe memory impairment. *J. Neurosci.* **9**, 4355–4370.

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18

Functional Brain Studies of the Neurometabolic Bases of Cognitive and Behavioral Changes in Alzheimer's Disease

I. Introduction

The development of sophisticated biochemical methodologies for the functional assessment of the brain has made it possible to determine the neural metabolic bases that underlie cognitive and behavioral activities in a noninvasive manner in the living human brain (Sokoloff *et al*., 1977; Sokoloff, 1981, 1982; Mazziotta and Phelps, 1986; Holcomb *et al*., 1989; Roland, 1993; Raichle, 1998; Pietrini *et al*., 1999a,b). Over the past two decades, positron emission tomography (PET) and, more recently, functional magnetic resonance imaging (fMRI) have been employed in conjunction with neuropsychological and pharmacological experimental paradigms to investigate brain activity under a variety of physiological conditions in healthy individuals as well as in those with neurological and psychiatric disorders. In the present chapter, we will review the main studies conducted with these functional brain methodologies to investigate the neurobiological bases of dementing illnesses, with particular reference to Alzheimer's disease (AD).

II. Metabolic Correlates of Neural Activity in the Brain

A brief summary of the main aspects of brain energy metabolism is necessary to understand better the functional meaning of the *in vivo* neurometabolic studies performed with PET to measure regional cerebral glucose utilization and blood flow or fMRI to assess blood-flow-related phenomena.

In physiological conditions, energy in the brain, in the form of adenosine triphosphate (ATP), is produced almost exclusively through the oxidative metabolism of glucose. Because in the mature central nervous system neurons do not replicate and neosynthetic activities are limited, the great majority of ATP is utilized to support the biochemical processes associated with the activity of the ATP-dependent sodium/potassium pumps located in the cell membrane. These pumps actively exchange Na^+ and K^+ ions between the external and the internal cell compartments (for each molecule of ATP used, three Na^+ ions get out for every two K^+ ions that get in) and thus are essential for maintaining the internally negative cell membrane potential. When neurons depolarize following an action potential, the activity of the Na^+/K^+ pumps increases significantly to restore the ionic gradients required to reestablish the negative membrane potential (Whittam, 1962; Jueptner and Weiller, 1995). Therefore, increases in functional electrical activity linked to the transmission of impulses from neuron to neuron increase the demand for ATP and, in turn, for glucose metabolism. Furthermore, as cell storage of glucose and oxygen are quite limited, increases in glucose oxidative metabolism are accompanied by parallel changes in capillary blood flow in the same brain regions as a means for supplying adequate amounts of glucose and oxygen and to remove metabolic products (Roy and Sherrington, 1890; Sokoloff, 1982; Roland, 1993). This functional link provides the basis for using measures of cerebral glucose metabolism as well as blood flow as indices of neuronal and synaptic activity.

The frequency of action potentials on one hand and the rate of glucose utilization and capillary blood flow on the other show a direct linear correlation (Sokoloff, 1981; Schwartz *et al*., 1979; Kadekaro *et al*., 1985, 1987; Jueptner and Weiller, 1995). Moreover, increments in glucose utilization or blood flow over a baseline resting state, observed when neurons are being stimulated, reflect almost exclusively increased energy demands to support the activity of the Na^+/K^+ pumps (Mata *et al*., 1980; Jueptner and Weiller, 1995) and are localized in the regions with dense synaptic contacts between axons and dendrites and not in the location of cell bodies (Kadekaro *et al*., 1985, 1987; Schwartz *et al*., 1979; Roland, 1993).

While metabolic increases indicate increased synaptic activity, conversely, decreases in cerebral glucose metabolism or blood flow represent reduced functional activity and are due

to a net decrease in regional synaptic metabolic demand. This view is supported by both PET studies in humans and autoradiographic studies in animals that demonstrated a decrease in local cerebral glucose metabolism and blood flow in relation to the inhibition of cortical neurons (Kelly and McCulloch, 1993; Roland and Friberg 1988). For instance, the intracarotid injection of the GABA-A agonist 4,5,6,7-tetrahydroisoxazolo (5,4)-pyridin-3-ol (THIP), which induces postsynaptic neuronal inhibition by increasing the membrane conductance to Cl^- and thereby enhancing the negative membrane resting potential, is associated with a dose-dependent decrease in regional cerebral blood flow (rCBF) in the cortical areas of the affected hemisphere (Roland and Friberg, 1988). These deactivations may be due to a decreased excitation (i.e., the deactivated area may receive less input from another brain structure) or to a local net increase in synaptic inhibition (Haxby *et al*., 1994; Kawashima *et al*., 1995). Therefore, changes in glucose utilization or blood flow represent valuable indices of regional changes in functional synaptic activity in the brain.

III. Cerebral Glucose Metabolism and Blood-Flow Studies in Alzheimer's Disease

Alzheimer's disease, the most common form of dementing illnesses in the elderly, typically begins with a subtle onset of memory disturbances that may represent the only symptoms for several years (Pietrini *et al*., 1993b; Linn *et al*., 1995) and that are often followed by impairments of attentional and executive functions, semantic memory, and visuoperceptual skills along with alterations in personality and behavior (Grady *et al*., 1998; Haxby *et al*., 1990; Mendez *et al*., 1990; Pietrini *et al*., 1996).

From a neuropathological perspective, AD is characterized by the presence of senile plaques, neurofibrillary tangles, and loss of neurons and their synaptic projections (Whitehouse *et al*., 1981; Terry and Katzman, 1983). Although the regional distribution of neuropathological lesions varies among patients, the areas most commonly affected include the association neocortical areas of the parietal, temporal, and frontal lobes and limbic regions (Braak and Braak, 1991; Terry and Katzman, 1983; Lewis *et al*., 1987; Hof *et al*., 1995). The progression of the neuropathological process is thought to produce the cognitive deficits associated with dementia by affecting first the medial temporal lobe structures, including the entorhinal cortex and the hippocampal formation, and subsequentely spreading to the neocortical association areas of the temporal, parietal, and frontal lobes (Lewis *et al*., 1987; Braak and Braak, 1991; Van Hoesen *et al*., 1991).

Since their first appearance in the early 1980s, functional brain imaging methodologies have been used extensively to investigate the neurophysiological correlates of cognitive decline in patients with AD at different stages of dementia severity (for reviews, see Rapoport, 1990, 1991, 1999; Grady and Rapoport, 1992; Mielke *et al*., 1998; Alexander and Pietrini, 2000; Pietrini *et al*., 2000b). Unlike postmortem examinations, which reflect only the end stage of disease and do not allow for concurrent assessment of cognitive or behavioral features, assessments by PET with 18-fluoro-D-deoxyglucose (18-FDG) provide *in vivo* metabolic measures of brain function. With PET, one can examine regional changes in metabolic indices of brain activity in disease during life and correlate such findings with clinical and cognitive features, even in the initial stages of the disease or, as we will discuss later, prior to the onset of symptoms in subjects at risk for developing the disease (Pietrini *et al*., 1993a, 1997a; Small *et al*., 1995, 2000; Reiman *et al*., 1996). Further, *in vivo* PET studies can be repeated over time to follow the development of the neuropathological process and to evaluate the brain response to potential therapeutic interventions (Grady *et al*., 1988; Haxby *et al*., 1990; Pietrini *et al*., 1993a; Nordberg, 1996).

A. Cerebral Glucose Metabolism in the "Resting State"

1. Metabolic Reductions Affect Mostly the Neocortical Association Areas

In agreement with the distribution of the neuropathological lesions revealed by the autopsy studies, the *in vivo* measurements of regional cerebral metabolic rates for glucose (rCMRglc) using PET in the "resting state" (i.e., eyes covered/ears plugged, minimal sensory stimulation) have been consistent in showing that rCMRglc reductions in patients with AD affect mostly the association neocortical areas, with a relative sparing of primary neocortical regions, subcortical structures, and cerebellum at least until the later stages of the disease (Duara *et al*., 1986; Kumar *et al*., 1991; DeCarli *et al*., 1992; Grady and Rapoport, 1992; Pietrini *et al*., 1996; Mega *et al*., 1999).

Grouping AD patients into those with mild, moderate, and severe dementia using the Mini-Mental State Exam (MMSE; Folstein *et al*., 1975) score has shown differences in cerebral metabolism as a function of dementia severity. Representative examples of brain PET scan examinations from AD patients in the different stages of dementia severity are shown in Fig. 18.1 (see color insert). Such cohort comparisons suggest that the first reductions in brain glucose utilization in mildly demented patients with AD relative to that seen in healthy elderly subjects typically appear in parietal and temporal neocortical association areas. As the disease progresses, resting state metabolism is reduced in additional neocortical regions, including frontal and occipital lobes until, in the most severe stages, only areas of sensorimotor and primary visual and auditory cortices and subcortical structures show some relative preservation of glucose metabolism (Duara *et al*., 1986; Kumar *et al*., 1991; Grady and Rapoport, 1992).

2. The Distribution of Metabolic Reductions is Heterogenous

Within this general framework of progression of the metabolic impairment, the regional distribution of metabolic lesions may differ markedly in individual patients. For example, some patients may show a greater involvement of the left cerebral hemisphere, whereas others may show a larger involvement of the right hemisphere. Some subjects may have a greater impairment of the frontal and temporal lobes and others of the posterior cortical regions (Haxby *et al*., 1988). This hetero-

geneous distribution of metabolic reductions allows for the determination of right/left asymmetry indices and anterior/posterior ratios that can be related to the distribution of the neuropsychological and behavioral impairment shown by individual AD patients (Haxby *et al*., 1985, 1988, 1990; Haxby, 1990; Grady *et al*., 1990). Interhemispheric metabolic asymmetries are greater in patients with AD than in healthy elderly people (Haxby *et al*., 1985; Duara *et al*., 1986; Waldemar *et al*., 1994). Further, these metabolic asymmetries appear to be stable over time, indicating that the pathological process maintains a relatively more selective effect on the same brain regions across the different stages of dementia progression (Grady *et al*., 1986, 1988; Haxby *et al*., 1988, 1990). In a study conducted in our laboratory (Grady *et al*., 1990), a principal component analysis applied to cerebral metabolic data from a large sample of mildly to moderately demented patients with AD revealed four subgroups characterized by distinct patterns of cerebral metabolic abnormalities (Fig. 18.2, see color insert).

The most common pattern involved metabolic reductions in superior and inferior parietal lobules and in the posterior medial temporal regions. In a second group, rCMRglc was reduced in orbitofrontal and anterior cingulate areas, with a relative sparing of parietal regions. Metabolic reductions primarily affected the left hemisphere in a third group of patients, and the fourth group had reduced metabolism in frontal, temporal, and parietal cortical areas. These subgroup differences in brain metabolic abnormalities were subsequently verified in 24 patients with postmortem histopathological confirmation of AD, indicating that the clinical, neuropsychological, and neurophysiological heterogeneity observed in AD patients is not the consequence of clinical misdiagnosis or of concomitant additional neuropathological abnormalities (Strassburger *et al*., 1996) but rather an intrinsic characteristic of the disease process itself.

In the study from Grady *et al*. (1990), each patient subgroup had a characteristic neuropsychological and behavioral profile that corresponded to the distribution of the metabolic deficits. Specifically, the group of patients showing reduced metabolism in orbitofrontal cortex, a brain region known to be important in the regulation of behavior including the modulation of aggressive response (Pietrini *et al*., 2000c), showed increased agitation, anger outbursts, inappropriate social behavior, and personality changes (Grady *et al*., 1990). Patients in Group 3, who had greater left hemisphere hypometabolism, showed more impaired verbal memory, verbal fluency, and calculating abilities than Group 1 patients (who had parietal and temporal deficits), but had better visuoperceptual performance and drawing skills. In agreement with these findings, another study that focused on the relation between intrahemispheric variability of metabolic lesion distribution and neuropsychological profiles, demonstrated that patients with greater parietal than frontal hypometabolism had greater impairment of verbal comprehension, calculations, visuospatial construction, and immediate visuospatial memory span, whereas patients with disproportionate frontal hypometabolism were more impaired on verbal fluency and attention (Haxby *et al*., 1988).

Additional independent studies in patients with AD have demonstrated regional differences in the distribution of brain metabolic abnormalities in the frontal and temporal lobes associated with specific behavioral disturbances, such as depression, agitation, disinhibition, delusions, and hallucinations (Sultzer, 1996), apathy (Craig *et al*., 1996), or delusional misidentification syndrome (Mentis *et al*., 1995).

3. Metabolic Reductions in Neocortical Areas May Precede and Predict Nonmemory Cognitive Impairment

Longitudinal studies that combined clinical, neuropsychological, and PET scan examinations have indicated that some patients in the very early stages of AD, who complain only of isolated amnesia, express abnormal patterns of glucose metabolism in neocortical areas that precede and predict the subsequent development of nonmemory cognitive impairments (Grady *et al*., 1988; Haxby *et al*., 1990). For instance, patients with memory impairment and predominant left hemisphere hypometabolism are subsequentely more likely to develop greater language impairment, while those with disproportionate right hemisphere hypometabolism will tend to show greater impairment of visuoconstructive abilities (Haxby *et al*., 1985). These metabolic alterations can be detected as much as 18–24 months prior to the clinical appearance of the cognitive signs, suggesting that compensatory mechanisms in the brain, likely related to the redundancy in neuronal connections, may maintain cognitive skills within a relatively normal range for quite a long time.

4. Interregional Functional/Metabolic Correlations Are Altered in Alzheimer's Disease

In addition to these regional metabolic abnormalities, patients with AD also have a decreased number of metabolic correlations in functional activity between the association neocortical areas as compared to age-matched healthy individuals. The loss of significant metabolic correlations involves particularly the correlations between homologous regions in the right and left hemispheres and the correlations between frontal and parietal cortical areas (Horwitz *et al*., 1987). These findings suggest that a disruption of the normal functional connectivity between brain regions accompanies the dementing neuropathological process (Horwitz *et al*., 1987). As we will discuss later, in some patients with AD such a disruption in functional/metabolic interregional correlations may be detected even before significant glucose metabolic reductions in any cortical region become evident (Pietrini *et al*., 1993a; Azari *et al*., 1993). Thus, determination of the patterns of interregional metabolic correlations may provide a sensitive method for identifying subtle functional changes in brain activity that can aid in early detection and in characterizing the neural systems preferentially affected throughout the clinical course of AD (Pietrini *et al*., 1993a; Alexander and Moeller, 1994; Azari and Pietrini, 1995).

5. Metabolic Reductions in Temporal and Parietal Cortex Can Be Found in Other Dementing Disorders

While metabolic reductions in the neocortical association areas of the parietal and temporal lobes are a typical finding

in patients with AD, they are not necessarily specific for AD. A pattern of parietal and temporal cortex hypometabolism was found in a patient initially diagnosed as having dementia of the Alzheimer type and then Parkinson's disease with dementia but who was found to have only Parkinson's disease on postmortem histopathological examination. The metabolic changes observed were similar in magnitude and regional distribution to what is seen in patients with probable AD (Schapiro *et al*., 1993). This may indicate that glucose metabolic reductions in parietal and temporal neocortical areas in patients with AD are related to the dementia state rather than being a specific characteristic of the disease. Indeed, glucose hypometabolism in parietal and/or temporal cortical regions has been demonstrated in some patients with Parkinson's disease (Kuhl *et al*., 1984; Arahata *et al*., 1999), normal pressure hydrocephalus (Friedland, 1989), and Creutzfeldt–Jakob disease (Friedland *et al*., 1984). Some mild reductions in glucose metabolism in temporal cortical areas appear also to be present in patients with sleep apnea syndrome, a disorder of breathing during sleep that is associated with cognitive disturbances, as compared to healthy control individuals. The degree of reduction in glucose utilization is strongly related to the severity of the breathing disorder and of the cognitive impairment (Pietrini *et al*., 1998).

6. Clinical Subgroups in Alzheimer's Disease: The Visual Variant

As discussed above, AD is characterized by some degree of clinical and neuropsychological heterogeneity that corresponds to a heterogenous distribution of brain metabolic alterations as revealed by *in vivo* PET examinations. Despite their distinctive features, however, these clinical subgroups commonly share disturbances of memory function as the presenting complaint at onset of the disease (Foster *et al*., 1983; Haxby *et al*., 1985; Mayeux *et al*., 1985; Martin *et al*., 1986; Grady *et al*., 1990). This seems to imply that the disease process originates in common brain structures in these subgroups but then progresses to involve different cortical regions, resulting in distinct sets of neuropathological and clinical features, such as those characterized by prominent language or visuospatial deficits (Foster *et al*., 1983; Haxby *et al*., 1985; Grady *et al*., 1990). Indeed, impariment of episodic memory appears to be a constant accompanying clinical feature also in the case of patients with focal cortical syndromes who later are shown to have AD (De Renzi, 1986; Mesulam, 1987).

In a relatively rare clinical subtype of AD, however, the onset of the disease is characterized by prominent disturbances of visual and visuospatial functions, which are not accompanied by any memory complaints. The first activities to be impaired usually are those requiring visuospatial processing, including driving and reading. As patients progressively lose their ability to judge the relative distance among objects, simple actions such as keeping the proper distance from other vehicles, parking between cars, or even driving in a straight line become increasingly difficult (Furey-Kurkjian *et al*., 1996; Pietrini *et al*., 1996). Similarly, these individuals lose the ability to keep track of a printed line so that reading books or newspapers is affected as well. These cognitive difficulties stand in isolation and in sharp contrast to the relative preservation of other cognitive domains, including memory function and personality (Furey-Kurkjian *et al*., 1996). Thus, for instance, while driving is impaired because of their visual difficulties, these subjects retain a normal capability to perform all the actions required to operate their cars in the proper sequence. This appears to be different from what is seen in patients with more typical AD, whose driving may be impaired because they cannot remember aspects of the task or become confused in unfamiliar environments.

Because of the purely visual nature of their complaints, it is common for individuals with this visual variant of AD to seek repeated ophthalmological evaluations, which fail to detect a lesion of the peripheral visual system that can explain their disturbances (Pietrini *et al*., 1996; Galton *et al*., 2000). Only several months after the appearance of the first disturbances when, with worsening of the disease, visual dysfunction progresses to include alexia, poor hand–eye coordination, visual agnosia, and Bálint's syndrome (oculomotor apraxia, optic ataxia, visual inattention, and simultagnosia), and even more so when additional nonvisual cognitive difficulties also begin to appear, is the possibility of a neurodegenerative process taken into account (Pietrini *et al*., 1996). Thus, while visual functions also may become impaired in patients with typical AD, the peculiarity of this atypical subgroup is that visual and visuospatial deficits appear as the first salient clinical symptoms and remain prominent until the end stage of disease (Pietrini *et al*., 1993b, 1996; Galton *et al*., 2000). On the other hand, memory, personality, behavior, critical judgment, moral restraints, and insight into their condition, which are consistently affected to some degree in patients with typical AD, remain relatively preserved until the most advanced stages of dementia (Pietrini *et al*., 1993b, 1996).

These distinctive features shown by patients with the visual variant of AD compared to patients with the typical form of AD on clinical and neuropsychological testing are accompanied by differential patterns of brain metabolic changes as measured by PET scan examinations. Relative to heathy control subjects, both the typical and the visual variant groups of AD patients revealed reduced glucose utilization at rest (eyes/ears covered) bilaterally in parietal neocortical association areas (Pietrini *et al*., 1996). The patients with typical AD showed additional metabolic reductions in limbic, temporal, and frontal cortical regions and relative preservation of visual and primary sensory areas. In contrast, the visual variant patients had greater metabolic deficits than the typical AD patients in parietal and occipital cortices (including primary visual cortex) with a relative sparing of inferior temporal, frontal, and limbic regions (Fig. 18.3, see color insert). This distinctive pattern of the regional distribution of brain metabolic impairment remains relatively stable as the disease progresses (Pietrini *et al*., 1993b).

Consistent with the *in vivo* metabolic measures obtained by PET, the postmortem distribution of histopathology in two of the visual variant patients that had come to autopsy from our group showed greater Alzheimer-like neuropathology in the posterior occipital association and primary visual cortex compared to reference values from a group of severity-matched demented patients with typical AD (Pietrini *et al*., 1997b). A similar prevalent distribution of senile plaques and neurofibrillary tangles in the occipitoparietal and posterior cingulate

regions with relative sparing of the temporal, frontal, and limbic regions has been described in a group of autopsy-confirmed AD patients with visual symptoms (Hof *et al*., 1990, 1993, 1997). A recent clinical, neuroradiological, and postmortem study demonstrated severe bilateral reduction in occipital lobe perfusion (as measured by single photon emission computed tomography, SPECT), posterior brain atrophy, and severe neuropathological involvement of occipital visual cortex at autopsy in a patient with AD who had a more than 3-year history of isolated and progressive visual difficulties prior to the appearance of additional cognitive complaints (Galton *et al*., 2000). Together, the clinical and neuropsychological features and the brain metabolic and histological findings in these patients with AD and early and prominent visual dysfunction indicate that a different underlying neuronal vulnerability to the disease process may occur in this clinical variant of AD. This visual variant, along with other atypical presentations of AD, such as progressive biparietal syndrome, provides evidence in support of the heterogeneity of the neuropathological process in AD and raises the question about the mechanisms that may underlie the expression of these different phenotypes. The progress in molecular pathology and genetic studies likely will lead to the understanding of the differential neuronal sensitivity to neuropathological processes and explain the heterogeneity of disease development (Galton *et al*., 2000).

7. Decreased Brain Glucose Utilization in Alzheimer's Disease Reflects Neuronal Dysfunction

Dementia in AD is accompanied by a 30–40% cell loss which leads to brain atrophy. Given that an increased partial volume effect secondary to atrophy may artifactually decrease *in vivo* metabolic measures by PET, for many years it has been debated whether glucose metabolic reductions in the brain of AD patients were true indices of impaired neural activity or simply the result of tissue loss. To address this issue, we employed high-resolution segmented structural magnetic resonance images to correct rCMRglc measures for the atrophy-related partial volume effects in a group of mildy to severely demented patients with AD and age-matched healthy controls (Ibáñez *et al*., 1998). The results demonstrated that rCMRglc reductions per gram of brain tissue in parietal, frontal, and temporal cortex remained significantly lower in the patients with AD than in controls after correction for partial volume effects (Fig. 18.4). Thus, rCMRglc reductions observed in AD represent a true biochemical index of neuronal synaptic dysfunction in the affected brain regions and are not an artifact due to the loss of tissue.

B. Stimulation Studies in the Assessment of the Neural Correlates of Cognitive Dysfunction in Patients with Alzheimer's Disease

The combination of sensory or neuropsychological stimulation paradigms with functional brain imaging methodologies has made it possible to determine the neurophysiological activity of the brain systems involved in distinct cognitive functions and the changes that occur in association with the development of the neuropathological process in patients with AD at different stages of dementia severity. Further, one can evaluate the effects of the pharmacological modulation of specific neurotransmitter systems on brain metabolic and cognitive functions by comparing behavioral performance and brain activity in response to a neuropsychological task prior to and during the administration of a selective neurotransmitter enhancer or suppressor (Furey *et al*., 1997, 2000).

The great majority of these functional brain studies has been conducted using the PET–$H_2{}^{15}O$ technique to measure rCBF, which, as rCMRglc, represents a reliable index of neuronal

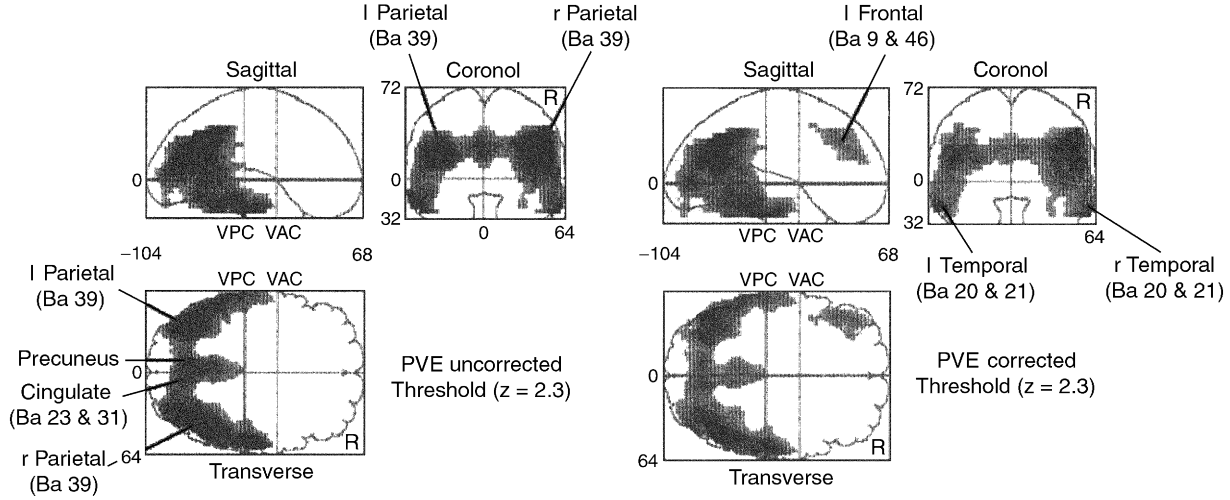

FIG. 18.4. Comparison of glucose metabolism between control subjects and AD patients before and after partial volume (atrophy) correction. Statistical parametric maps of orthogonal projections for significant voxels are shown. The intercommissural line is set to 0 mm. The anterior commissure (VAC) and the posterior commissure (VPC) are represented in the sagittal and transverse projections. The projections are presented with a z threshold of 2.3 for uncorrected (left) and partial volume effect (PVE)-corrected (right) values. No area with significant reduction in metabolism became normal after correction. Further, reductions in glucose metabolism in a left frontal area of AD patients became significant only after correction. Ba, Brodmann areas; l, left; r, right (adapted from Ibáñez *et al*., 1998).

synaptic activity (Jueptner and Weiller, 1995). Unlike PET–rCMRglc exams, though, which require approximately 1 hr to be completed and allow for no more than two scans in the same PET session (Duara *et al*., 1990, 1992; Kessler *et al*., 1991; Pietrini *et al*., 1997a, 1999b, 2000a), rCBF determinations last from 1 to 4 min so that several scans can be repeated sequentially only a few minutes apart, while subjects are at rest or engage in a variety of cognitive activities (Grady *et al*., 1993; Mentis *et al*., 1996, 1998). In general, these studies are conducted by contrasting brain functional responses during a certain stimulation condition with a baseline or control condition to determine those brain regions that show a significantly increased or decreased rCBF from baseline during a specific cognitive or sensory stimulation paradigm.

1. Studies with Cognitive Probes

Several studies have sought to investigate the brain functional responsiveness in patients with AD in comparison with matched healthy control subjects during the performance of distinct cognitive tasks, including memory, language, and visual abilities. In Table 18.1 are reported the experimental designs and major findings from rCMRglc and rCBF studies in AD patients performed with the use of cognitive probes.

In a study conducted in our laboratory, Grady and colleagues (1993) used PET–rCBF to determine how the brain functional response in AD patients with mild to moderate dementia differed from healthy elderly controls on a visual discrimination task that involved the perceptual matching of faces as compared to a sensorimotor control task. The rCBF during the control task (which required pressing a button alternately with right and left thumbs in response to a nonsensical visual stimulus) was subtracted from the rCBF during the face-matching task (subjects had to indicate whether the right or the left face matched the target by pressing the button in the corresponding hand) to obtain the mean difference rCBF value associated with the perceptual task. Response accuracy was similar in the two groups, although the AD patients had significantly slower reaction times. Significant rCBF increases occurred in equivalent occipitotemporal and occipitoparietal visual cortical regions in the AD patients and the control group. Further, despite the fact that rCBF in occipitotemporal regions during the control task was significantly lower in the AD patients than in the control subjects, the mean increment rCBF did not differ between the two groups (Grady *et al*., 1993). These findings suggest that the ability to recruit visual association areas during the performance of a visual perceptual discrimination task is preserved despite the presence of reduced rCBF in the baseline state in the mild stages of dementia in patients with AD (Rapoport and Grady, 1993).

Functional studies in young healthy individuals have shown that face matching is a complex cognitive task that relies on a widely distributed set of brain regions important for object perception and attentional processing that extends from occipital and occipitotemporal areas through frontal association cortical areas (Haxby *et al*., 1994). In addition, individual differences in task performance during the perceptual matching of faces are associated with a differential modulation of the functional interactions among the cortical and subcortical brain regions implicated in object perception and attentional processing (Alexander *et al*., 1999). In patients with AD, these functional regional interactions may be altered by the neuropathological process even in the early stages of the disease and prior to more evident changes in brain responsiveness. Horwitz and colleagues (1995) applied network path analysis to the same face-matching PET–rCBF data reported in the above study by Grady *et al*., (1993) to determine whether the patterns of brain functional regional interactions during the perceptual task differed between the AD patients and healthy controls. Indeed, the results indicated that although the AD patients had the same performance accuracy and mean rCBF increase in occipital visual association areas as controls (Table 18.1), they appeared to use a different network of brain regions while performing the face-matching task (Horwitz *et al*., 1995).

In a study by Becker *et al*. (1996), rCBF was assessed with PET during the performance of auditory–verbal memory tasks in a group of seven AD patients and seven healthy controls. They found that the AD group activated brain regions important for auditory and verbal memory processing to a greater extent than the healthy elderly subjects and, further, that additional brain regions were activated in the AD patients during the cognitive task conditions compared to that seen in the elderly controls. The results of this study and those of Horwitz and colleagues (1995) support the idea that networks important for cognitive functions are altered by the neuropathological process in AD and that the brain may compensate for the effects of neurodegeneration through a functional reorganization of neurocognitive resources.

2. Studies with Sensory Stimulation Paradigms

The *in vivo* assessment of the brain metabolic activity underlying distinct cognitive functions in patients with AD is limited to individuals with mild or mild to moderate levels of dementia severity, as all of the above neuropsychological paradigms require that subjects perform and actively engage in a given memory, language, or visuospatial task. Unfortunately, with worsening of dementia, patients lose their ability to perform even simple cognitive tasks, making the interpretation of the functional data questionable (i.e., AD subjects may not activate a certain brain region found activated in controls because that region is dysfunctional or simply because they did not engage in the task). Further, their compliance with the PET experimental procedure, which requires some restriction of movement, progressively diminishes during the scanning session. In an effort to overcome these limitations and thus be able to investigate brain functional activity across the whole range of dementia severity, including patients in the very advanced stages, we have developed some passive sensory stimulation paradigms that do not require any active participation on the patient's side.

We employed the double injection PET–FDG technique (Brooks *et al*., 1987), which allows for two rCMRglc measures to be performed sequentially during the same PET scan session, to determine the brain functional metabolic response elicited by audiovisual stimulation (presentation of a color movie) as compared to a resting state (i.e., eyes closed, ears occluded, no sensory stimulation) condition. The study was conducted in a group of 15 patients with AD who ranged from the mild to severe stage of dementia and 14 healthy

TABLE 18.1 Functional Brain Imaging Studies in Alzheimer's Disease

Reference	Subjects	Procedure	Results and comments
Duara *et al.*, 1987	7 healthy subjects (2M/5F; mean age 69; range 52–80 years) and 5 AD patients (3M/2F; mean age 69 years; range 52–80 years); careful medical screening	18-FDG i.v. bolus PET; PETT V (FWHM 15 mm); resting state (quiet and darkened environment) compared to picture viewing task condition	The variability of CMRglc measures can be reduced by performing studies during behaviour activation in normal and demented subjects; i.e., a specific behavioral state would be expected to induce more comparable levels of functional activity in the same or in different individuals.
Miller *et al.*, 1987	7 AD patients (68 ± 6 years) and 7 controls (67 ± 8 years); careful medical screening	11C-2-DG i.v. bolus PET; PETT VI; recognition memory task	AD patients were unable to recognize repeated items above a chance level. An abnormal temporal lobe metabolic response was associated with impairment on the memory task: all AD patients demonstrated a right temporal lobe asymmetry, while five of seven controls showed a left one.
Mubrin *et al.*, 1989	26 AD patients (6M/20F; 76 ± 8 years)	^{133}Xe by inhalation, Cerebrograph 32B, (low resolution, planar imaging) was used to assess the effect of pyritinol during mental activation [Word Pair Learning and Recall test (WPLR)] on cognitive disturbance and rCBF	Before treatment, during mental activation, AD patients showed a larger area of CBF increase associated with a poorer WPLR performance. After placebo, the mean hemispheric blood flow at rest remained unchanged, but a diminished number of activated areas was found in the placebo group with a significant increase in WPLR performance. After pyritinol, the resting blood flow level was unchanged, while during activation, a smaller number of activated areas was found in the drug group, which showed a better performance than the placebo group.
Duara *et al.*, 1990	(1) 26 young (33 ± 9 years) and 30 elderly healthy subjects (64 ± 9 years); 17 AD patients (74 ± 9 years) (2) 3 young (42 ± 6 years) and 19 elderly healthy subjects (63 ± 10 years); 21 AD patients (69 ± 9 years) (3) 8 young (38 ± 7 years) and 12 elderly healthy subjects (65 ± 11 years); 6 AD patients (60 ± 11 years)	18-FDG i.v. bolus PET; PETT V (FWHM 15 mm); (1) picture preference task (2) reading memory task (3) verbal fluency task	(1) AD patients showed rCMRglc increases in primary and association visual cortex, similar to elderly normals. (2) During the task, left superior parietal regions, already hypometabolic at rest, were less activated. (3) AD patients demonstrated no rCMRglc increases, but only significant decreases in the right and left occipital regions.
Buchsbaum *et al.*, 1991	6 AD patients (4M/2F; mean age 71; range 65–74 years) and 6 age-matched healthy controls (3M/3F; mean age 69 ± 5 years); careful medical screening	18-FDG i.v. bolus PET; NeuroECAT (FWHM 7.6 mm); olfactory match-to-sample task	Patients had lower metabolic rates in the anterior portion of the medial temporal cortex; this rCMRglc difference was greater during the olfactory memory task.
Kessler *et al.*, 1991	21 ''possible'' AD patients (6 M/15 F; 65 ± 7 years) and 9 age-matched healthy controls (6 M/3F; 62 ± 7 years); careful medical and neuropsychological screening	18-FDG i.v. bolus PET; Scanditronix PC384; a continuous visual recognition task with different degrees of difficulty	The visual task induced significant increase in global CMRglc. CMRglc increase was significantly larger in controls (+21%) than in AD patients (+6%); increases were mainly in visual cortex.
Duara *et al.*, 1992	20 AD patients (6M/14F; 69 ± 9 years) and 14 controls (9M/5F; 61 ± 11 years)	18-FDG i.v. bolus PET; PETT V (FWHM 15 mm); reading memory task versus a resting state condition	No significant difference in the degree of rCMRglc change was found between the AD patients and normal controls. Thus the task did not allow for a better discrimination of AD patients from normal controls on the basis of regional metabolic deficits, despite that the mean global CMRglc change in normals was about 33% greater than in the AD subjects.

(*continues*)

TABLE 18.1 (*Continued*)

Reference	Subjects	Procedure	Results and comments
Grady *et al.*, 1993	7 "possible" AD male patients (64 ± 7 years) and 8 age-matched healthy male controls (65 ± 9 years); careful medical screening	15-Oxygen water i.v. bolus PET; Scanditronix PC1024-7B (FWHM 6.5 mm); a face matching task versus a sensorimotor control task	The pattern of rCBF response to the face-matching task found in controls was seen also in mildly to moderately demented AD patients. Both groups showed bilateral increases in occipitotemporal and occipitoparietal extrastriate cortex, despite lower rCBF in these regions during the sensorimotor control task in the AD group.
Herholz *et al.*, 1993	43 AD patients (19M/24F; 68 ± 7 years) and 8 controls (6M/2F; 61 ± 9 years); careful medical screening	18-FDG PET; resting state versus continuous visual recognition task	Global increase in glucose metabolism was significantly greater in normals than in AD patients. Task-related activation was found in visual cortex, temporo-parietal regions, and cerebellum.
Riddle *et al.*, 1993	10 "possible" AD patients (72 ± 12 years) and 9 healthy controls (69 ± 11 years)	99mTc-exametazime SPECT (Multi-X810) during a verbal memory task	Performance on recognition was worse and more variable in patients than controls. During the recognition task, controls had greater CBF increases in left dorsolateral frontal cortex and anterior cingulate.
Kessler *et al.*, 1996	35 AD patients divided into two groups of 17 patients (68 ± 8 years) and 18 patients (65 ± 8 years)	18-FDG i.v. bolus; Siemens CTI, ECAT EXACT (FWHM 5 mm); resting state versus a continuous auditory recognition task (first group); or versus continuous visual recognition task (second group)	Comparison of task versus resting conditions showed significant rCMRglc increases only in thalamus and cerebellum in the first group and in occipital regions in the second one.
Mentis *et al.*, 1996a,b	10 AD patients (5M/5F; 72 ± 7 years) and 12 controls (7M/5F; 64 ± 12 years)	15-Oxygen water i.v. bolus PET; Scanditronix PC2048-15B; monochromatic red flashing lights administered at different frequencies	The rCBF responses in striate (at 7 and 14 Hz) and in bilateral middle temporal neocortical association areas (at 1 Hz) were signficantly greater in the control subjects than in AD patients.
Becker *et al.*, 1996	7 AD patients (2M/5F; 69 ± 11 years) and 7 age-matched healthy controls (2M/5F; 66 ± 8 years)	15-Oxygen water i.v. bolus PET; Siemens 951/31 PET; auditory–verbal memory task	Performance of automatic cognitive operations activated a similar pattern of cortical activity in both groups. Increasing task demand showed in the AD patients a specific activation of dorsolateral prefrontal cortex and parietal temporal borders, demonstrating that the AD brain retains a significant degree of functional plasticity.
Buck *et al.*, 1997	29 AD patients and 17 age-matched healthy controls	HMPAO-SPECT; cued reaction time task	The cognitive profile associated with AD included both spatial- (right superior parietal lobe hypoperfusion) and object-based (left inferior parietal lobe hypoperfusion) attentional impairments.
Hock *et al.*, 1997	8 healthy subjects (60 ± 15 years) and 10 AD patients (63 ± 13 years)	Near-infrared spectroscopy (NIRO 500, Hamamatsu Photonics K.K.) to assess Hb oxygenation in the frontal and parietal cortex during a verbal fluency task	Elderly subjects showed increases in hemoglobin oxygenation in both parietal and frontal regions, while AD patients had decreases in parietal and increases in frontal cortical regions. In AD patients, a marked reduction in rCBF and hemoglobin oxygenation may accompany cognitive activation, probably mainly in degenerating brain areas, such as the parietal cortex.
Pietrini *et al.*, 1997a	16 healthy subjects with trisomy 21 (Down syndrome): 8 younger (35 ± 2 years) and 8 older (50 ± 7 years) adults; careful medical screening	18-FDG i.v. bolus PET; Scanditronix PC2048-15B (FWHM = 6 mm); resting state condition (eyes patched, ears plugged, minimal room noise) versus a passive audiovisual stimulation paradigm	No significant differences in rCMRglc at rest between the two groups. In contrast, during audiovisual stimulation older nondemented subjects with Down syndrome showed significantly lower rates of rCMRglc than young subjects in parietal and temporal neocortical areas. The differential increases in rCMRglc could reflect an intrinsic functional limitation of the brain to respond to increased neural stimulation in older Down syndrome subjects prior to the onset of dementia, as a result of the effects of the pathological process in the preclinical stages of AD.
Cardebat *et al.*, 1998	17 AD patients (9M/6F; 66 ± 8 years) and 20 healthy controls (7M/13F; 64 ± 5 years)	^{133}Xe SPECT; Tomomatic 64 Medimatic;	No significant rCBF differences between the two groups in the memory versus listening to words comparison. A significant correlation between memory

		verbal episodic memory activation task (rest, passive listening, and memorizing)	performance and rCBF was found in the right lateral frontal cortex in AD patients only.
Mentis *et al.*, 1998	10 mildly demented ASD patients (3M/7F; 69 ± 5 years) and 11 severely demented AD patients (5M/6F; 73 ± 12 years) were compared to 19 healthy controls (11M/8F; 65 ± 11 years)	15-Oxygen water i.v. bolus PET; Scanditronix PC2048-15B; flashing monochromatic red lights at different frequencies	Functional failure was shown in brain regions that had normal function at rest and that typically show modest (frontal and extrastriate cortex, Brodmann's areas 18/19) or minimal (striate cortex) AD neuropathology. Stimuli that resulted in large changes in rCBF in controls were necessary to elicit functional failure in mildly demented patients, whereas stimuli resulting in intermediate and small changes in rCBF were sufficient to elicit failure in patients with moderate to severe dementia.
Woodard *et al.*, 1998	6 healthy older subjects (3M/3F; 70 ± 8 years) and 6 mildly demented AD patients (3M/3F; 69 ± 7 years)	15-Oxygen water i.v. bolus PET; Siemens 951 or 921; word rehearsal task versus a reading control task	Performance accuracy did not differ significantly between the two groups. During task, controls revealed prominent activation of dorsolateral prefrontal cortex (Brodmann's areas 46/9), middle frontal gyrus, precuneus, and precentral gyrus, whereas AD patients demonstrated additional involvement of left dorsolateral prefrontal cortex, left inferior parietal lobule, and left medial frontal gyrus.
Backman *et al.*, 1999	8 AD patients (3M/5F; 63 ± 4 years) and 8 healthy controls (4M/4F; 60 ± 7 years)	15-Oxygen water PET (GEMS PC2048-15B) during verbal episodic retrievial	AD patients showed a marked performance deficit in cued recall. During task, both groups showed increased rCBF in bilateral orbital and dorsolateral prefrontal cortex, left precuneus and right cerebellum, and reduced rCBF in left temporal regions. Healthy elderly subjects only activated the left parietal cortex and left hippocampus, while AD patients showed higher rCBF in the left orbital prefrontal cortex and left cerebellum than controls.
Johannsen *et al.*, 1999	16 AD patients (6M/10F; 68 ± 5 years) with mild to moderate dementia and 16 healthy age-matched control subjects (7M/9F; 65 ± 7 years)	15-Oxygen water PET; ECAT Exact HR-47 PET-camera; visual sustained and divided attention tasks	Compared to the healthy elderly subjects, activations in the right medial, superior, and inferior frontal gyri, and the right middle temporal and left lingual gyri were significantly lower in the AD patients; these differences became statistically greater during the divided attention task.
Saykin *et al.*, 1999	Mildly demented AD patients and healthy controls	Functional MRI BOLD; auditory stimulation tasks requiring semantic or phonological decisions	On the semantic task, inferior and middle frontal gyri were activated in both groups, but AD patients recruited locally expanded foci and other remote regions, suggesting functional compensation.
Smith *et al.*, 1999	26 healthy women were sudivided into a high-risk group ($N=14$, 52 ± 5 years) and a low-risk group ($N=12$, 53 ± 6 years) that differed only in their risk for developing AD (evaluated by positive family history or presence of APOE ϵ_4)	Functional MRI scan (1.5-T Siemens Magnetom Vision Scanner); visual naming and letter fluency tasks	The high-risk group showed areas of significantly reduced activation in the mid- and posterior inferotemporal regions bilaterally during both tasks, as compared to the low-risk group.
Pietrini *et al.*, 1999b	15 AD patients (70 ± 10 years); careful medical screening	18-FDG i.v. bolus; two sequential scans; Scanditronix PC2048-15B PET scanner; a resting state condition (eyes patched, ears plugged, minimal room noise) versus an audiovisual stimulation (watching and listening to a movie)	rCMRglc in the parietal neocortical areas correlated with dementia severity at rest and during audiovisual stimulation; in contrast metabolism in the visual and auditory regions was correlated with dementia severity during stimulation but not at rest.
Pietrini *et al.*, 2000a	15 AD patients (70 ± 10 years), 14 matched healthy subjects; careful medical screening	18-FDG i.v. bolus; two sequential scans; Scanditronix PC2048-15B PET scanner; same experimental paradigm as above (Pietrini *et al.*, 1999b)	Brain metabolic/functional response to stimulation in primary and association visual cortical areas was within the normal control values in the mildly demented AD patients but progressively declined with worsening of dementia up to point where no response could be elicited any longer.

elderly control subjects (Pietrini *et al*., 1999b, 2000a). As a group, the AD patients showed rCMRglc increases in response to the audiovisual stimulation paradigm in similar visual and auditory cortical brain regions as the healthy controls. Compared to controls, the AD patients also showed higher metabolism during stimulation than at rest in additional brain regions, including in the neocortical association areas of the parietal lobes, despite the fact that these areas were hypometabolic at rest. This suggests that more cortical networks may have been recruited in an attempt to compensate for the failure of others (Grady *et al*. 1993).

Consistent with the findings of the rCBF study by Grady *et al*. (1993) examined above, the subgroup of AD patients with mild dementia had rCMRglc increases in visual and auditory brain regions that did not differ significantly from those in healthy controls (i.e., they were within 2 standard deviations); in contrast, the patients with moderate to severe dementia showed significantly diminished rCMRglc responses. Further, in the AD patient group, the decline in the magnitude of metabolic response in primary and association visual cortical regions was highly correlated with dementia severity: virtually no metabolic response to audiovisual stimulation could be elicited in the severely demented patients (Fig. 18.5). The inability of the brain at this late stage of disease to respond to audiovisual stimulation implies a loss or otherwise severe dysfunction of its synaptic elements and neurotransmitter integrity, as shown by biopsy and postmortem studies (DeKosky and Scheff, 1990; Rapoport, 1999).

In addition to the metabolic increments in visual and auditory cortical areas, significant rCMRglc reductions in frontal and limbic regions during audiovisual stimulation were seen in the healthy controls but not in the AD patients (Pietrini *et al*., 2000a). Reductions in brain activity relative to baseline

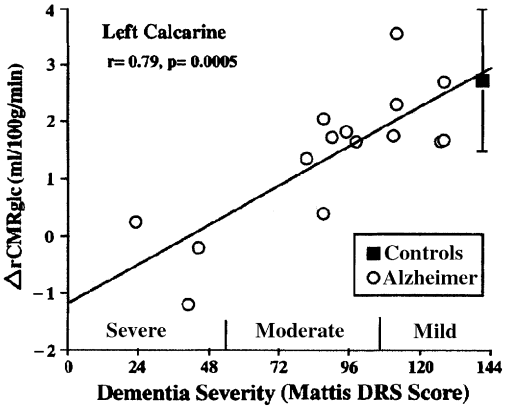

FIG. 18.5. Relation between dementia severity and brain metabolic response to audiovisual stimulation in patients with AD. The abscissa reports dementia severity, measured by the Mattis Dementia Rating Scale (DRS) score (maximum normal score 144). Regional differences in absolute rCMRglc in mg per 100 g of brain tissue per minute between audiovisual stimulation and the resting state for the AD patients are reported on the ordinate. The mean ± SD bar for controls is shown on the right. Mildly demented patients had rCMRglc increases in visual cortical areas within two standard deviations from the control mean. Brain metabolic response to audiovisual stimulation progressively declines with worsening dementia and is almost null in the severe stage of disease (from Pietrini *et al*., 2000a).

in areas not directly involved in a task have been shown in other sensory and cognitive activation studies in healthy subjects (Seitz and Roland, 1992; Haxby *et al*., 1994) and have been interpreted as regional depression of synaptic activity in relation to cross-model inhibition during selective attentional processing. The absence of such regional metabolic reductions in the AD patient group may reflect disruption of corticocortical connectivity (Morrison *et al*., 1990; Azari *et al*., 1992), consistent with the results of Horwitz *et al*. (1995) discussed above and with the neuropathological evidence that large pyramidal neurons with long intracortical and intercortical axons are most affected in the neocortex of patients with AD.

To examine the relation between dementia severity and brain synaptic efficiency in response to neural stimulations of increasing intensity, Mentis *et al*. (1996, 1998) in our laboratory employed PET–$H_2{}^{15}O$ to measure rCBF during the passive presentation of a graded visual stimulus. In this paradigm, goggles were used to present flashing red lights that alternated between left and right eyes to a group of healthy elderly controls and AD patients with mild and moderate-to-severe dementia. The flash frequency of the lights was parametrically increased from 0 (i.e., eyes open in darkness) to 1, 2, 4, 7, and 14 Hz, so that a stimulus–rCBF response curve could be obtained for the distinct primary and association visual cortical areas involved in the processing of these stimuli (Mentis *et al*., 1996, 1998). In the healthy control subjects, rCBF response to increasing flash frequency included a biphasic rising (with a peak at 7 Hz) and then falling in striate visual cortex, a linear increase in visual association cortical regions and a linear decrease in frontal cortical regions (Fig. 18.6). The AD patients showed similar curves but they had significantly smaller rCBF responses than controls at one or more levels of stimulation in many cortical regions (see example in Fig. 18.6). Specifically, in the mildly demented patients, abnormal brain responses were elicited only at the frequency producing the greatest changes in the healthy controls, whereas in the patients in the more advanced stage of dementia, a functional failure was evident also with stimuli that induced moderate rCBF changes in the controls (Mentis *et al*., 1998). Thus, despite the fact that rCBF at rest and rCBF changes at low frequency were not different from those in controls, patients with AD showed a severity-related decrease in the brain's ability to respond to incremental neural stimulation.

Together, results from the above studies by Pietrini *et al*., (2000a) and Mentis *et al*. (1996, 1998), along with those of Grady *et al*. (1993), demonstrate that synaptic viability and thus functional responsiveness in the brain are relatively maintained in the mild and to a lesser extent in the moderate stage of dementia in patients with AD, whereas they are greatly impaired in the later phases. Biopsy and postmortem studies of the density and size of synaptic elements in the brain of AD patients suggest stages of loss that may correspond to two stages of responsiveness in life (DeKosky and Scheff, 1990; Rapoport, 1999). In the first stage, the initial loss of presynaptic terminals is compensated by the relative enlargement of the remaining presynaptic terminals so that the net contact area between pre- and postsynaptic elements remains in the normal range. In the second stage, this hypertrophic mechanism is no longer sufficient to compensate for the additional presynaptic dropout, producing a net decrease in the total synaptic

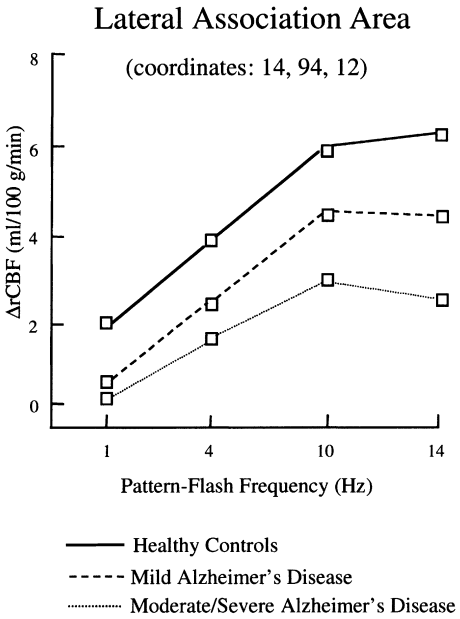

FIG. 18.6. Mean rCBF changes in response to graded neural stimulation induced by a flashing red light at different frequencies in a representative local maxima of the lateral visual association cortical area in healthy controls and in AD patients with mild and with moderate-to-severe dementia. Coordinates are according to the Tailarach and Tournoux atlas (1988). $^{*}P < 0.05$; $^{**}P < 0.001$ (from Mentis *et al*., 1998, modified).

contact area (Terry *et al*., 1991; DeKosky and Scheff, 1990; Rapoport, 1999). These results agree with other postmortem evidence that early neurofibrillary changes in pyramidal cortical neurons in the AD brain are associated with selective downregulation of mitochondrial gene expression (mRNA levels) for enzymes involved with mitochondrial oxidative phosphorylation. Such a downregulation may represent a "physiological" response to the decrease in energy demand associated with the reduced synaptic activity (Rapoport, 1999). In the later stage of the disease, in contrast, higher degrees of neurofibrillary tangle accumulation and cell death are accompanied by a general reduction in gene transcription (Chandrasekaran *et al*., 1996).

The greatly diminished synaptic efficiency observed in the later stage of AD could have a role in the lack of response to pharmacological treatment shown by patients with advanced dementia. Indeed, as available treatments for AD aim at increasing neurotransmitters in the synaptic cleft, viable synapses are needed for them to be effective (Pietrini *et al*., 2000a).

C. Functional Brain Studies in the Diagnosis of Alzheimer's Disease

1. Differential Diagnosis

From a clinical standpoint, the *in vivo* brain metabolic measurements obtained by PET may aid in the differential diagnosis of neurodegenerative diseases by demonstrating relatively distinctive patterns of changes in cerebral glucose utilization and blood flow among the different forms of progressive dementia. Although the distribution of metabolic changes in the brain is heterogeneous and may vary from patient to patient, as we discussed previously, dysfunction of parietal and temporal cortical regions is usually present in the early phase of AD. Further, with progression of dementia, additional metabolic impairments appear in other association regions, including the neocortical areas of the frontal and occipital lobes.

As reduced glucose metabolism and blood flow in several neocortical association areas, including those in the parietal and temporal lobes, can be observed also in other dementing disorders, it becomes important to consider ways for improving the diagnostic specificity and sensitivity of PET measures for AD (Azari and Pietrini, 1995; Small and Leiter, 1998). Integrating information obtained from laboratory and clinical examinations, together with relevant risk factors, may significantly enhance the ability to diagnose AD earlier and with a high degree of accuracy. For example, such an integrated approach has the potential to shorten time to diagnosis by several months or even years in patients with subtle cognitive complaints, thereby allowing for earlier, and potentially more effective, therapeutic interventions (Small and Leiter, 1998).

2. Early Detection

The patterns of abnormal changes in cerebral glucose metabolism and blood flow described in the previous sections of this paper have been reported in individuals who already met established research criteria for the diagnosis of "*possible*" or "*probable*" AD (McKhann *et al*., 1984). That is, significant cognitive impairment or dementia in these subjects was already evident on clinical examination.

In the past few years, several research groups have developed new experimental strategies aimed at detecting subtle alterations in brain metabolic function that could be employed for the preclinical identification of subjects who are likely to develop dementia. To pursue this finality, different experimental approaches can be used in various combinations.

The longitudinal evaluation of healthy individuals who have an established genetic risk for AD is a valuable strategy for identifying brain metabolic dysfunction predictive of development of dementia. Small *et al*. (1995) examined brain glucose metabolism at rest in subjects with mild memory complaints who had family history of AD and showed that those with the APOE ϵ4 allele had lower rCMRglc in the parietal areas than the subjects without APOE ϵ4. In a subsequent study, the same authors found that individuals with a single copy of the APOE ϵ4 allele, followed over 2 years, showed greater declines in glucose metabolism from baseline than noncarriers of the ϵ4 allele (Small *et al*., 2000). Further, they found that the baseline metabolic abnormalities predicted cognitive decline after the 2-year period of follow-up.

Reiman *et al*. (1996) showed altered patterns of resting glucose utilization in posterior cingulate, parietal, temporal, and prefrontal regions in cognitively normal subjects (without memory complaints) with a family history of AD and who were homozygous for the APOE ϵ4 allele. Studies of yet clinically unaffected family members in families with mutations on chro-

mosome 14 or mutations in the amyloid precursor protein (APP) gene have also shown evidence of resting state hypometabolism in parietotemporal brain regions (Kennedy *et al*., 1995).

Individuals with Down syndrome (or trisomy 21), who have a 100% risk for the development of AD pathology (Holland and Oliver, 1995), represent a unique human population for investigating brain function in the preclinical stages of AD and during the transition to dementia (Schapiro *et al*., 1992). All subjects with Down syndrome over 40 years of age show some neuropathological and neurochemical abnormalities postmortem that are indistinguishable from those observed in AD (Holland and Oliver, 1995). Further, up to 75–90% of individuals with Down syndrome develop dementia after 60 years of age.

In a longitudinal study of nondemented and otherwise healthy adults with Down syndrome examined at yearly intervals for up to 12 years, we showed that measures of cognitive abilities and brain metabolism at rest remained normal and stable until the onset of dementia. After the clinical diagnosis of dementia, both cognitive functions and parietotemporal glucose metabolism began to rapidly decline. These findings suggested that neurons maintain the ability to produce energy and perform biochemical activities essential for their survival until a threshold level of AD-like neuropathology is reached and a profound disruption of both cognitive and metabolic brain functions is triggered (Dani *et al*., 1996). However, the accumulation of AD-like neuropathology in the years preceding clinical onset of dementia is likely to impair the neuronal capability to respond to increased functional demands on the brain. Indeed, cerebral metabolic response to the same passive audiovisual stimulation paradigm described above (Pietrini *et al*., 1999b, 2000a) was impaired in parietal and temporal cortical areas of older nondemented Down subjects as compared to young control Down subjects (Pietrini *et al*., 1997a). Thus, despite normal biochemical activities and brain glucose utilization at rest, the functional damage associated with the progressive development of pathology prevents neurons in the parietal and temporal cortical areas, the first and most affected regions in the brains of patients with AD, from responding normally when the neuronal ''workload'' is maximized (Pietrini *et al*., 1997a).

In agreement with data from activation studies in AD patients (Pietrini *et al*., 1999b, 2000a; Mentis *et al*., 1996, 1998; Smith *et al*., 1999), these results indicate that stimulation paradigms with functional brain imaging methodologies may offer greater sensitivity than resting studies to the earliest effects of AD, acting as a neurophysiological stress test for dementia.

The use of sophisticated statistical methods can further enhance our ability to identify subtle metabolic changes to aid in early detection and to relate patterns of cerebral metabolic dysfunction to clinical manifestations (Alexander and Moeller, 1994; Azari *et al*., 1993; Azari and Pietrini, 1995; Pietrini *et al*., 1993a). Rather than relying on *t*-test comparisons of mean metabolic values to demonstrate significant differences between two subject groups (e.g., healthy controls and patients with AD), these analytical methods can uncover subtle abnormalities in the patterns of metabolic and functional correlations among different brain regions, and can be applied to study individual patients (Azari and Pietrini, 1995; Pietrini *et al*., 1993a). Using a multivariate discriminant function method, we identified an AD-like pattern of brain metabolic interregional correlations at rest in a nondemented subject with a family history of autosomal dominant AD, one year before conventional methods of analysis could demonstrate any cerebral metabolic reduction or cognitive decline in comparison to a group of healthy control subjects (Fig. 18.7A, see color insert, and 18.7B; Pietrini *et al*., 1993a).

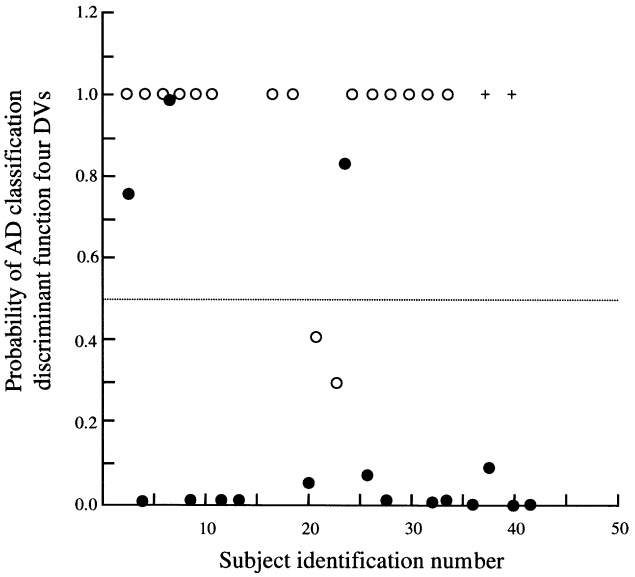

FIG. 18.7B. Probability of being classified as an AD patient based on a discriminant function analysis of rCMRglc data for patients with AD (open circles), healthy control subjects (filled circles), and the subject at risk (crosses). The vertical axis indicates the probability of being classified as AD obtained by applying a jackknife procedure to the discriminant analysis function. The great separation between the healthy control and the AD patient groups can be appreciated from the graph. Note that the at-risk subject was classified as having a 100% probability of being an AD patient at both times, despite the lack of any significant group-mean rCMRglc abnormality at time 1. PET scanning parameters are the same as in Fig. 1 (from Pietrini *et al*., 1993a).

In conclusion, the development of functional brain imaging methodologies has made it possible to evaluate, *in vivo*, the neurometabolic consequences of AD throughout its clinical course, from the earliest to the end stages of disease. In addition to PET studies of glucose metabolism and blood flow at rest, the combination of experimental paradigms which include sensory or cognitive stimulation as well as pharmacological modulation of neurotransmitter systems (Furey *et al*., 1997, 2000) with increasingly more sophisticated data analytic techniques is likely to further enhance our ability to detect subtle alterations in brain function in the earliest, presymptomatic phases of AD. Studying the preclinical and early phases of this disease in individuals with increased genetic risk may greatly advance our understanding of the neurobiological mechanisms involved in AD, individual differences in the progression of distinct clinical subtypes, and which individuals might benefit most from preventive or curative treatments as they become available.

References

Alexander, G. E., and Moeller, J. R. (1994). Application of the Scaled Subprofile Model to functional imaging in neuropsychiatric disorders: A principal component approach to modeling regional patterns of brain function in disease. *Hum. Brain Mapp.* **2**, 79–94.

Alexander, G. E., and Pietrini, P. (2000). Functional neuroimaging: SPECT and PET. *In* ''Early Onset Dementia'' (J. Hodges, ed.) Oxford University Press, Oxford, UK, (in press).

Alexander, G. E., Mentis, M. J., Van Horn, J. D., Grady, C. L., Berman, K. F., Furey, M. L., Pietrini, P., Schapiro, M. B., Rapoport, S. I., and Moeller, J. R. (1999). Individual differences in PET activation of object perception and attention systems predict face matching accuracy. *NeuroReport* **10**, 1965–1971.

Arahata, Y., Hirayama, M., Ieda, T., Koike, Y., Kato, T., Tadokoro, M., Ikeda, M., Ito, K., and Sobue, G. (1999). Parieto-occipital glucose hypometabolism in Parkinson's disease with autonomic failure. *J. Neurol. Sci.* **163**, 119–126.

Azari, N. P., and Pietrini, P. (1995). Preclinical stages in subjects at risk for neurological disorders: Can PET-FDG tell us more? *J. Neurol.* **242**, 112–114.

Azari, N. P., Rapoport, S. I., Salerno, J. A., Grady, C. L., Gonzalez-Aviles, A., Schapiro, M. B., and Horwitz, B. (1992). Interregional correlations of resting cerebral glucose metabolism in old and young women. *Brain Res.* **589**, 279–290.

Azari, N. P., Pettigrew, K. D., Schapiro, M. B., Haxby, J. V., Grady, C. L., Pietrini, P., Salerno, J., Heston, L. L., Rapoport, S. I., and Horwitz, B. (1993). Early detection of Alzheimer's disease: A statistical approach using positron emission tomographic data. *J. Cereb. Blood Flow Metab.* **13**, 438–447.

Backman, L., Andersson, J. L., Nyberg, L., Winblad, B., Nordberg, A., and Almkvist, O. (1999). Brain regions associated with episodic retrieval in normal aging and Alzheimer's disease. *Neurology* **52**, 1861–1870.

Beckerm, J. T., Mintun, M. A., Aleva, K., Wiseman, M. B., Nichols, T., and DeKosky, S. T. (1996). Compensatory reallocation of brain resources supporting verbal episodic memory in Alzheimer's disease. *Neurology* **46**, 692–700.

Braak, H., and Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* **82**, 239–259.

Brooks, R. A., Di Chiro, G., Zukerberg, B. W., Bairamian, D., and Larson, S. M. (1987). Test-retest studies of cerebral glucose metabolism using fluorine-18 deoxyglucose: Validation of method. *J. Nucl. Med.* **28**, 53–59.

Buchsbaum, M. S., Kesslak, J. P., Lynch, G., Chui, H., Wu, J., Sicotte, N., Hazlett, E., Teng, E., and Cotman, C. W. (1991). Temporal and hippocampal metabolic rate during an olfactory memory task assessed by positron emission tomography in patients with dementia of the Alzheimer type and controls. Preliminary studies. *Arch. Gen. Psychiatry* **48**, 840–847.

Buck, B. H., Black, S. E., Behrmann, M., Caldwell, C., and Bronskill, M. J. (1997). Spatial- and object-based attentional deficits in Alzhemier's disease. Relationship to HMPAO-SPECT measures of parietal perfusion. *Brain* **120**, 1229–1244.

Cardebat, D., Demonet, J. F., Puel, M., Agniel, A., Viallard, G., and Celsis, P. (1998). Brain correlates of memory processes in patients with dementia of Alzheimer's type: A SPECT activation Study. *J. Cereb. Blood Flow Metab.* **18**, 457–462.

Chandrasekaran, K., Hatanpää, K., Brady, D. R., and Rapoport, S. I. (1996). Evidence for physiological down-regulation of brain oxidative phosphorylation in Alzheimer's disease. *Exp. Neurol.* **142**, 80–88.

Craig, A. H., Cummings, J. L., Fairbanks, L., Itti, L., Miller, B. L., Li, J., and Mena, I. (1996). Cerebral blood flow correlates of apathy in Alzheimer disease. *Arch. Neurol. (Chicago)* **53**, 1116–1120.

Dani, A., Pietrini, P., Furey, M. L., McIntosh, A. R., Grady, C. L., Horwitz, B., Freo, U., Alexander, G. E., and Schapiro, M. B. (1996). Brain metabolism and cognition in Down syndrome adults associated with development of dementia. *NeuroReport* **7**, 2933–2936.

DeCarli, C. S., Atack, J. R., Ball, M. J., Kay, J. A., Grady, C. L., Fewster, P., Pettigrew, K. D., Rapoport, S. I., and Schapiro, M. B. (1992). Postmortem regional neurofibrillary tangle densities but not senile plaque densities are related to regional cerebral metabolic rates for glucose during life in Alzheimer's disease patients. *Neurodegeneration* **1**, 113–121.

DeKosky, S. T., and Scheff, S. W. (1990). Synapse loss in frontal cortex biopsies in Alzheimer's disease: Correlation with cognitive severity. *Ann. Neurol.* **27**, 457–464.

De Renzi, E. (1986). Slowly progressive visual agnosia or apraxia without dementia. *Cortex* **22**, 171–180.

Duara, R., Grady, C. L., Haxby, J. V., Sundaram, M., Cutler, N. R., Heston, L., Moore, A., Schlageter, N. L., Larson, S., and Rapoport, S. I. (1986). Positron emission tomography in Alzheimer's disease. *Neurology* **36**, 879–887.

Duara, R., Gross-Glenn, K., Barker, W. W., Chang, J. Y., Apicella, A., Loewenstein, D., and Boothe, T. (1987). Behavioral activation and the variability of cerebral glucose metabolic measurements. *J. Cereb. Blood Flow Metab.* **7**, 266–271.

Duara, R., Loewenstein, D. A., and Barker, W. W. (1990). Utilization of behavioral activation paradigms for positron emission tomography studies in normal young and elderly subjects and in dementia. *In* ''Positron Emission Tomography in Dementia'' (R. Duara, ed.), pp. 131–148. Wiley-Liss, New York.

Duara, R., Barker, W. W., Chang, J., Yoshii, F., Loewenstein, D. A., and Pascal, S. (1992). Viability of neocortical function shown in behavioral activation state PET studies in Alzheimer disease. *J. Cereb. Blood Flow Metab.* **12**, 927–934.

Folstein, M. F., Folstein, S. E., and McHugh, P. R. (1975). Mini mental state. A practical method for grading the cognitive state of patients for the clinician. *J. Psychiatry Res.* **12**, 189–198.

Foster, N. L., Chase, T. N., Fedio, P., Patronas, N. J., Brooks, R., and DiChiro, G. (1983). Alzheimer's disease: Focal cortical changes shown by positron emission tomography. *Neurology* **33**, 961–965.

Friedland, R. P. (1989). 'Normal'-pressure hydrocephalus and the saga of the treatable dementias. *JAMA, J. Am. Med. Assoc.* **262**, 2577–2581.

Friedland, R. P., Prusiner, S. B., Jagust, W. J., Budinger, T. F., and Davis, R. L. (1984). Bitemporal hypometabolism in Creutzfeldt-Jakob disease measured by positron emission tomography with [18F]-2-fluorodeoxyglucose, *J. Comput. Assist. Tomogr.* **8**, 978–981.

Furey, M. L., Pietrini, P., Haxby J. V., Alexander, G. E., Lee, H. C., VanMeter, J., Grady, C. L., Shetty, U., Rapoport, S. I., Schapiro, M. B., and Freo, U. (1997). Cholinergic stimulation alters performance and task-specific regional cerebral blood flow during working memory. *Proc. Nat. Acad. Sci. U.S.A.* **94**, 6512–6516.

Furey, M. L., Pietrini, P., Alexander, G. E., Schapiro, M. B., and Horwitz, B. (2000). Cholinergic enhancement improves performance on working memory by modulating the functional activity in distinct brain regions: a positron emission tomography regional cerebral blood flow study in healthy humans. *Brain Res. Bull.* **51**, 213–218.

Furey-Kurkjian, M. L., Pietrini, P., Graff-Radford, N. R., Alexander, G. E., Freo, U., Szczepanik, J., and Schapiro, M. B. (1996). Visual variant of Alzheimer's disease: Distinctive neuropsychological features. *Neuropsychology* **10**, 294–300.

Galton, C. J., Patterson, K., Xuereb, J. H., and Hodges, J. R. (2000). Atypical and typical presentations of Alzheimer's disease: A clinical neuropsychological, neuroimaging and pathological study of 13 cases. *Brain* **123**, 484–498.

Grady, C. L., and Rapoport, S. I. (1992). Cerebral metabolism in aging and dementia. *In* ''Handbook of Mental Health and Aging'' (J. Birren, R. B. Sloane, and G. Cohen, eds.), pp. 201–228. Academic Press, San Diego, CA.

Grady, C. L., Haxby, J. V., Schageter, N. L., Berg, G., and Rapoport, S. I. (1986). Stability of metabolic and neuropsychological asymmetries in dementia of the Alzheimer type. *Neurology* **36**, 1390–1392.

Grady, C. L., Haxby, J. V., Horwitz, B., Sundaram, M., Berg, G., Schapiro, M. B., Friedland, R. P., and Rapoport, S. I. (1988). Longitudinal study of the early neuropsychological and cerebral metabolic changes in dementia of the Alzheimer type. *J. Clin. Exp. Neuropsychol.* **10**, 576–596.

Grady, C. L., Haxby, J. V., Schapiro, M. B., Gonzalez-Aviles, A., Kumar, A., Ball, M. J., Heston, L., and Rapoport, S. I. (1990). Subgroups in dementia of the Alzheimer type using positron emission tomography. *J. Neuropsychiatry Clin. Neurosci.* **2**, 373–384.

Grady, C. L., Haxby, J. V., Horwitz, B., Gillette, J., Salerno, J. A., Gonzalez-Aviles, A., Carson, R. E., Hersocovitch, P., Schapiro, M. B., and Rapoport, S. I. (1993). Activation of cerebral blood flow during a visuoperceptual task in patients with Alzheimer-type dementia. *Neurobiol. Aging* **14**, 35–44.

Haxby, J. V. (1990). Resting state regional cerebral metabolism in dementia of the Alzheimer type. *In* ''Positron Emission Tomography in Dementia'' (R. Duara, ed.), pp. 93–116. Wiley-Liss, New York.

Haxby, J. V., Duara, R., Grady, C. L., Cutler, N. R., and Rapoport, S. I. (1985). Relations between neuropsychological and cerebral metabolic asymmetries in early Alzheimer's disease. *J. Cereb. Blood Flow Metab.* **5**, 193–200.

Haxby, J. V., Grady, C. L., Koss, E., Horwitz, B., Schapiro, M., Friedland, R. P., and Rapoport, S. I. (1988). Heterogenous anterior-posterior metabolic patterns in dementia of the Alzheimer type. *Neurology* **38**, 1853–1863.

Haxby, J. V., Grady, C. L., Koss, E., Horwitz, B., Heston, L., Schapiro, M., Friedland, R. P., and Rapoport, S. I. (1990). Longitudinal study of cerebral metabolic asymmetries and associated neuropsychological patterns in early dementia of the Alzheimer type. *Arch. Neurol. (Chicago)* **47**, 753–760.

Haxby, J. V., Horwitz, B., Ungerleider, L. G., Maisog, J. M., Pietrini, P., and Grady, C. L. (1994). The functional organization of human extrastriate cortex: A PET-rCBF study of selective attention to faces and locations. *J. Neurosci.* **14**, 6336–6353.

Herholz, K., Perani, D., Salmon, E., Franck, G., Fazio, F., Heiss, W. D., and Comar, D. (1993). Comparability of FDG PET studies in probable Alzheimer's disease. *J. Nucl. Med.* **34**, 1460–1466.

Hock, C., Villringer, K., Muller-Spahn, F., Wenzel, R., Heekeren, H., Schuh-Hofer, S., Hofmann, M., Minoshima, S., Schwaiger, M., Dirnagl, U., and Villringer, A. (1997). Decrease in parietal cerebral hemoglobin oxygenation during performance of a verbal fluency task in patients with Alzheimer's disease monitored by means of near-infrared spectroscopy (NIRS)—correlation with simultaneous rCBF-PET measurements. *Brain Res.* **755**, 293–303.

Hof, P. R., Bouras, C., Constantinidis, J., and Morrison, J. H. (1990). Selective disconnection of specific visual association pathways in cases of Alzheimer's disease presenting with Balint's syndrome. *J. Neuropathol. Exp. Neurol.* **49**, 168–184.

Hof, P. R., Archin, N., Osmand, A. P., Dougherty, J. H., Wells, C., Bouras, C., and Morrison, J. H. (1993). Posterior cortical atrophy in Alzheimer's disease: Analysis of a new case and revaluation of a historical report. *Acta Neuropathol.* **86**, 215–223.

Hof, P. R., Giannakopoulos, P., Vickers, J. C., Bouras, C., and Morrison, J. H. (1995). The morphologic and neurochemical basis of dementia: Aging, hierarchical patterns of lesion distribution and vulnerable neuronal phenotype. *Rev. Neurosci.* **6**, 97–124.

Hof, P. R., Vogt, B. A., Bouras, C., and Morrison, J. H. (1997). Atypical form of Alzheimer's disease with prominent posterior cortical atrophy: A review of lesion distribution and circuit disconnection in cortical visual pathways. *Vision Res.* **37**, 3609–3625.

Holcomb, H. H., Links, J., Smith, C., and Wong, D. (1989). Positron emission tomography: Measuring the metabolic and neurochemical characteristics of the living human nervous system. *In* ''Brain Imaging: Applications in Psychiatry'' (N. Andreasen, ed.), pp. 235–370. American Psychiatric Press, Washington, DC.

Holland, A. J., and Oliver, C. (1995). Down's syndrome and the links with Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* **39**, 111–114.

Horwitz, B., Grady, C. L., Schlageter, N. L., Duara, R., and Rapoport, S. I. (1987). Intercorrelations of regional cerebral glucose metabolic rates in Alzheimer's disease. *Brain Res.* **31**, 294–306.

Horwitz, B., McIntosh, A. R., Haxby, J. V., Furey, M., Salerno, J. A., Schapiro, M. B., Rapoport, S. I., and Grady, C. L. (1995). Network analysis of PET-mapped visual pathways in Alzheimer type dementia. *NeuroReport* **6**, 2287–2292.

Ibáñez, V., Pietrini, P., Alexander, G. E., Furey, M. L., Teichberg, D., Rajapakse, J. C., Rapoport, S. I., Schapiro, M. B., and Horwitz, B. (1998). Abnormal metabolic patterns in Alzheimer's disease after correction for partial volume effects. *Neurology* **50**, 1585–1593.

Johannsen, P., Jakobsen, J., Bruhn, P., and Gjedde, A. (1999). Cortical responses to sustained and divided attention in Alzheimer's disease. *NeuroImage* **10**, 269–281.

Jueptner, M., and Weiller, C. (1995). Review: Does measurement of regional cerebral blood flow reflect synaptic activity? Implications for PET and fMRI. *NeuroImage* **2**, 148–156.

Kadekaro, M., Crane, A. M., and Sokoloff, L. (1985). Differential effects of electric stimulation of sciatic nerve on metabolic activity in spinal cord and dorsal root ganglion in the rat. *Proc. Natl. Acad. Sci. U.S.A.* **82**, 6010–6013.

Kadekaro, M., Vance, W. H., Terrell, M. L., Gary, H. Jr., Eisenberg, H. M., and Sokoloff, L. (1987). Effects of antidromic stimulation of the ventral root on glucose utilization in the ventral horn of the spinal cord in the rat. *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5492–5495.

Kawashima, R., O'Sullivan, B. T., and Roland, P. E. (1995). Positron-emission tomography studies of cross-modality inhibition in selective attentional tasks: Closing the ''mind's eye.'' *Proc. Natl. Acad. Sci. U.S.A.* **92**, 5969–5972.

Kelly, P. A., and McCulloch, J. (1993). The effects of the GABAergic agonist muscimol upon the relationship between local cerebral blood flow and glucose utilization. *Brain Res.* **258**, 338–342.

Kennedy, A. M., Frackowiak, R. S. J., Newman, S. K., Bloomfield, P. M., Seaward, J., Roques, P., Lewington, G., Cunningham, V. J., and Rossor, M. N. (1995). Deficits in cerebral glucose metabolism demonstrated by positron emission tomography in individuals at risk of familial Alzheimer's disease. *Neurosci. Lett.* **186**, 17–20.

Kessler, J., Herholz, K., Grond, M., and Heiss, W.-D. (1991). Impaired metabolic activation in Alzheimer's disease: A PET study during continuous visual recognition. *Neuropsychologia* **29**, 229–243.

Kessler, J., Ghaemi, M., Mielke, R., Herholz, K., and Heiss, W. D. (1996). Visual versus auditory memory stimulation in patients with probable Alzhemier's disease. A PET study with 18 FDG. *Ann. N. Y. Acad. Sci.* **777**, 233–238.

Kuhl, D. E., Metter, E. J., Riege, W. H., and Markham, C. H. (1984). Patterns of cerebral glucose utilization in Parkinson's disease and Huntington's disease. *Ann. Neurol.* **15** (Suppl.), S119–S125.

Kumar, A., Schapiro, M. B., Grady, C. L., Haxby, J. V., Wagner, E., Salerno, J. A., Friedland, R. P., and Rapoport, S. I. (1991). High-resolution PET studies in Alzheimer's disease. *Neuropsychopharmacology* **4**, 35–46.

Lewis, D. A., Campbel, M. J., Terry, R. D., and Morrison, J. H. (1987). Laminar and regional distributions of neurofibrillary tangles and neuritic plaques in Alzheimer's disease: A quantitative study of visual and auditory cortices. *J. Neurosci.* **7**, 1799–1808.

Linn, R. T., Wolf, P. A., Bachman, D. L., Knoedel, J. E., Cobb, J. L., Bélanger, A. J., Kaplan, E. F., and D'Agostino, R. B. (1995). The 'preclinical phase' of probable Alzheimer's disease. *Arch. Neurol. (Chicago)* **52**, 485–490.

Martin, A., Brouwers, P., Lalonde, F., Cox, C., Teleska, P., Fedio, P., Foster, N. L., and Chase, T. N. (1986). Towards a behavioral typology of Alzheimer's patients. *J. Clin. Exp. Neuropsychol.* **8**, 594–610.

Mata, M., Fink, D. G., Gainer, H., Smith, C. B., Davidsen, L., Savaki, H., Schwartz, W. J., and Sokoloff, L. (1980). Activity-dependent energy metabolism in rat posterior pituitary reflects sodium pump activity. *J. Neurochem.* **34**, 213–215.

Mayeux, R., Stern, Y., and Spanton, S. (1985). Heterogeneity in dementia of the Alzheimer type: Evidence of subgroups. *Neurology* **35**, 453–461.

Mazziotta, J. C., and Phelps, M. E. (1986). Positron emission tomography studies of the brain. *In* ''Positron Emission Tomography and Autoradiography: Principles and Applications for the Brain and Heart'' (M. E. Phelps, J. C. Mazziotta, and H. R. Schelbert, eds.), pp. 493–580. Raven Press, New York.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., and Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA work group under the auspices of Department of Health and Human Services task force on Alzheimer's disease. *Neurology* **34**, 939–944.

Mega, M. S., Chu, T., Mazziotta, J. C., Trivedi, K. H., Thompson, P. M., Shah, A., Cole, G., Frautschy, S. A., and Toga, A. W. (1999). Mapping biochemistry to metabolism: FDG-PET and amyloid burden in Alzheimer's disease. *NeuroReport* **10**, 2911–2917.

Mendez, M. F., Mendez, M. A., Martin, R., Smyth, K. A., and Whitehouse, P. J. (1990). Complex visual disturbances in Alzheimer's disease. *Neurology* **40**, 439–443.

Mentis, M. J., Weinstein, E. A., Horwitz, B., McIntosh, A. R., Pietrini, P., Alexander, G. E., Furey, M. L., and Murphy, D. G. M. (1995). Abnormal brain glucose metabolism in the delusional misidentification syndromes: A positron emission tomography study in Alzheimer disease. *Biol. Psychiatry* **38**, 438–449.

Mentis, M. J., Horwitz, B., Grady, C. L., Alexander, G. E., VanMeter, J. W., Maisog, J. M., Pietrini, P., Schapiro, M. B., and Rapoport, S. I. (1996). Visual cortical dysfunction in Alzheimer's disease evaluated with a temporally graded ''stress test'' during PET. *Am. J. Psychiatry* **153**, 32–40.

Mentis, M. J., Alexander, G. E., Krasuski, J., Pietrini, P., Furey, M. L., Schapiro, M. B., and Rapoport, S. I. (1998). Increasing required neural response to expose abnormal brain function in mild versus moderate or severe Alzheimer's disease: PET study using parametric visual stimulation. *Am. J. Psychiatry* **155**, 785–794.

Mesulam, M. M. (1987). Primary progressive aphasia—differentiation from Alzheimer's disease. *Ann. Neurol.* **22**, 533–534.

Mielke, R., Kessler, J., Szelies, B., Herholz, K., Wienhard, K., and Heiss, W. D. (1998). Normal and pathological aging—findings of positron-emission-tomography. *J. Neural Transm.* **105**, 821–837.

Miller, J. D., de Leon, M. J., Ferris, S. H., Kluger, A. George, A. E., Reisberg, B., Sachs, H. J., and Wolf, A. P. (1987). Abnormal temporal lobe response in Alzheimer's disease during cognitive processing as measured by ^{11}C-2-deoxy-D-glucose and PET. *J. Cereb. Blood Flow Metab.* **7**, 248–251.

Morrison, J. H., Hof, P. R., Campbell, M. J., DeLima, A. D., Voigt, T., Bouras, C., Cox, K., and Young, W. G. (1990). Cellular pathology in Alzheimer's disease: Implications for corticortical disconnection and differential vulnerability. *In* ''Imaging, Cerebral Topography and Alzheimer's Disease'' (S. I. Rapoport, H. Petit, D. Leys, and Y. Christen, eds.), pp. 19–40. Springer-Verlag, Berlin.

Mubrin, Z., Knezevic, S., Spilich, G., Risberg, J., Gubarev, N., Wannenmacher, W., and Vucinic, G. (1989). Normalization of rCBF pattern in senile dementia of the Alzheimer's type. *Psychiatry Res.* **29**, 303–306.

Nordberg, A. (1996). Functional studies of new drugs for the treatment of Alzheimer's disease. *Acta Neurol. Scand.* **165** (Suppl.), 137–144.

Pietrini, P., Azari, N. P., Grady, C. L., Salerno, J. A., Gonzales-Aviles, A., Heston, L. L., Pettigrew, K. D., Horwitz, B., Haxby, J. V., and Schapiro, M. B. (1993a). Pattern of cerebral metabolic interactions in a subject at risk for Alzheimer's disease. *Dementia* **4**, 94–101.

Pietrini, P., Freo, U., Grady, C. L., Haxby, J. V., Salerno, J. A., Gonzales-Aviles, and Schapiro, M. B. (1993b). A longitudinal PET study of cerebral glucose metabolism in patients with Alzheimer's disease and prominent visuospatial impairment. *Adv. Biosci.* **87**, 69–70.

Pietrini, P., Furey, M. L., Graff-Radford, N., Freo, U., Alexander, G. E., Grady, C. L., Dani, A., Mentis, M. J., and Schapiro, M. B. (1996). Preferential metabolic involvement of visual cortical areas in a subtype of Alzheimer's disease: Clinical implications. *Am. J. Psychiatry* **153**, 1261–1268.

Pietrini, P., Dani, A., Furey, M. L., Alexander, G. E., Freo, U., Grady, C. L., Mentis, M. J., Mangot, D., Simon, E., Horwitz, B., Haxby, J. V., and Schapiro, M. B. (1997a). Low glucose metabolism during brain stimulation in older Down's syndrome subjects at risk for Alzheimer's disease prior to dementia. *Am. J. Psychiatry* **154**, 1063–1069.

Pietrini, P., Hof, P., Graff-Radford, N. R., Furey, M. L., Alexander, G. E., Mentis, M. J., Nichelli, P., Dani, A., Brown, C. A., and Schapiro, M. B. (1997b). Altered regional distribution of brain metabolic and pathological lesions in the visual variant of Alzheimer disease. *Soc. Neurosci. Abst.* **23**, 2170.

Pietrini, P., Dani, A., Raphaelson, M., Furey, M. L., Alexander, G. E., Levine, B., Guazzelli, M., Rapoport, S. I. and Schapiro, M. B. (1998). Cerebral glucose metabolic and neuropsychological dysfunction in patients with untreated Sleep Apnea Syndrome. *Sleep* **21**(Suppl.), 82.

Pietrini, P., Furey, M. L., and Guazzelli, M. (1999a). In vivo biochemistry of the brain in understanding human cognition and emotions: Toward a molecular psychology. *Brain Res. Bull.* **50**, 417–418.

Pietrini, P., Furey, M. L., Alexander, G. E., Mentis, M. J., Dani, A., Guazzelli, M., Rapoport, S. I., and Schapiro, M. B. (1999b). Association between brain functional failure and dementia severity in Alzheimer's disease: Resting versus stimulation PET study. *Am. J. Psychiatry* **156**, 470–473.

Pietrini, P., Alexander, G. E., Furey, M. L., Dani, A., Mentis, M. J., Horwitz, B., Guazzelli, M., Schapiro, M. B., and Rapoport, S. I. (2000a). Cerebral metabolic response to passive audiovisual stimulation in Alzheimer's disease patients and healthy controls assessed by positron emission tomography. *J. Nucl. Med.* **41**, 575–583.

Pietrini, P., Alexander, G. E., Furey, M. L., Hampel, H., and Guazzelli, M. (2000b). The neurometabolic landscape of cognitive decline: In vivo studies with positron emission tomography in Alzheimer's disease. *Int. J. Psychophysiol.* **37**, 87–98.

Pietrini, P., Guazzelli, M., Basso, G., Jaffe, K., and Grafman J. (2000c). The neurometabolic bases of aggressive behavior assessed by positron emission tomography in humans. *Am. J. Psychiatry* **157**, (in press).

Raichle, M. E. (1998). Imaging the mind. *Semin. Nucl. Med.* **28**, 278–289.

Rapoport, S. I. (1990). Topography of Alzheimer's disease: Involvement of association neocortices and connected regions: pathological, metabolic and cognitive correlations; relation to evolution. *In* ''Imaging, Cerebral Topography, and Alzheimer's Disease'' (S. I. Rapoport, H. Petit, D. Leys, and Y. Christen, eds.), pp. 1–17. Springer-Verlag, Berlin.

Rapoport, S. I. (1991). Positron emission tomography in Alzheimer's disease in relation to disease pathogenesis: A critical review. *Cerebrovasc. Brain Metab. Rev.* **3**, 297–335.

Rapoport, S. I. (1999). Functional brain imaging in the resting state and during activation in Alzheimer's disease. Implications for disease mechanisms involving oxidative phosphorylation. *Ann. N. Y. Acad. Sci.* **893**, 341–344.

Rapoport, S. I., and Grady, C. L. (1993). Parametric in vivo brain imaging during activation to examine pathological mechanisms of functional failure in Alzheimer disease. *Int. J. Neurosci.* **70**, 39–56.

Reiman, E. M., Caselli, R. J., Yun, L. S., Chen, K., Bandy, D., Minoshima, S., Thibodeau, S. N., and Osborne, D. (1996). Preclinical evidence of Alzheimer's disease in persons homozygous for the ϵ4 allele for apolipoprotein E. *N. Engl. J. Med.* **334**, 752–758.

Riddle, W., O'Carroll, R. E., Dougall, N., Van Beck, M., Murray, C., Curran, S. M., Ebmeier, K. P., and Goodwin, G. M. (1993). A single photon emission computerised tomography study of regional brain function underlying verbal memory in patients with Alzheimer-type dementia. *Br. J. Psychiatry* **163**, 166–172.

Roland, P. E. (1993), "Brain Activation." Wiley-Liss, New York.

Roland, P. E., and Friberg, L. (1988). The effect of the GABA-A agonist THIP on regional cortical blood flow in humans. A new test of hemispheric dominance. *J. Cereb. Blood Flow Metab.* **8**, 314–323.

Roy, C. S., and Sherrington, C. S. (1890). On the regulation of blood supply of the brain. *J. Physiol. (London)* **11**, 85–108.

Saykin, A. J., Flashman, L. A., Frutiger, S. A., Johnson, S. C., Mamourian, A. C., Moritz, C. H., O'Jile, J. R., Riordan, H. J., Santulli, R. B., Smith, C. A., and Weaver, J. B. (1999). Neuroanatomic substrates of semantic memory impairment in Alzheimer's disease: Patterns of functional MRI activation. *J. Int. Neuropsychol. Soc.* **5**, 377–392.

Schapiro, M. B., Grady, C. L., and Haxby, J. V. (1992). Nature of mental retardation and dementia in Down's syndrome: Study with PET, CT, and neuropsychology. *Neurobiol. Aging* **13**, 723–734.

Schapiro, M. B., Pietrini, P., Grady, C. L., Ball, M. J., DeCarli, C., Kumar, A., Kaye, J. A., and Haxby, J. V. (1993). Reductions in parietal and temporal cerebral metabolic rates for glucose are not specific for Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* **56**, 859–864.

Schwartz, W. J., Smith, C. B., Davidsen, L., Savaki, H., Sokoloff, L., Mata, M., Fink, D. J., and Gainer, H. (1979). Metabolic mapping of functional activity in the hypotalamo-neurohypophysial system of the rat. *Science* **205**, 723–725.

Seitz R. J., and Roland, P. E. (1992). Vibratory stimulation increases and decreases the regional cerebral blood flow and oxidative metabolism: A positron emission tomography (PET) study. *Acta Neurol. Scand.* **86**, 60–67.

Small, G. W., and Leiter, F. (1998). Neuroimaging for diagnosis of dementia. *J. Clin. Psychiatry* **59**, 4–7.

Small, G. W., Mazziotta, J. C., Collins, M. T., Baxter, L. R., Phelps, M. E., Mandelkern, M. A., Kaplan, A., La Rue, A., Adamson, C. F., Chang, L., Buze, B. H., Corder, E. H., Saunders, A. M., Haines, J. L., Pericak-Vance, M. A., and Roses, A. D. (1995). Apolipoprotein E type 4 allele and cerebral glucose metabolism in relatives at risk for familial Alzheimer disease. *JAMA, J. Am. Med. Assoc.* **273**, 942–947.

Small, G. W., Ercoli, L. M., Silvermann, D. H. S., Huang, S.-C., Komo, S., Bookheimer, S. Y., Cummings, J. L. K., Lavretsky, H., Miller, K., Mazziotta, J. C., Saxena, S., Wu, H. M., Mega, M. S., Saunders, A. M., Pericak-Vance, M. A., Roses, A. D., Barrio, J. R., and Phelps, M. E. (2000). Cerebral metabolic and cognitive decline in persons at genetic risk for Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 6037–6042.

Smith, C. D., Andersen, A. H., Kryscio, R. J., Schmitt, F. A., Kindy, M. S., Blonder, L. X., and Avison, M. J. (1999). Altered brain activation in cognitively intact individuals at high risk for Alzheimer's disease. *Neurology* **53**, 1391–1396.

Sokoloff, L. (1981). Relationship among local functional activity, energy metabolism and blood flow in the central nervous system. *Fed. Proc.* **40**, 2311–2316.

Sokoloff, L. (1982). The radioactive deoxyglucose method, theory, procedure and application for the measurement of local cerebral glucose utilization in the central nervous system. *Adv. Neurochem.* **4**, 1–82.

Sokoloff, L., Reivich, M., Ken, C., Des Rosiers, M. H., Patlak, C. S., Pettigrew, K. D., Sakurada, O., and Shinohara, M. (1977). The [^{14}C]-deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure and normal values in the conscious and anesthetized albino rat. *J. Neurochem.* **28**, 897–916.

Strassburger, T., Alexander, G. E., Brady, D. R., Mangot, D., Pietrini, P., Mentis, M., Schapiro, M. B., and Grady, C. L. (1996). Heterogeneity of regional cerebral glucose metabolic (rCMRglc) patterns in neuropathologically confirmed Alzheimer disease (AD). *Neurology* **46**, A134.

Sultzer, D. L. (1996). Behavioral syndromes in dementia: Neuroimaging insights. *Semin. Clin. Neuropsychiatry* **1**, 261–271.

Tailarach, J., and Tounoux, P. (1988). Co-planar Steretaxic Atlas of the Human Brain. Thieme, New York.

Terry, R. D., and Katzman, R. (1983). Senile dementia of the Alzheimer type. *Ann. Neurol.* **14**, 497–506.

Terry, R. D., Masliash, E., Salmon, D. P., Butters, N., De Teresa, R., Hill, R., Hansen, L. A., and Katzman, R. (1991). Physical basis of cognitive alterations in Alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. *Ann. Neurol.* **30**, 572–580.

Van Hoesen, G. W., Hyman, B. T., and Damasio, A. R. (1991). Entorhinal cortex pathology in Alzheimer's disease. *Hippocampus* **1**, 1–8.

Waldemar, G., Bruhn, P., Kristensen, M., Johnsen, A., Paulson, O. B., and Lassen, N. A. (1994). Heterogeneity of neocortical cerebral blood flow deficits in dementia of the Alzheimer type: A [99mTc]-d,1-HMPAO-SPECT study. *J. Neurol. Neurosurg. Psychiatry* **57**, 285–295.

Whitehouse, P. J., Price, D. L., Clark, A. W., Coyle, J. T., and DeLong, M. R. (1981). Alzheimer disease: Evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann. Neurol.* **10**, 122–126.

Whittam, R. (1962). The dependence of the respiration of brain cortex on active cation transport. *Biochem. J.* **82**, 205–212.

Woodard, J. L., Grafton, S. T., Votaw, J. R., Green, R. C., Dobraski, M. E., and Hoffman, J. M. (1998). Compensatory recruitment of neural resources during overt rehearsal of word lists in Alzheimer's disease. *Neuropsychology* **12**, 491–504.

Elliott J. Mufson and Jeffrey H. Kordower

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Cholinergic Basal Forebrain Systems in the Primate Central Nervous System: Anatomy, Connectivity, Neurochemistry, Aging, Dementia, and Experimental Therapeutics

The cholinergic basal forebrain is composed of a collection of magnocellular hyperchromic neurons located within the septal/diagonal band complex and nucleus basalis of Meynert. These neurons provide the major cholinergic innervation to the hippocampus and neocortical mantle. Empirical investigations since the 1980s have established a role for the cholinergic basal forebrain in higher order cognitive processes, although its exact role in attentional versus memory processes remains an area of debate. The fact that the cholinergic basal forebrain degenerates in dementing illnesses such as Alzheimer's disease (AD) underscores the importance for understanding the structural and functional aspects of this region. Further, the cholinergic basal forebrain system is intimately linked with trophic factor systems as these neurons contain the appropriate receptors for, and are exquisitely sensitive to, neurotrophins such as nerve growth factor (NGF) and brain-derived neurotrophic factor. This chapter focuses on the neuroanatomical and neurochemical organization of the primate cholinergic basal forebrain, with special reference where appropriate to the organizational properties of the human brain and its relation to aging and dementia. The importance of understanding human cholinergic basal forebrain function is featured as this region displays a unique species-specific organization, especially as it pertains to its relationships with peptidergic systems and the expression of the estrogen receptor within the primate cholinergic basal forebrain as compared to nonprimates. Whether these species differences underlie the vulnerability of cholinergic basal forebrain neurons in diseases of higher order cognitive function remains a mystery. Finally, experimental therapeutic strategies aimed at augmenting the survival of cholinergic basal forebrain neurons, including NGF administration or fetal cholinergic grafting, are discussed.

I. Introduction

A heterogeneous collection of telencephalic structures located on the medial and ventral regions of the cerebral hemispheres collectively make up the basal forebrain in the primate brain. These structures lack a true cortical organization, despite their location on the surface of the brain. They can best be described as having a ''corticoid'' architecture. This area is complex and contains structures such as the septal area and the vertical and the horizontal limbs of the diagonal band of Broca, as well as the region termed the substantia innominata (Beccari, 1911), which is the most anatomically complicated. Historically, this later region has been termed the nucleus of the ansa lenticularis (Meynert, 1872) and later renamed the nucleus basalis (Kölliker, 1896). Although this nomenclature is the commonly most used, other terms have been applied to this region (see Mesulam *et al*., 1983a). Part of the difficulty in understanding this region is the complexity of its chemoanatomical projections. These regions have a diversity of cells containing different neurotransmitters, morphology, and projection patterns (see de Lacalle and Saper, 1997, for review). Among the great diversity of neuronal populations within the basal forebrain (e.g., calbindin, GABAergic, NADPH-d/nitrous oxide, and galaninergic cells), the cholinergic corticopetal projection neurons have received particular attention due to their reduction in Alzheimer's disease (AD) and their intimate relationship with the neurotrophic substance nerve growth factor (NGF) and its high (trkA) and low ($p75^{NTR}$) affinity receptors.

A large body of evidence has accumulated over the years indicating that NGF is involved in the survival and maintenance of the cholinergic neurons of the basal forebrain by an interaction with its receptors (see Mufson *et al*., 1997a; Mufson and Kordower, 1999, for reviews). In fact, virtually all choline acetyltransferase (ChAT)-immunopositive basal forebrain neurons composing the cholinergic subgroups termed Ch1-4 by Mesulam and colleagues (1983b) contain the protein and gene for trkA and $p75^{NTR}$ (Kordower *et al*., 1988; Mufson *et al*., 1989a,b; Sobreviela *et al*., 1994; Gibbs and Pfaff, 1992; Kordower *et al*., 1994b; Mufson *et al*., 1996, 1997a,b) and transport NGF retrogradely (Mufson *et al*., 1994, 1995). Both NGF receptors are excellent markers for cholinergic neurons of the basal forebrain. These NGF receptors have not been revealed within the other brain stem cholinergic cell groups (Schwab *et al*., 1979; Mufson *et al*., 1989a,b; Mesulam and Geula, 1988). The use of genetically engineered cells that secrete NGF has been proposed as a treatment approach for slowing of the progression of cholinergic basal forebrain neuron degeneration in AD (Tuszynski *et al*., 1990a,b, 1991, 1996, 1999; Kordower *et al*., 1994b). This chapter focuses primarily on the ascending cholinergic projection neurons of the basal forebrain and, to a lesser degree, the mesopontine magnocellular cholinergic cell groups. In contrast, we will only mention briefly the intrinsic cholinergic interneurons located within the striatum, the various cholinergic motor neurons, the cerebellum, or brain stem reticular formation. Furthermore, we will not concentrate on the cortical cholinergic interneurons that have been reported only in the rodent (Wainer *et al*., 1993). This review surveys the central cholinergic projection neurons in the primate brain and their relation to development, aging, disease, and therapeutics. For a more complete historical discussion of the definition of the basal forebrain cholinergic system, the reader is refered to the review by de Lacalle and Saper (1997).

II. Embryogenesis of Magnocellular Basal Forebrain

Although many studies have examined the cytoarchitecture, chemoarchitecture, and connections of the cholinergic basal forebrain, there have been relatively few studies examining the development of this system. Initial studies were performed using tritiated thymidine either alone or combined with choline acetyltransferase immunohistochemistry in rats (Bayer, 1985; Semba and Fibiger, 1988). These studies revealed that cholinergic basal forebrain neurons in rats are generated along a caudal to rostral gradient between gestational days 12 and 17. In mice, ChAT immunohistochemistry alone has been used to identify cholinergic basal forebrain neurons. Using this technique, Schambra and co-workers (1989) found immunoreactive cholinergic basal forebrain neurons on days, 14 and 15 of mouse gestation. It needs to be realized, however, that immunohistochemistry does not reveal the genesis of cells just the time at which the cholinergic phenotype is expressed. Thus, it remains possible that these cells were born at an earlier time point but did not express cholinergic markers until embryonic day 14 of gestation.

III. Embryogenesis of the Cholinergic Basal Forebrain in Monkey

In nonhuman primates, the development of the rhesus monkey magnocellular basal forebrain complex was studied using the archival collection of tritiated thymidine-labeled sections from the collection of Dr. P. Rakic (Kordower and Rakic, 1990). When radiolabeled magnocellular neurons are charted, an early burst of neurogenesis is seen at embryonic day 30 of the rhesus monkey 165-day gestation period followed by a short quiescent period (Figs. 19.1 and 19.2). Then, in a manner similar to that seen in rats, the development of the basal forebrain is completed across a caudal to rostral gradient between embryonic days 36 and 45, with peak neurogenesis seen between embryonic days 40 and 42. In this regard, the posterior subdivision develops completely between embryonic days 33 and 36. The intermediate subdivision displays peak neurogenesis between embryonic days 36 and 40, and development of this region is completed by embryonic day 43. The anterior division of the magnocellular basal forebrain, including the septal diagonal band complex, develops last, between embryonic days 35 and 45, with peak neurogenesis seen between embryonic days 40 and 43. In contrast to the caudal to rostral gradient of development, a neurogenic gradient is not observed in the radial direction. The development of the magnocellular basal forebrain between embryonic days 30 and 45 makes this cluster of neurons among the earliest developing cells within the telencephalon. Like other cortically projecting cells located in the thalamus and those giving rise to corticocortical connections, this early development puts these cells in a strategic position to potently influence the development of their target neurons located within the cortical mantle that are generated later in gestation.

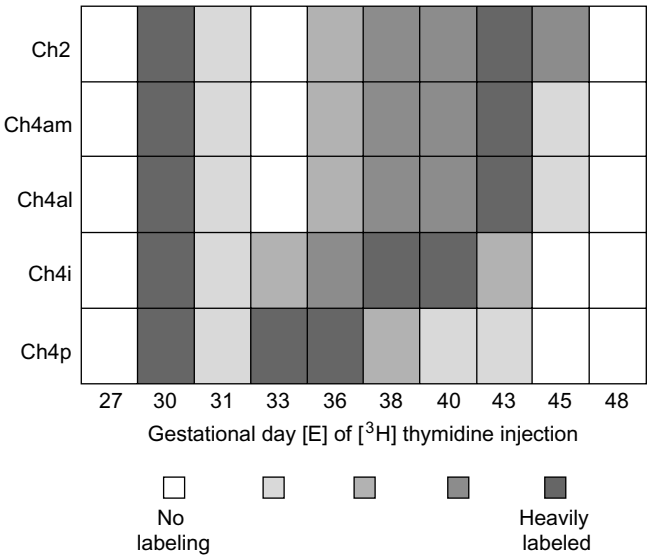

FIG. 19.1. Summary schematic illustrating the developmental tempo for each subregion of the basal forebrain. Black represents abundant neurogenesis whereas white represents little or no neurogenesis. From Kordower and Rakic (1990), with permission.

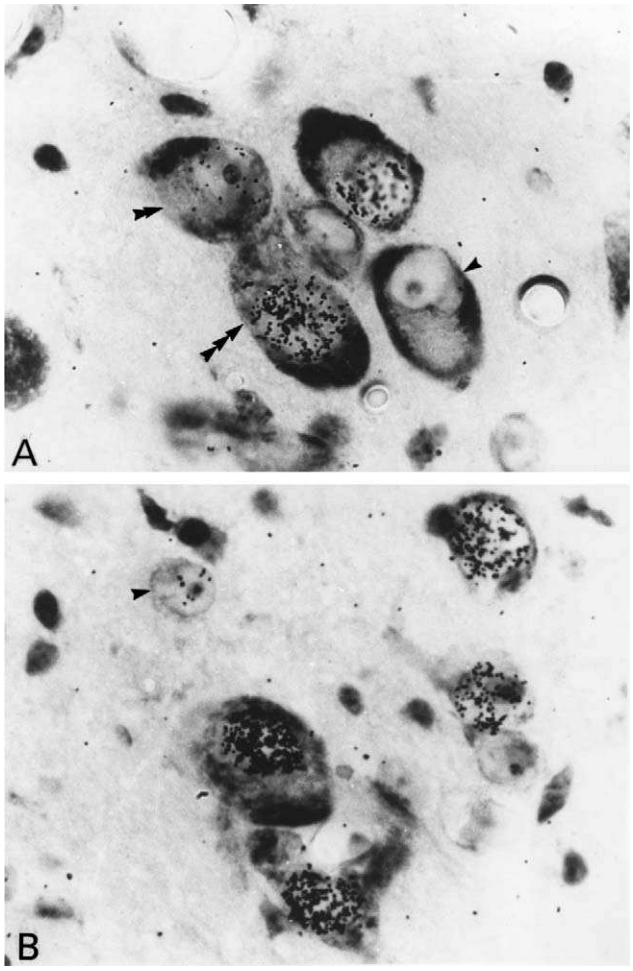

FIG. 19.2. (A) High-power photomicrographs demonstrating typical [^{3}H]thymidine-labeling profiles in the nucleus basalis. In this region, unlabeled (one arrowhead), lightly labeled (double arrowhead), and heavily labeled (triple arrowhead) magnocellular basal forebrain neurons can be observed. (B) Labeled neurons within the Ch4 region. The arrow points to a lightly labeled parvicellular neuron, which, due to its small size, would be excluded from the data analysis (A, B: 400X). From Kordower and Rakic (1990), with permission.

IV. Embryogenesis of the Cholinergic Basal Forebrain in Humans

In humans, the genesis of the cholinergic basal forebrain can only be established using morphological criteria for the presence of magnocellular neurons and the presence of phenotypic markers for this cell population. Thus the birthdating of these cells employing these techniques must be regarded as indirect. Using Nissl and acetylcholinesterase-stained material (Kracun and Rösner, 1986; Kostovic, 1986), the genesis of human cholinergic basal forebrain neurons has been established to be between weeks 9 and 15 of gestation. This early development in the first trimester is similar to what has been seen in nonhuman primates (Kordower and Rakic, 1986) and differs from what is seen in rodents in which this region develops relatively late in gestation (Bayer, 1985; Semba and Fibiger, 1988). This time window of human basal forebrain development is partially confirmed by our group, which examined the development of the human brain using immunohistochemical probes directed against either the low-affinity p75^{NTR} receptor or the high-affinity tropomycin-related kinase (trk) receptor, two receptors known to extensively colocalize with cholinergic basal forebrain neurons in primates (Hefti *et al*., 1986; Kordower *et al*., 1988; 1989b,c; Mufson *et al*., 1989a; see Mufson *et al*., 1997b, for review). Using these tools, we found that the cholinergic basal forebrain expresses both markers in their normal cytoarchitectonic position within the septal/diagonal band complex (Ch1–2; Fig. 19.3) and throughout the nucleus basalis (Ch4; Fig.19.4) by embryonic week 14, the earliest specimen available for analysis. At embryonic week 14, trk-immunoreactive neurons are scattered lightly throughout the Ch1–Ch4 subfields. In addition to the localization of these cells within the classic cholinergic basal forebrain subfields, trk-immunoreactive interstitial neurons are distributed in the external and internal medullary laminae of the globus pallidus, as well as within the internal capsule. The intensity of trk staining, as well as the number of stained neurons, increases appreciably between embryonic weeks 20 and 22. The general pattern of staining for low-affinity p75^{NTR}-immunoreactive neurons is generally the same as that observed for trk immunoreactivity. However, the intensity of staining and the number of trk-immunoreactive perikarya are greater relative to p75^{NTR}-immunoreactive neurons. The discordance between these two basal forebrain markers appears greatest early in gestation, whereas similar staining patterns are seen later in development.

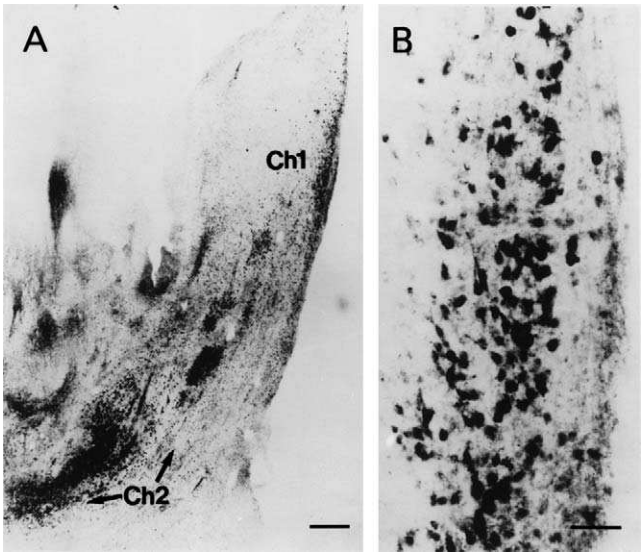

FIG. 19.3. (A) Low-power photomicrograph illustrating the distribution of trk-immunoreactive neurons in the Ch1/Ch2 (septal/diagonal band) complex of a 20-week human fetus. (B) High-power photomicrograph through Ch1 illustrating the morphology of trk-immunoreactive medial septum neurons. Scale bars: 400 (A) and 50 (B) μm. From Kordower *et al*. (1996), with permission.

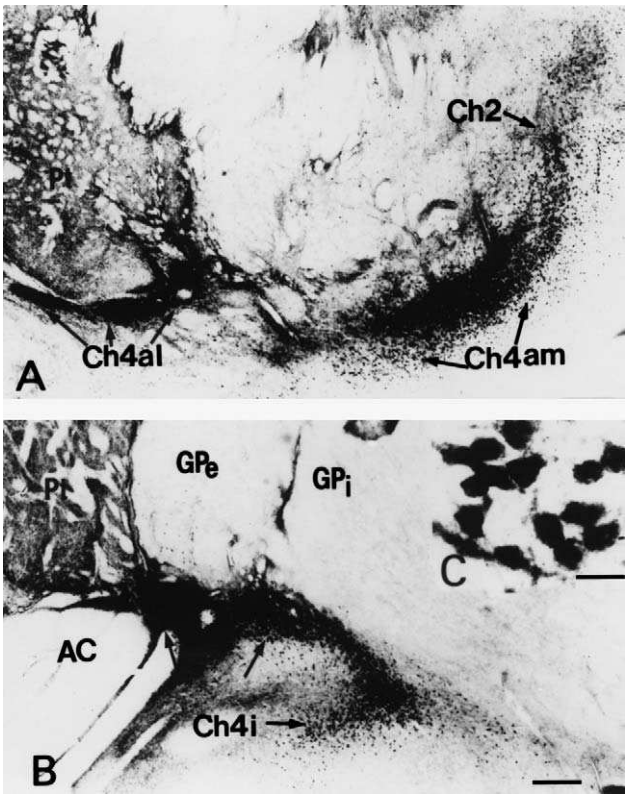

FIG. 19.4. (A and B) Low-power photomicrographs illustrating the distribution of trk-immunoreactive neurons within the subfields of basal forebrain. (A) Anterior (Ch4am-al) nucleus basalis and diagonal band (Ch2). (B) Intermediate (Ch4i) subdivision of nucleus basalis. (C) High-power photomicrograph illustrating the morphology of trk-immunoreactive neurons within the Ch4i subdivision of nucleus basalis. AC, anterior commissure; Pt, putamen; GPe, external segment of globus pallidus; GPi, internal segment of globus pallidus. Scale bars: 400 (A and B) and 20 (C) μm. From Kordower *et al.* (1996), $p75^{NTR}$.

V. Anatomy of Adult Cholinergic Basal Forebrain Subgroups

Initial studies used both the nonspecific cholinergic marker acetylcholinesterase (AChE) and the synthesizing enzyme for acetycholine, choline acetyltransferase, to delimit the location of the primate central cholinergic neurons (Mesulam and Van Hoesen, 1976; Mesulam *et al*., 1983a; Mufson *et al*., 1989a; Mesulam and Geula, 1988). Although various nomenclatures have been applied to the area of the primate basal forebrain [see earlier discussion; we will mainly employ the Ch nomenclature proposed by Mesulam and co-workers (1983a, 1984, 1986; Mufson *et al*., 1989a)] to designate the ascending cholinergic subgroups in the monkey and human brain. These histochemical investigations revealed a continuum of cholinergic neurons within the basal forebrain extending from the olfactory tubercle to the level of the lateral geniculate body (Fig. 19.5). The length of the basal forebrain is approximately 15–19 mm (Zaborszky *et al*., 1999). The cholinergic basal forebrain can be divided into four subdivisions: The cholinergic neurons of the medial septum (Ch1) lie along the midline of the septum and also along the outer edge of the nucleus (Figs. 19.6 and 19.7). The cells are relatively small (25–30×25–30 μm) and generally ovoid and many are embedded within the fibers of the precommissural fornix (Mesulam *et al*., 1983a). Approximately 10% of the Ch1 neurons are cholinergic and most contain both AChE and ChAT (Mesulam *et al*., 1983b; 1986; Mufson *et al*., 1989a). There are very few cholinergic neurons in the monkey and human Ch1 (Figs. 19.6 and 19.7; see Mesulam *et al*., 1983b, 1984; Mufson *et al*., 1989a; de Lacalle and Saper, 1997). The dorsal boundary of the vertical limb of the diagonal band (Ch2) is defined less clearly in the primate (Mesulam *et al*., 1983a, 1986) as compared to the rodent where Ch2 can be divided from Ch1 by a line drawn between the two limbs of anterior commissure (Mesulam *et al*., 1983a; Rye *et al*., 1984). This region appears as a boomerang-shaped structure that lies along the ventral lateral border of Ch1 in rat (Mesulam *et al*., 1983a) and, to a lesser degree, in the primate (Mesulam *et al*., 1986). These Ch2 neurons are somewhat larger ($20–25 \times 30$–40 μm) in the primate and are embedded within the vertical limb of the diagonal band of Broca. These neurons are mostly fusiform in shape, with their long axis parallel to the diagonal band fibers (Mesulam *et al*., 1986). Sections dual stained for ChAT and thionine indicate that approximately 70% of the perikarya in this subfield are cholinergic. At the medial edge of the olfactory tubercle the Ch2 adjoins the nucleus of the horizontal limb of the diagonal band (Ch3), which lies dorsal to the olfactory tubercle. The Ch3 cell group has the most diffuse boundaries in the primate brain (Fig. 19.8; Mesulam *et al*., 1983a, 1986; Mufson *et al*., 1989a). It extends between the septal-preoptic region medially and the amygdaloid region laterally. Very few (approximately 1–2%) of these fusiform-shaped, medium-sized ($15–20 \times 40$–50 μm) neurons contain cholinergic markers (Mesulam *et al*., 1983a, 1986; Mufson *et al*., 1989a,b). The largest group of cholinergic neurons of the basal forebrain is found within nucleus basalis (Ch4; Figs. 19.6, 19.9, 19.10, and 19.11). These neurons constitute the Ch4 sector. The Ch4 subgroup extends from the rostral aspect of the anterior commissure to the lateral geniculate nucleus and comes into close contact with many different structures. Throughout most of its rostral–caudal extent, this region is always in juxtaposition to the globus pallidus (Mesulam *et al*., 1983a, 1986; Mesulam and Geula, 1988; Mufson *et al*., 1989a, 1997a,b). These ChAT-positive neurons are relatively large ($40–50 \times 60$–70 μm) and range in shape from fusiform to complex multipolar cells. Approximately 90% of the perikarya in the nucleus basalis are ChAT positive. Topographically, ChAT-positive perikarya constituting Ch4 can be separated into anterior (Ch4a), intermediate (Ch4i), and posterior (Ch4p) subfields. Moreover, the anterior portion can be divided into medial (Ch4am) and lateral (Ch4al) sectors, whereas the intermediate subfield is divided by the ansa peduncularis into dorsal (Ch4id) and ventral (Ch4iv) regions (Figs. 19.9 and 19.11). The posterior subfield (Ch4p) is located caudal to the ansa peduncularis and extends up to the level of the lateral geniculate nucleus. The total number of cholinergic neurons located within the nucleus basalis using modern sterological counting techniques is approximately 210,000 in a single hemisphere (Gilmore *et al*., 1999). At each level, the Ch4 group has not

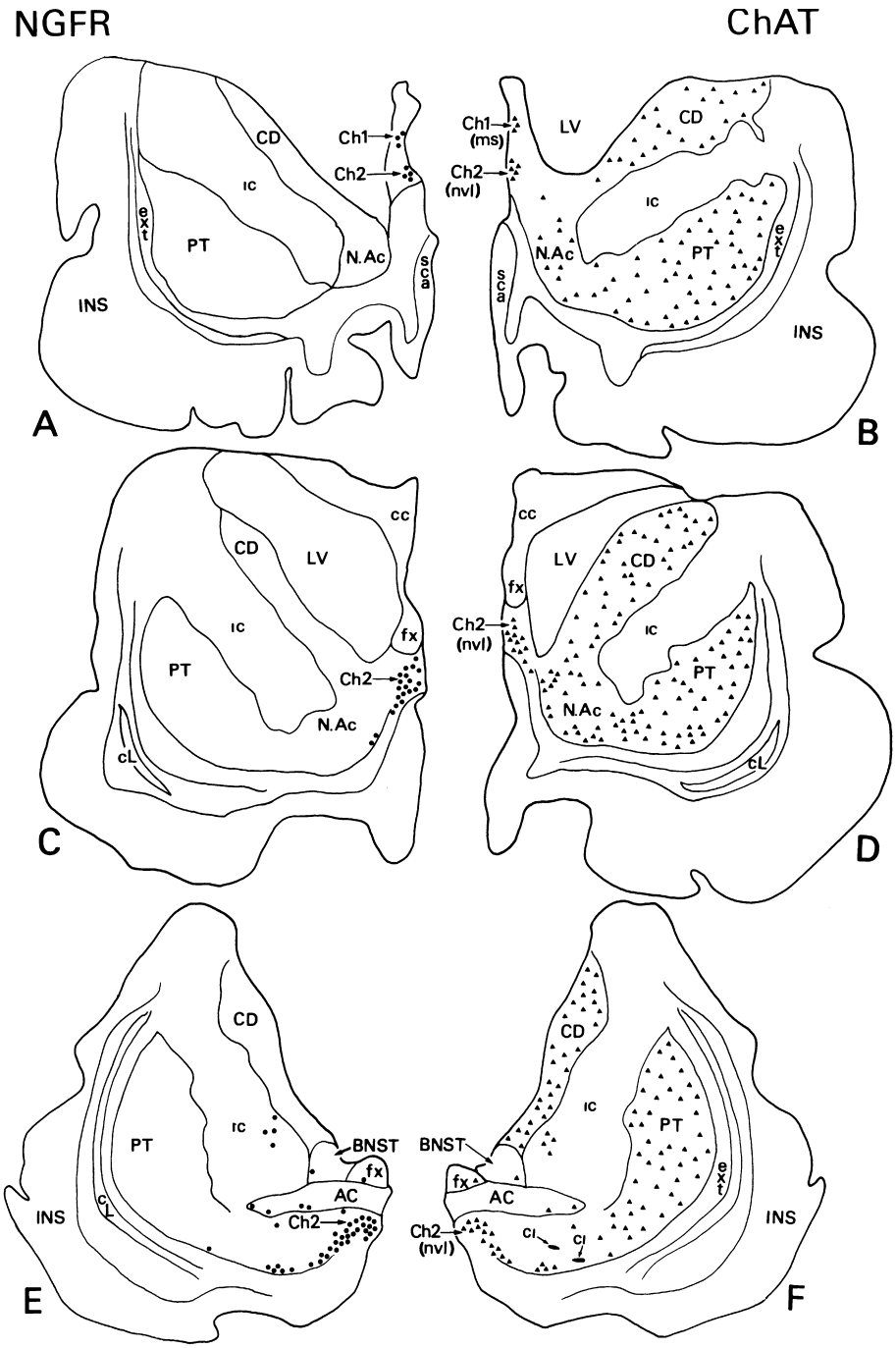

FIG. 19.5. Distribution of $p75^{NTR}$-immunoreactive (black dots) and ChAT-containing (triangles) neurons within the normal, aged human basal forebrain. Note the concordance of the distribution of these markers within the basal forebrain and the lack of such concordance within the striatum. Each section is separated by 720 mm. The distribution of $p75^{NTR}$ -containing neurons was mapped from the brain of a 77-year-old normal male patient. ChAT-containing neurons were mapped from a 78-year-old normal female patient. Each section was matched for level. Curved arrow indicates interstitial elements of the basal forebrain. The blacked-in areas in F indicate islands of Calleja. From Mufson *et al.* (1989b), with permission.

only a compact but also an interstitial component consisting of perikarya, which penetrate nearby fiber bundles, including the anterior commissure, internal capsule, internal and external medullary lamainae of the globus pallidus, stria terminalis, inferior thalamic peduncle, and the ansa lenticularis (Mesulam *et al.*, 1983a, 1986; Saper and Chelimsky, 1984; Satoh and Fibiger, 1985; Kordower *et al.*, 1988; Mufson *et al.*, 1989a,b). These are considered interstitial components of the Ch4 group rather than independent interstitial nuclei of the individual fiber bundles (Fig. 19.12).

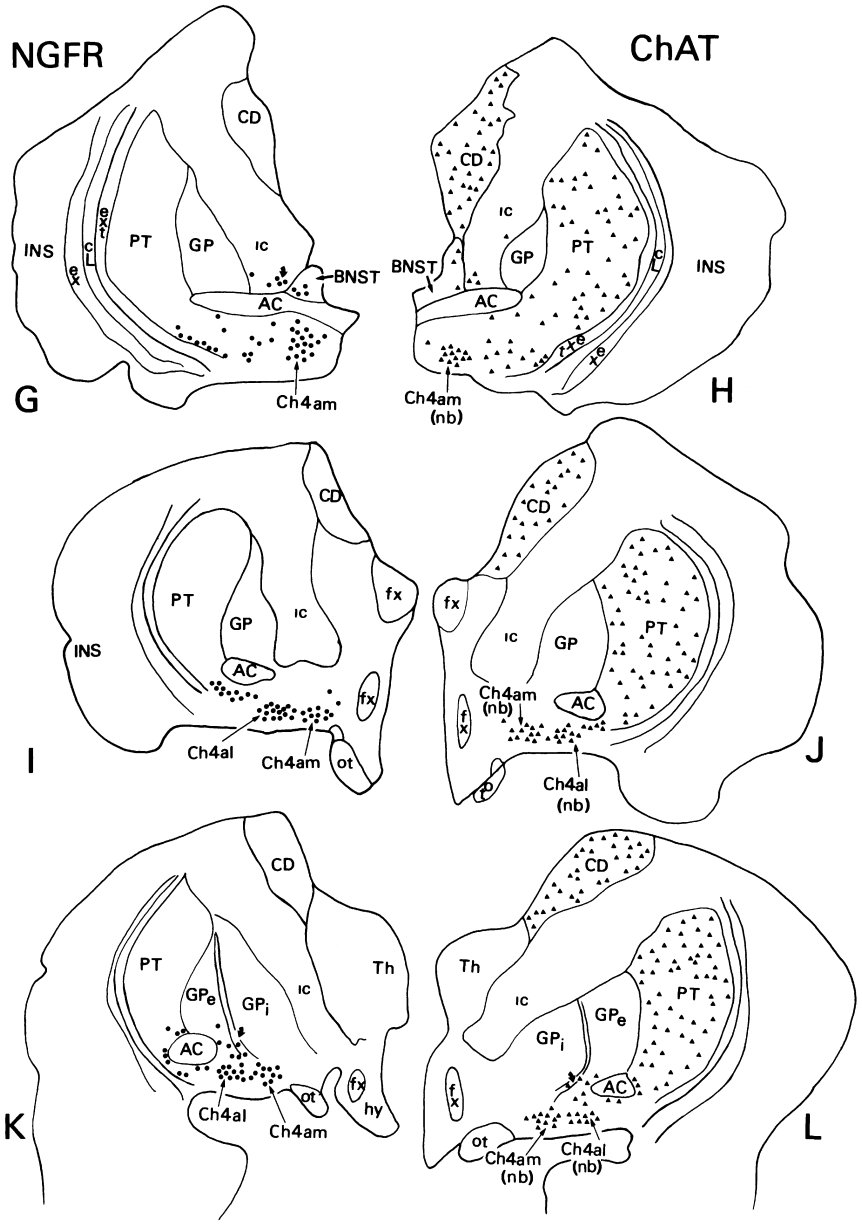

FIG. 19.5. (*Continued*)

VI. Anatomy of Thalamic and Brain Stem Cholinergic Subgroups

Within the upper brain stem, a continuous band of ChAT-containing neurons extends from the rostral midbrain to the midpontine levels (Fig. 19.13; Mesulam *et al*., 1983a, 1986; Satoh and Fibiger, 1985; Mufson *et al*., 1989a). These perikarya can be subdivided into two major fields: Ch5 and Ch6. The cholinergic neurons of Ch5 remain mostly within the pedunculopontine nucleus (PPN) of Olszewski and Baxter (1982) and are analogous to the Ch5 group identified in rodents (Mesulam *et al*., 1983a). This anterior sector of this region appears just lateral to the substantia nigra. More caudally, this subgroup displays its maximum extent within the region of the pedunculopontine nucleus, they are relatively large (75–80 × 40–45 μm), and range in shape from multipolar to fusiform. At the level of the PPN this sector exhibits a compact lateral component, which abuts on the lateral lemniscus, and a more diffuse medial portion, which is interdigitated with the superior cerebellar peduncle, the medial longitudinal fasciculus, and the central tegmental tract (Fig. 19.13). Some Ch5 neurons extend into the region of the cuneiform nucleus and more caudally into the parabrachial region. Moreover, the main portion of the parabrachial area is devoid of ChAT (see de Lacalle and Saper, 1997, for details). As the Ch5 sector gradually disappears at the level of the midpons, the more caudal Ch6 subgroup occupies a lateral position in the periventricular gray, at a location that corresponds to the doral tegmental nucleus (see de Lacalle and Saper, 1997). These cholinergic neurons are relatively smaller than Ch6 (40–45 × 50–55 μm).

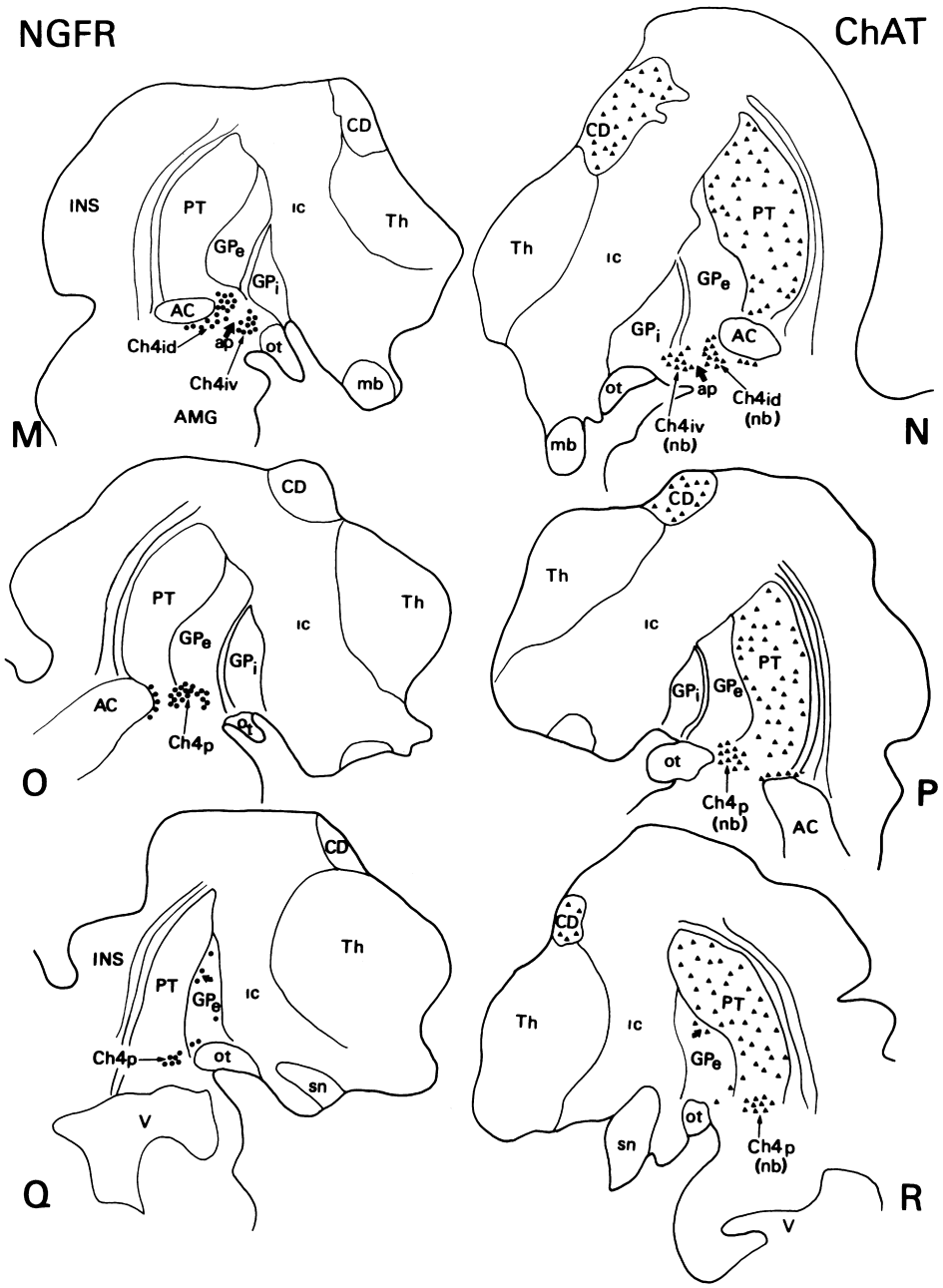

FIG. 19.5. (*Continued*)

Cholinergic markers are confined to 90% of the larger neurons in this region (Mesulam *et al*., 1986). The medial habenula, which is a component of the epithalamus, contains densely packed cholinergic neurons termed the Ch7 subgroup. These cholinergic neurons are oval shaped and $30 \times 35\,\mu m$ in size (Fig. 19.14; Wainer *et al*., 1993). A compact cell group lies along the lateral margin of the mesopontine region just ventral to the brachium of the inferior colliculus termed the parabigeminal nucleus, called Ch8. ChAT immunohistochemical preparations revealed that approximately 80–90% of the perikarya of this region are cholinergic (Mufson *et al*., 1986). A summary schematic diagram of the central cholinergic projections neurons (Ch1–8) is shown in Fig. 19.14.

VII. Other Cholinergic Regions

This section briefly reviews the regions containing cholinergic neurons that are outside the Ch1–8 ascending projection systems. These include the striatal complex interneurons, cranial nerve nuclei, reticular neurons, lateral hypothalamus, and scattered small intercortical cells seen in rodents (see Wainer *et al*., 1993). The latter has been reported in primates (Mesulam *et al*., 1986; Mufson *et al*., 1989a,b). The striatal complex may be subdivided into four major sectors: the caudate, the putamen, the nucleus basalis, and the olfactory tubercle. Each of these regions contains ChAT-immunoreactive neurons. ChAT-positive neurons are larger ($35–40 \times 50\,\mu m$) and less

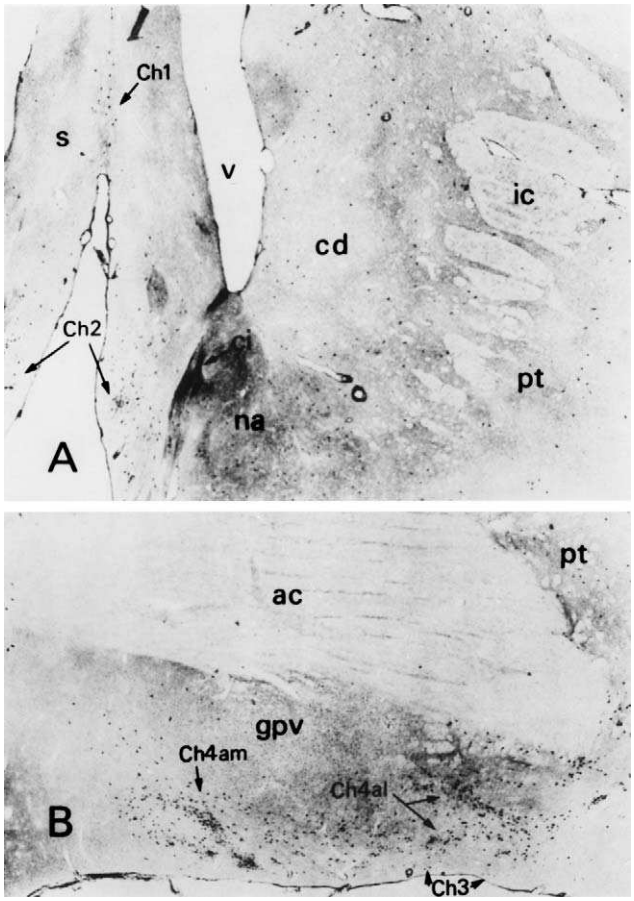

FIG. 19.6. Choline acetyltransferase immunohistochemistry in the brain of a female *Macaca nemestrina* showing Ch1, Ch4am, and Ch4al (arrows) cell groups. The sections are from progressively more caudal levels of the brain. Medial is to the left and dorsal toward the top. Curved arrowheads point to interstitial elements of Ch4. A nonspecific punctate deposit is occasionally seen in the globus pallidus. Magnification 24X. From Mesulam *et al*. (1986), with permission.

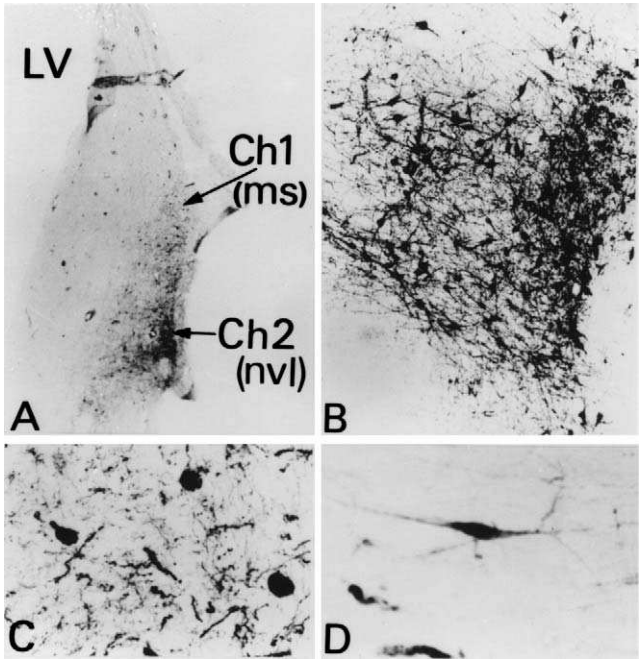

FIG. 19.7. $P75^{NTR}$ receptor immunoreactivity in human basal forebrain of a 77-year-old normal male patient. (A) NGFR-containing neurons within the medial septum (Ch1) merging with the vertical limb nucleus (Ch2). 12X. (B–D) NGFR-containing neurons within Ch2, Ch1, and Ch3, respectively. B, 80X. C,D, 800X. From Mufson *et al*. (1989b), with permission.

densely packed than those of the olfactory tubercle (Figs. 19.5, 19.9, and 19.13). In addition, the islands of Calleja also contain numerous cholinergic neurons, although the granule cells do not contain ChAT (Mesulam *et al*., 1986). The striatum and olfactory tubercle display the high (trkA) but not the low ($p75^{NTR}$) affinity NGF receptor staining in the monkey and human (Kordower *et al*., 1994c; Mufson *et al*., 1997a). Interestingly, the interpeduncular nucleus contains an extensive array of ChAT-positive fibers but lacks cholinergic neurons (Mufson *et al*., 1987) (Fig. 19.15).

VIII. Neurotrophin Receptor Expression and Cholinergic Basal Forebrain Neurons

A. Historical Overview

The classic research of Levi-Montalcini (for review, see Levi-Montalcini and Angeletti, 1954; Thoenen and Barde, 1980) first demonstrated that NGF was an important trophic substance in the development and maintenance of noradrenergic peripheral sympathetic neurons. Investigations of mammalian brain have demonstrated the responsiveness of cholinergic neurons to NGF. For example, NGF increases ChAT levels in cultured septal and striatal cholinergic perikarya (Hefti *et al*., 1986; Martinez-Serrano *et al*., 1995). Intraventricular injections of NGF in neonatal rats increase ChAT activity in the basal forebrain, hippocampus, cortex, and striatum (Gnahn *et al*., 1983). In addition, evidence has been accumulating

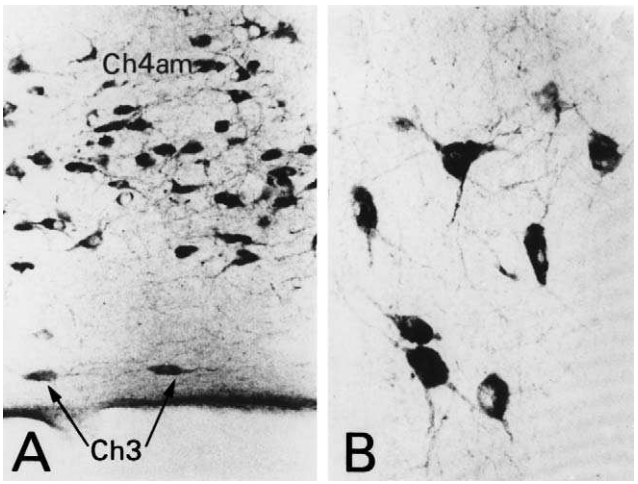

FIG. 19.8. (A) Choline acetyltransferase immunohistochemical stain of the anteromedial division of Ch4 and Ch3. Magnification 150X. (B) Detail of neurons from the anteromedial division of Ch4. Magnification 300X.

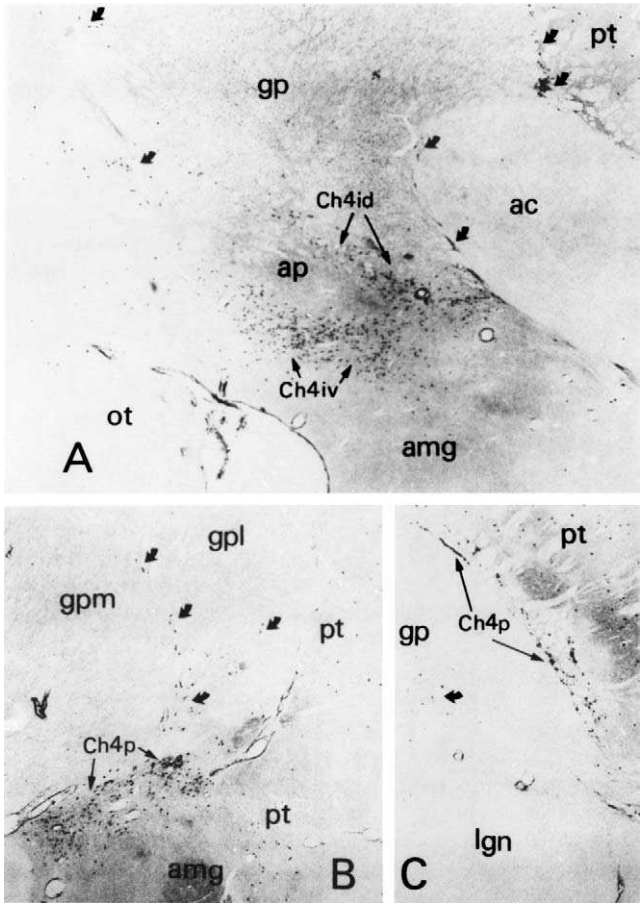

FIG. 19.9. Choline acetyltransferase immunohistochemistry in the brain of a female *Macaca nemestrina* showing Ch4id and Ch4iv cell groups. Medial is to the left and dorsal toward the top. Curved arrowheads point to interstitial elements of Ch4. A nonspecific punctate deposit is occasionally seen in the globus pallidus. Magnification 24X. From Mesulam *et al.* (1986), with permission.

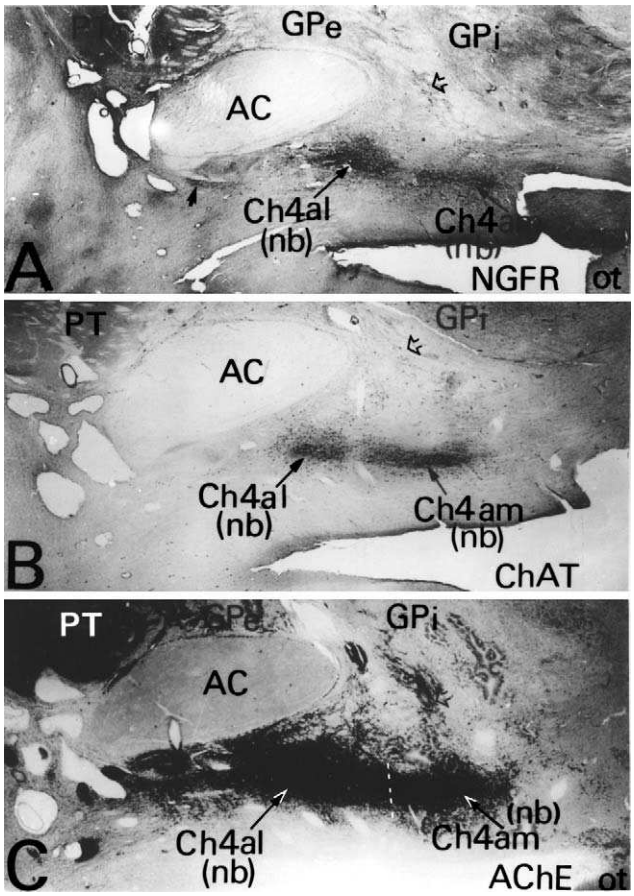

FIG. 19.10. Adjacent sections processed histochemically for $p75^{NTR}$ (A), ChAT (B), and AChE (C) at the level of the emergence of the anterior commissure showing the concordance of all three markers within the anteromedial and anterolateral subsectors of the human nucleus basalis (Ch4) from a 77-year-old normal female patient. The dotted line in C indicates the approximate demarcation between Ch4am and Ch4al, which is obscured by the intense AChE neuropil staining. Magnification 10X. From Mufson *et al.* (1989b), with permission.

that NGF may modulate regenerative events within the primate cholinergic basal forebrain. For example, administration of NGF or transplantation of NGF rescues medial septal neurons that undergo degeneration after transection of the fimbria-fornix system in primates (Tuszynski *et al.*, 1990a,b; Kordower *et al.*, 1994b). Early studies using ^{125}I-labeled NGF infused into rat hippocampus resulted in retrograde transport of this injectate to cholinergic perikarya located within the medial septum and vertical limb nucleus of the diagonal band continuum, but not to catecholaminergic neurons of the locus coeruleus or substantia nigra (Schwaab *et al.*, 1979). Cortical injections of iodinated NGF produce exclusive retrograde labeling of neurons located within the nucleus basalis (Seiler and Schwaab, 1984). These studies further supported the view that NGF may serve as a trophic factor for cholinergic basal forebrain neurons. Converging lines of evidence have supported the suggestion that NGF modulates the development, survival, and maintenance of cholinergic basal forebrain neurons in mammals, including primates (Kordower *et al.*, 1994b). There are high levels of NGF protein (Hayashi *et al.*, 1990) and mRNA (Hayashi *et al.*, 1993) in the developing primate brain. In the adult brain, the highest levels of NGF are found in the projection zones of the basal forebrain, including the cerebral cortex, hippocampus, and olfactory bulb (Conner *et al.*, 1992; Conner and Varon, 1992). Radiolabeled NGF-binding sites are found in the human basal forebrain (Strada *et al.*, 1992), and autoradiographic investigations have reported that perikarya that bind ^{125}I-labeled NGF codistribute with cholinergic basal forebrain neurons (Richardson *et al.*, 1986). In fact, NGF immunoreactivity was observed within the cholinergic basal forebrain neurons of monkeys and humans, suggesting that it is transported from the cortex retrogradely (Mufson *et al.*, 1994, 1995).

B. NGF Receptors within Cholinergic Subgroups

1. Overview of NGF Receptors

Localization of receptors that specifically bind, internalize, and transport NGF retrogradely to the cholinergic basal fore-

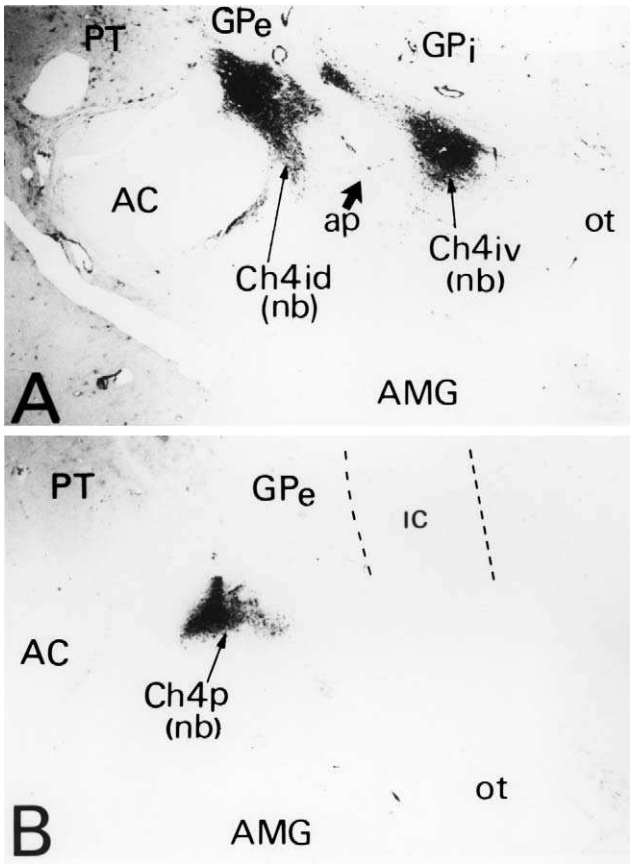

FIG. 19.11. p75^{NTR} immunoreactivity in the brain of a 77-year-old normal male patient showing the intermediate and posterior subfields of the nucleus basalis. (A) NGFR immunoreactivity within the intermediodorsal (Ch4id) and intermedioventral (Ch4iv) subsectors of the nucleus basalis. (B) Posterior subfield (Ch4p) of the nucleus basalis. Dashed lines indicate position of the internal capsule. Magnification 10X. From Mufson *et al*. (1989b), with permission.

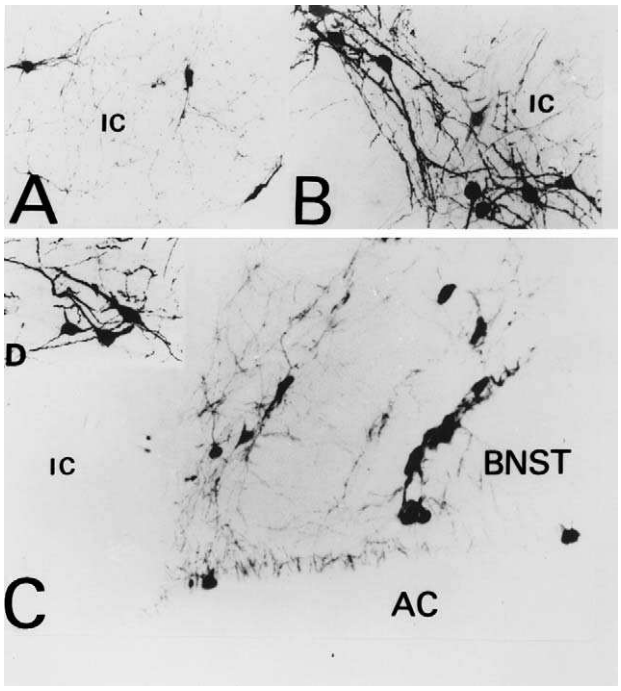

FIG. 19.12. Photomicrographs of cholinergic/p75^{NTR}-immunoreactive interstitial neurons within the human brain. (A) Internal capsule (lC), (B) higher magnification of internal capsule interstitial neurons, (C) bed nucleus of the stria terminalis (BNST) neurons located above the anterior commissure (AC), and (D) higher magnification of BNST neurons.

brain consumer neurons to induce signal transduction have been under investigation for many years. Since the mid-1990s, evidence has accumulated demonstrating that NGF recognizes at least two classes of cell surface receptors: (1) a fast-dissociating, low-affinity NGF receptor and (2) a slow-dissociating, high-affinity NGF receptor (Figure 19.1). The low-affinity receptor is a transmembrane glycoprotein of molecular weight 75 kDa (Bothwell, 1991). Because this receptor binds to virtually all members of the NGF family (Bothwell, 1991), it has been termed the p75 neurotrophin receptor (p75^{NTR}). In the classic retrograde model of NGF/p75^{NTR} interactions, it was hypothesized that this complex is internalized and transported retrogradely to cholinergic basal forebrain neurons where signal transduction occurs (Schwabb *et al*., 1977). Although studies using molecular biologic methods indicate that the physiological actions of NGF requires complexing to its high-affinity receptor (trkA), several important actions for p75^{NTR} have been suggested, such as presentation or recruitment of NGF to the trkA receptor (Jing *et al*., 1993), signaling through a G-protein-mediated mechanism, and a relationship with molecules capable of signaling or providing substrates for the high-affinity receptor, trkA (Parada *et al*., 1992; see Mufson and Kordower, 1997, for review). The trkA receptor is a 140-kDa transmembrane glycoprotein with a cytoplasmic protein kinase domain (see Bothwell, 1991, for review). The trkA receptor is essential for NGF signal transduction and mediates its biological effects (Chao, 1992). Although it was at first controversial whether coexpression of both the low-affinity p75^{NTR} and the high-affinity trkA receptor was necessary for the biological activity of NGF (see Mufson and Kordower, 1997, for review), it is now clear that the trkA receptor alone is sufficient to mediate cellular responses to NGF (Riccio *et al*., 1997) within the cholinergic basal forebrain neurons. TrkA, like most kinase growth factor receptors, signals through receptor oligomerization (Heldin, 1995) and can active gene expression (Riccio *et al*., 1997).

2. Colocalization of trkA and p75^{NTR} within the Primate Cholinergic Basal Forebrain

Immunocytochemical and *in situ* hybridization experiments have determined that trkA and p75^{NTR} are contained within the cholinergic basal forebrain neurons in the monkey and the human (Figs. 19.10 and 19.16; Hefti, 1983; Hefti *et al*., 1986; Kordower *et al*., 1988, 1989b,c; Schatteman *et al*., 1988; Hefti and Mash, 1989; Mufson *et al*., 1989a, 1996, 1997b; Strada *et al*., 1992; Gibbs and Pfaff, 1994; Boissière *et al*., 1997). In the adult and aged monkey using an antibody that reacts primarily with the trkA receptor (Steininger *et al*., 1993; Kordower *et al*., 1994a), an extensive, but incomplete colocalization between trk- and p75^{NTR}-immunoreactive neurons was found within the basal forebrain (Kordower *et al*.,

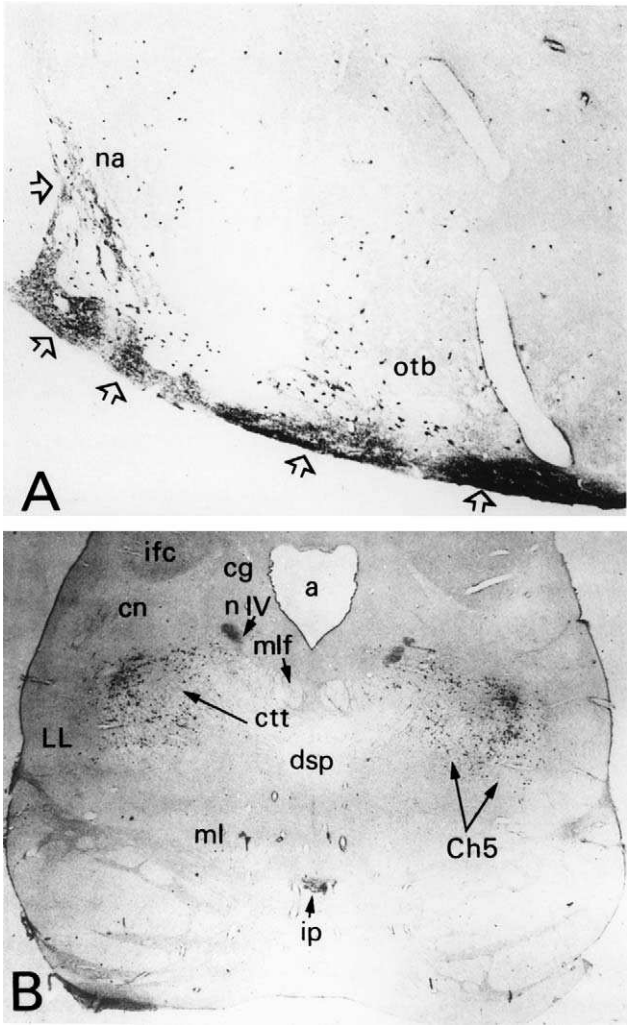

FIG. 19.13. (A) Choline acetyltransferase immunohistochemical stain of the ventral striatum. Open arrowheads point to the islands of Calleja where the neuropil give a positive ChAT reaction. ChAT-positive perikarya are concentrated more densely in the nucleus accumbens and the olfactory tubercle, especially in the spatial relationship to the islands of Calleja. Magnification 33X. (B) Choline acetyltransferase immunohistochemical stain of the upper pons showing the distribution of Ch5 neurons. Magnification 10X. From Mufson *et al.* (1986), with permission.

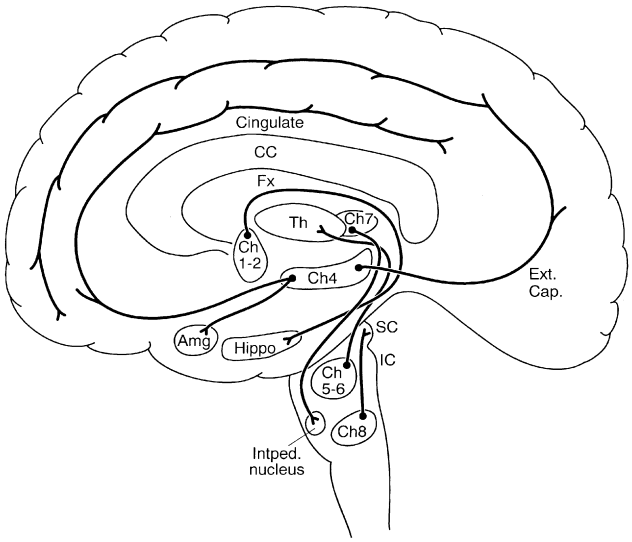

FIG. 19.14. Schematic diagram showing the trajectory of the central cholinergic projections system Ch1–8 (see text for details).

1994a). In addition to dual-labeled perikarya, many basal forebrain neurons only expressed trk immunoreactivity. Conversely, a few basal forebrain neurons expressed only $p75^{NTR}$. Colocalization experiments revealed that between 68 and 73% of all basal forebrain neurons (septal/diagonal band complex and nucleus basalis) colocalized trk and $p75^{NTR}$. In contrast, between 23 and 28% of basal forebrain neurons expressed only the trk receptor whereas approximately 4% of the basal forebrain neurons expressed only the $p75^{NTR}$. Although there was some individual variability, a similar degree of trk/$p75^{NTR}$ colocalization was observed in all subdivisions of the basal forebrain. Separate quantitation of double-labeled neurons was performed for hippocampal projecting septal/diagonal band (Ch1/Ch2) neurons and the cortical and amygdaloid projecting neurons of the nucleus basalis (Ch4) subfields. Between 42 and 75% of the neurons within the septal diagonal band complex colocalized trk and $p75^{NTR}$, whereas 66–76% of neurons within the nucleus basalis subfield also displayed both trk and $p75^{NTR}$ immunoreactivity. This nonhuman

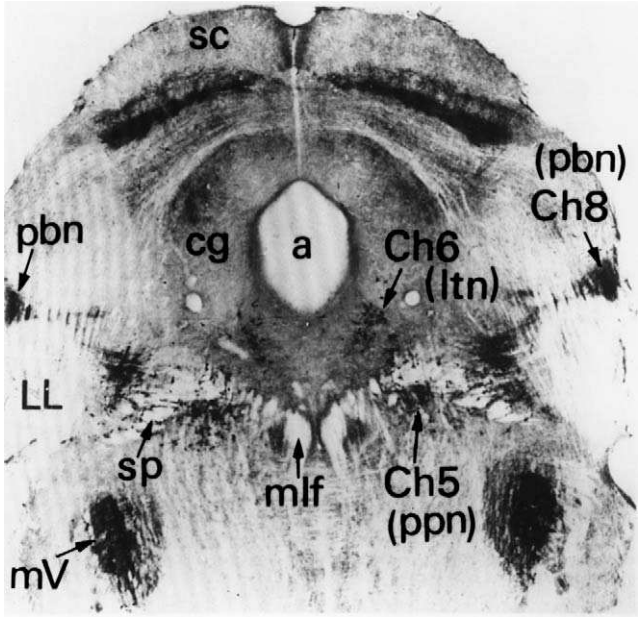

FIG. 19.15. Section stained for the immunohistochemical demonstration of ChAT-like immunoreactivity. Heavy ChAT staining is seen in the region of the parabigeminal nucleus (Ch8), as well as within the pedunculopontine nucleus (Ch5), lateral dorsal tegmental nucleus (Ch6), in the motor nucleus of the fifth cranial nerve, and in the intermediate layers of the superior colliculus. a, cerebral aqueduct; cg, central gray matter; Ch5, cholinergic cell group 5; Ch6, cholinergic cell group 6; Ch8, cholinergic cell group 8; LL, lateral lemniscus; ltn, lateral dorsal tegmental nucleus; mlf, medial longitudinal fasciculus; mV, motor nucleus of the fifth cranial nerve; pbn, parabigeminal nucleus; ppn, pedunculopontine nucleus; sc, superior colliculus; sp, superior cerebellar peduncle. Magnification 27X. From Mufson *et al.*, (1986), with pernission.

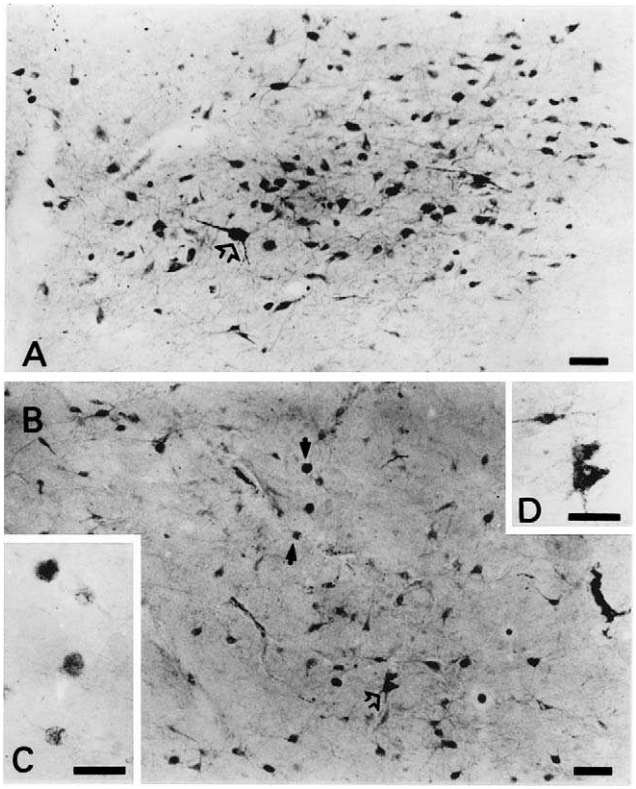

FIG. 19.16. Comparison of trkA immunostaining within the anteromedial portion of the nucleus basalis in an aged control (A) and an AD (B) case. Note in A that virtually all neurons are trkA positive. Open arrow points to the same trkA-positive perikarya shown in Fig. 19.1A. In AD (B), many neurons are shrunken (black arrows) and express decreased trkA immunoreactivity (C). Open arrow in B points to the cluster of trkA neurons shown in D, demonstrating that some neurons appear healthy and continue to express moderate immunoreactivity for trkA in AD. Bars: 100 (A and B) and 50 (C and D) μm. From Mufson *et al*. (1997a), with permission.

primate study also revealed that there was no age-related changes in the degree of colocalization. In humans, various reports using postmortem samples reported that all $p75^{NTR}$ containing cells with the basal forebrain were cholinergic based on the fact that they stained positive for the indirect cholinergic marker AChE (Mesulam *et al*., 1983a). An extensive topographic colocalization study of ChAT and $p75^{NTR}$ revealed that virtually all (>95%) of $p75^{NTR}$-containing cholinergic basal forebrain neurons contain ChAT and AChE within the Ch4 (nucleus basalis) where only about 10% colocalized these markers in Ch1 and 70% in Ch2 (Mesulam *et al*., 1983a; Mufson *et al*., 1989a). Ch3 is virtually nonexistent in the human and therefore was not included in this study. Mesulam *et al*. (1988) supported this finding by showing a topographic overlap between the distribution of ChAT and $p75^{NTR}$ positive neurons in the nucleus basalis. Neither of these neurotrophin markers has been shown to colocalize with ChAT containing neurons in the other brain stem cholinergic subfields. Monoclonal antibodies raised against extra- and intracellular domains of the trkA receptor have been shown to stain the magnocellular neurons of the Ch4 in humans (Fig. 19.16; Mufson *et al*., 1997a; Sendera *et al*., 2000). Studies using mRNA probes for trkA have also revealed a strong codistribution between genes for trkA and $p75^{NTR}$ in the human Ch4 (Mufson *et al*., 1995, 1999; Boissière *et al*., 1997).

IX. m2 Muscarinic Acetylcholine Receptor Neurons within the Primate Cholinergic Basal Forebrain

Several muscarinic receptor subtypes have been identified by differential affinities for antagonists, and it is now established that five distinct genes (m1–m5) encode highly related muscarinic receptor subtypes (Bonner *et al*., 1987; Hulme *et al*., 1990). It is often assumed that m2 is the gene product that functions as a presynaptic autoreceptor to inhibit ACh release (Mash *et al*., 1985, Mash and Potter, 1986; Pohorecki *et al*., 1988; Levey *et al*., 1991) and, like ''M2'' radioligand-binding sites, m2-immunoreactive protein is reduced in AD brain (Flynn *et al*., 1995). However, only a subset of cholinergic neurons coexpress the m2 protein within the rodent basal forebrain (Levey *et al*., 1995), suggesting that these neurons are not the only source of the m2 receptor protein seen in the hippocampus, cortex, and other terminal zones. In support of this concept, lesions of the rodent septohippocampal and monkey nucleus basalis cortical projection systems result in little, if any, decrease in m2 receptor protein in the hippocampus and cortex, respectively (Wall *et al*., 1994; Levey *et al*., 1995; Mufson *et al*., 1997b; Mrzljak *et al*., 1998). In humans, using an m2-specific monoclonal antibody (Levey *et al*., 1995), we found that m2 receptor protein is expressed primarily in noncholinergic multipolar neurons located mainly outside the main aggregate of cholinergic nucleus basalis (Ch1–4) subfields (Mufson *et al*., 1998). Despite this nonoverlap of cell populations, there was a virtually complete overlap between the distribution of cholinergic neurons and m2 neuropil staining in the human (Figs. 19.17–19.19; Mufson *et al*., 1998). Double-labeled sections from the septal/diagonal band complex revealed that m2 is detected in only 14% of ChAT-labeled neurons, whereas only 13% of m2-stained neurons colocalize ChAT. Within the nucleus basalis, ChAT is found in 35% of the m2-labeled neurons, whereas only 6% of ChAT-stained perikarya are double-labeled for m2. A similar distribution of m2 receptor cells was seen in the monkey basal forebrain (Mrzljak *et al*., 1993, 1998). Colocalization of m2-positive/ChAT-immunonegative neurons and the cholinergic inhibitory neuropeptide galanin was not observed, although galaninergic fibers coursed in close apposition to m2-immunoreactive cells. This suggests that galaninergic fibers may modulate m2 receptor activity within the basal forebrain of humans. Cell counts demonstrated that 90% of ChAT-immunolabeled striatal neurons express the m2 receptor. Despite the extensive reduction in cholinerigc basal forebrain neurons, cell counts of the relative number of m2-immunoreactive neurons within the nucleus basalis complex from aged controls and AD patients revealed that the m2 neurons are spared (Fig. 19.20; Mufson *et al*., 1998). Finally, these findings suggest that most m2 receptors in the cholinergic basal forebrain are located on noncholinergic small neurons and, therefore, are not the major source of m2 receptors seen in the cortex. There is, however, a population

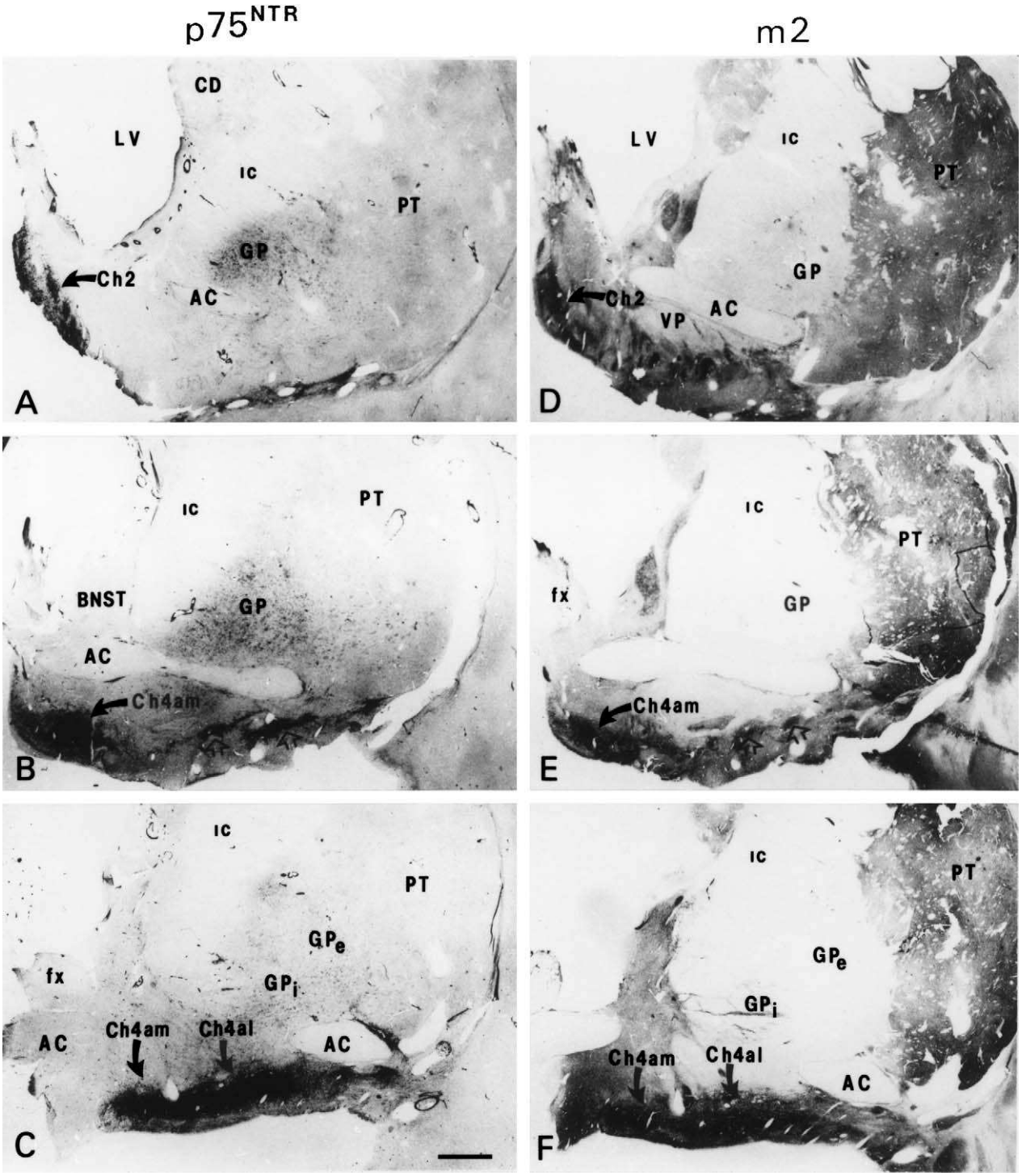

FIG. 19.17. Low-magnification photomicrographs of adjacent stained sections comparing the distribution of cholinergic (A–C) and m2 (D–F) immunoreactivity within the aged normal human basal forebrain. Cholinergic profiles were visualized by using an antibody against the low-affinity p75 neurotrophin receptor ($p75^{NTR}$; see text for details). There was a virtual complete overlap between the distribution of $p75^{NTR}$ and m2 neuropil staining within the diagonal band (Ch2) and the anteromedial (Ch4am) and anterolateral (Ch4al) subfields of the basal forebrain. In contrast, only m2 immunoreactivity was seen in the caudate (CD) and putamen (PT), as well as the bed nucleus of the stria terminals (BNST). AC, anterior commissure; fx, fornix; GR, globus pallidus; GPe, globus pallidus external; GPi, globus pallidus internal; ic, internal capsule; LV, lateral ventricle. Scale bar: 3 mm. From Mufson *et al*. (1998), with permission.

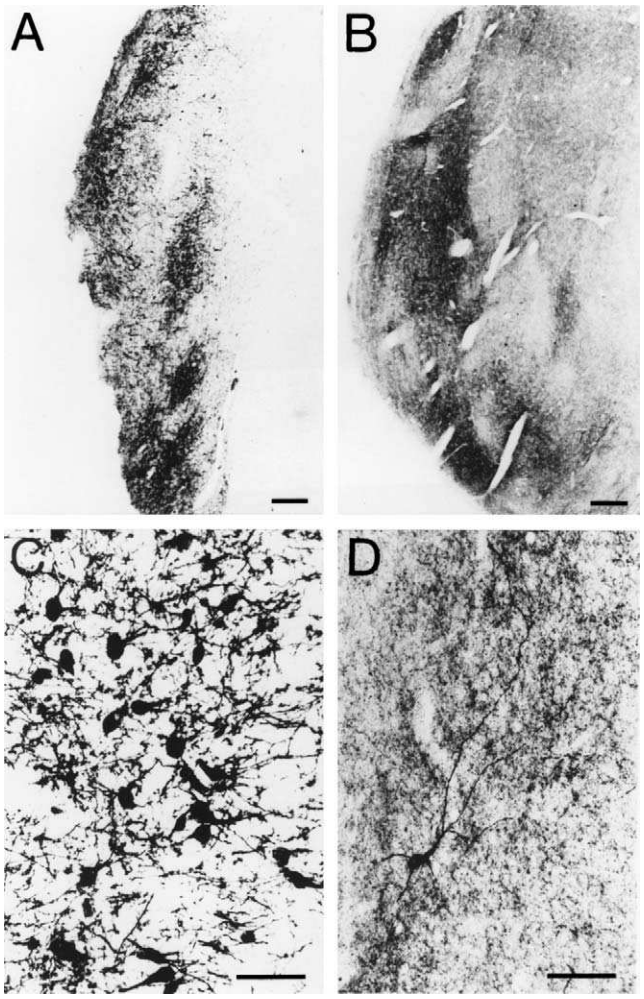

FIG. 19.18. (A and B) High-power photomicrographs of the diagonal band region shown in Figs. 19.1A and 19.1D immunostained for $p75^{NTR}$ and m2, respectively. In A, note that the cholinergic marker distinguishes the medial and lateral divisions of the diagonal band more clearly. (C) Photomicrograph of numerous $p75^{NTR}$-immunostained cholinergic diagonal band neurons and processes. (D) In contrast, very few m2 neurons were seen embedded within the immunoreactive neuropil of the diagonal band. Note the extensive dendritic process emanating from the m2 neuron. Scale bars: 500 (A and B) and 100 (C and D) μm. From Mufson *et al.* (1998), with permission.

of m2-immunoreactive small interneurons scattered throughout the cortex in both monkeys and humans (Mufson *et al.*, 1997b; Mrzljak *et al.*, 1998).

X. Relationship of Noncholinergic to ChAT-Contain ing Neurons within the Primate Cholinergic Basal Forebrain

A. Overview

In addition to the magnocellular cholinergic neurons located within the Ch4 subfields, there are many smaller noncholinergic perikarya (see de Lacalle and Saper, 1997). In the rhesus monkey the novel neuropeptide galanin coexists with ChAT in large neurons throughout the cholinergic basal forebrain (Kordower and Mufson, 1990; Kordower and Rakic, 1990; Walker *et al.*, 1991; Kordower *et al.*, 1992). Other perikarya stained for somatostatin, neuropeptide Y (NPY), enkephalin, or neurotensin interdigitate with cholinergic neurons in limited areas of the basal forebrain. De Lacalle and Saper (1997) indicate that these neuronal types are concentrated most heavily in the anterior and intermediate aspects of the nucleus basalis. However, these cells are relatively small interneurons that are not cholinergic. Within the various subregions of the monkey basal forebrain there are reports of fibers and/or putative terminals that display several peptides, including enkephalin (Haber and Elde, 1982; Candy *et al.*, 1985; Haber and Watson, 1985), NPY (Smith *et al.*, 1985; E. J. Mufson, personal observation), proopiomelanocortic peptides (Candy *et al.*, 1985), somatostatin (Fig. 19.21; Candy *et al.*, 1985; Mufson *et al.*, 1988a; Desjardins and Parent, 1992), substance P, cholecystokinin, vasoactive intestinal polypeptide, and oxytocin (Candy *et al.*, 1985). These observations suggest that peptidergic neurons can modulate the actions of cholinergic neurons within the basal forebrain.

1. Galanin Species Differences

Galanin is a 29 amino acid peptide that is cleaved from a 123 amino acid precursor, preprogalanin (Tatemoto *et al.*, 1983). Galanin inhibits acetycholine release in the ventral hippocampus (Fisone *et al.*, 1987). Galanin protein and mRNA have been shown to be widely distributed throughout the mammalian central nervous system and, in particular, galanin is intimately associated with basal forebrain cholinergic neurons (Melander and Staines, 1986; Chan-Palay, 1988; Kordower and Mufson, 1990; Walker *et al.*, 1991; Kordower *et al.*, 1992; Bowser *et al.*, 1997). Several studies have demonstrated that the distribution of galanin protein varies within the basal forebrain across a variety of mammalian species. For example, immunohistochemical investigations in rats have shown that galanin immunoreactivity exists within cholinergic neurons of the medial septal/vertical limb of the diagonal band complex, but not within the nucleus basalis (Melander *et al.*, 1986). In Old and New World primates, galanin protein colocalizes with virtually all magnocellular cholinergic neurons within each basal forebrain subfield (Melander *et al.*, 1986; Melander and Staines, 1986; Walker *et al.*, 1991; Kordower and Mufson, 1990; Kordower *et al.*, 1992). In contrast, neither galanin protein nor mRNA exists within the magnocellular cholinergic basal forebrain neurons in lesser or greater apes and humans, although a small population of parvicellular neurons within this region does express galanin (Figs. 19.22 and 19.23; Chan-Palay, 1988; Kordower and Mufson, 1990; Walker *et al.*, 1991; Kordower *et al.*, 1992; Mufson *et al.*, 1993; Benzing *et al.*, 1993). These observations are in conflict with others who have indicated that galanin immunostaining occurs in the different subfields of the human basal forebrain (Kowall and Beal, 1989; Vogels *et al.*, 1989; Gentleman *et al.*, 1989). The few neurons that do express galanin consist of a small population of parvicellular neurons within these regions that do not express either the protein or mRNA for galanin (Chan-Palay, 1988; Kordower and Mufson, 1990; Walker *et al.*, 1991; Kordower *et al.*, 1992; Mufson *et al.*, 1993; Benzing *et al.*, 1993).

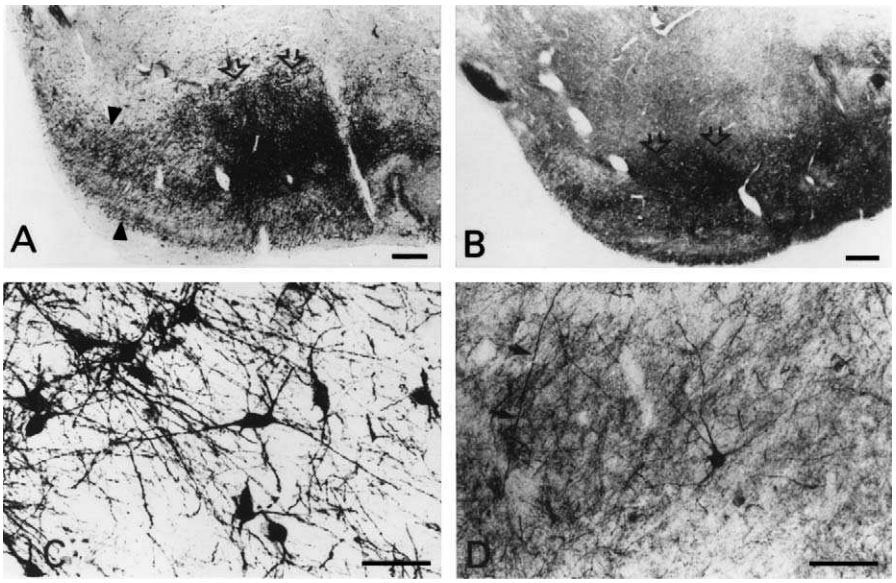

FIG. 19.19. (A and B) High-power photomicrographs of the anterior medial region of the nucleus basalis shown in Figs. 19.2B and 19.2E immunostained for $p75^{NTR}$ and m2, respectively. Arrows in A and B indicate the overlap between cholinergic and m2 staining in this region. Arrowheads in A indicate the caudal extension of the diagonal band nucleus. (C) Photomicrograph of numerous $p75^{NTR}$-immunostained cholinergic nucleus basalis neurons. Note their magnocellular appearance and extensive dendritic processes. (D) In contrast, very few m2 neurons were seen embedded within the immunoreactive neuropil. Arrows in D indicate the location of m2-immunostained fibers. Scale bars: 500 (A and B) and 100 (C and D) μm. From Mufson *et al*. (1998), with permission.

Although human magnocellular basal forebrain neurons do not synthesize galanin, they are innervated by a galanin-immunostained fiber system that courses through the basal forebrain and is closely apposed to the cholinergic perikarya mainly in the anterior portion of the nucleus basalis (Ch4a) (Fig. 19.24; Chan-Palay, 1988; Kordower and Mufson, 1990; Mufson *et al*., 1993). The potential importance of this unique galanin basal forebrain innervation pattern is underscored by observations that this galanin-immunoreactive fiber network is hypertrophied in AD (Chan-Palay, 1988; Mufson *et al*., 1993; Bowser *et al*., 1997). These findings, coupled with experimental evidence indicating that galanin is inhibitory to acetylcholine (Fisone *et al*., 1987), have led to the hypothesis that galanin modulates cholinergic tone within the magnocellular basal forebrain system (Chan-Palay, 1988). More recently, it has been suggested that galanin may exhibit trophic activity (Wynick *et al*., 1998), aiding in the survival of cholinergic neurons in disease states. In general, the distribution of neuroactive substances is organized along a general mammalian plan (Felten and Sladek, 1983). Available data indicate that galanin deviates from this rule.

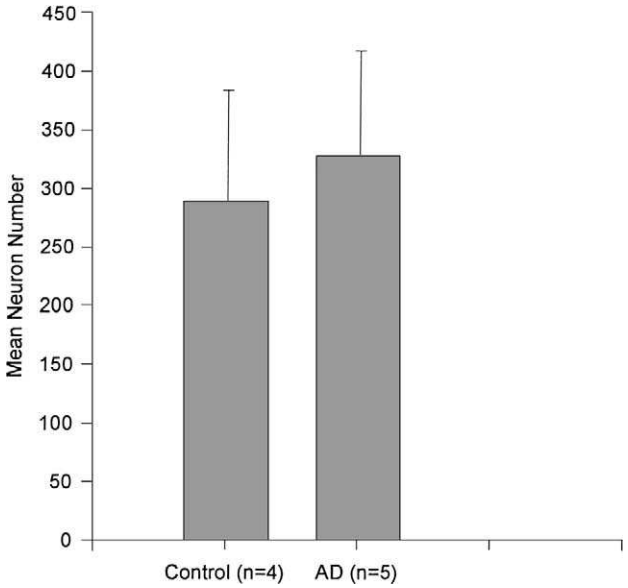

FIG. 19.20. Histogram showing that m2-immunoreactive neurons are not reduced significantly within the nucleus basalis in AD compared with aged controls. From Mufson *et al*. (1998), with permission.

2. Calcium-Binding Proteins

Findings in the squirrel monkey revealed large numbers of (35 μm mean diameter) calbindin-immunoreactive neurons in the septum (Ch1) and within the diagonal band (Ch2; Côté *et al*., 1991). Calbindin-immunoreactive neurons are also scattered within the nucleus basalis (Ch4) with an average diameter of 40 μm. Dual immunohistochemical experiments showed that ChAT and calbindin are colocalized within the basal forebrain of the rhesus monkey (Fig. 19.25; Geula *et al*., 1993; Côté and Parent, 1992). Calbindin and ChAT have been shown within the cholinergic neurons of the human nucleus basalis (Ch4) (Geula *et al*., 1993). de Lacalle and Saper (1997) have also reported that calbindin-immunoreactive neurons are intensely stained in the human nucleus basalis, but only light staining is seen in the nucleus of the diagonal band (Ch2). Geula and colleagues (1993) have shown an age-related

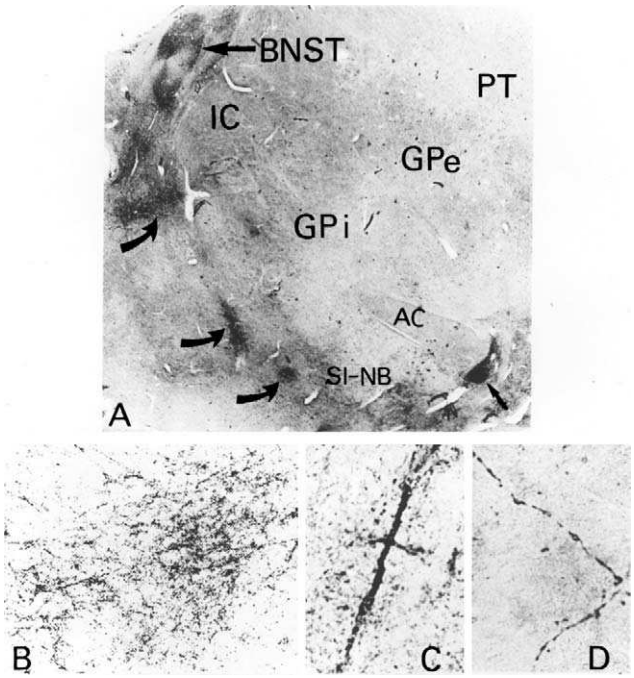

FIG. 19.21. Photomicrographs of tissue stained for somatostatin (SOM) with the S309 antibody. (A) Woolly fibers (curved black arrows) coursing dorsomedially beneath the anterior commissure at the level of the substantia innominata–nucleus basalis complex en route to the bed nucleus of the stria terminalis (large black arrow). Terminal-like staining is located adjacent to the lateral edge of the anterior commissure (small black arrow). Tissue obtained from a 68-year-old female AD case (X10). (B) Terminal-like and fine fiber staining in the substantia innominata–nucleus basalis complex (X30). (C) Wooly fiber in the substantia innominata-nucleus basalis complex (X40). (D) Slender beaded fiber with regularly dispersed varicosities located within the substantia innominata–nucleus basalis complex (X50). Tissue shown in B–D was obtained from a 70-year-old male neurologically normal case. From Mufson *et al*. (1988a), with permission.

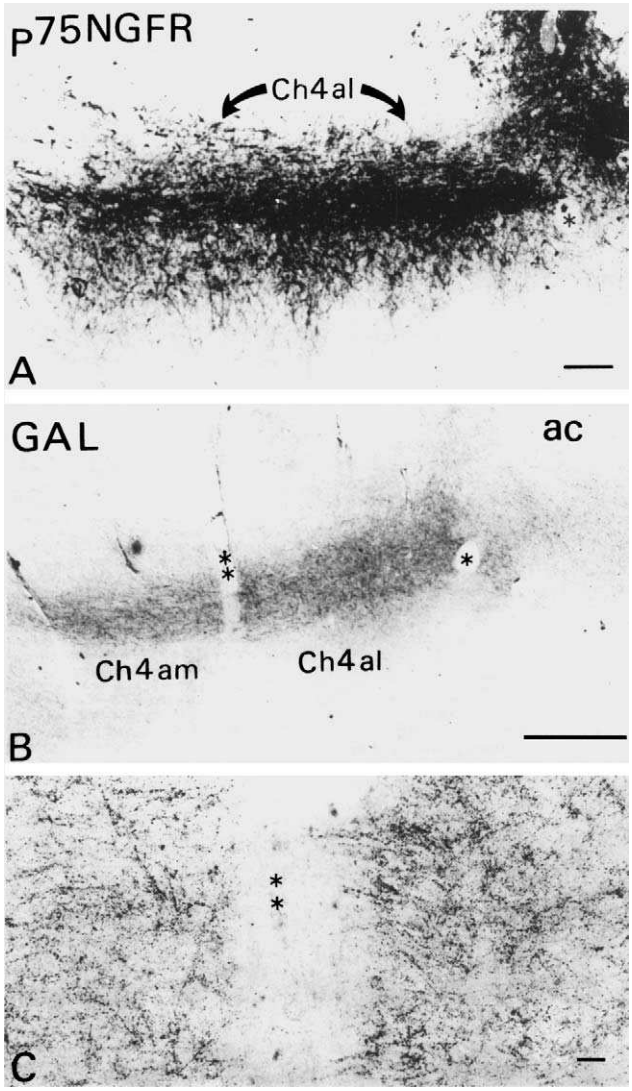

FIG. 19.22. $p75^{NTR}$ and galanin (GAL)-immunoreactivity within the gorilla nucleus basalis. (A) Intense p75 $p75^{NTR}$-immunoreactive perikarya and fiber staining within Ch4al. (B) Adjacent section stained for galanin-immunoreactivity showing only a dense band of immunoreactivity within the anteromedial (Ch4am) and anterolateral (Ch4al) divisions of the nucleus basalis. Single asterisks indicate the same blood vessel in A and B. (C) High-power photomicrograph of galanin-immunoreactive fiber staining within Ch4am and Ch4al subfields of the nucleus basalis. Asterisks in B and C indicate the position of the blood vessel separating Ch4am and Ch4al. ac, anterior commissure. Scale bars: 50 (A), 1000 (B), and 10 (C) μm. From Benzing *et al*. (1993), with permission.

loss of calbindin-containing neurons in the cholinergic basal forebrain in normal humans. They suggested that the loss of pheontypic expression of calbindin may be a potential mechanism for the selective loss of cholinergic neurons in aging and neurodegenerative disorders such as AD.

3. Nicotinamide Adenine Dinucleotide Phosphate Diaphorase (NADPH-d) in the Primate Cholinergic Basal Forebrain

Similar to galanin, several studies indicate major species differences in the expression of NADPH-d, a novel neurotransmitter that may be neurotoxic in the cholinergic basal forebrain (Dawson *et al*., 1991). Studies have shown that NADPH-d staining occurs in as many as 20–30% of the cholinergic basal forebrain neurons in the rat, but virtually none of these neurons contain this enzyme in the rhesus monkey (Geula *et al*., 1993). Similarly, in the human, NADPH-d did not colocalize with ChAT/$p75^{NTR}$ neurons within the nucleus basalis (Ch4). Ellison *et al*. (1987) reported a subset of neurons characterized by the presence of NADPH-d in human Ch4. We have shown that NADPH-d histochemistry revealed intensely stained (type 1; Fig. 19.26a), moderately stained (type 2; Fig. 19.26b), and lightly stained (type 3) (Fig. 19.26c) neurons. Types 1 and 2 are the primary neurons in the aged human, whereas type 3 is the least prevalent. Interestingly, only a few scattered NADPH-d-positive perikarya are interdigitated within the dense aggregates of the cholinergic basal forebrain neurons, although NADPH-d-positive processes course within and around aggregates of these perikarya (Benzing and Mufson, 1995). NADPH-d histochemistry labels neurons containing nitric oxide synthase, the synthesizing for nitric oxide.

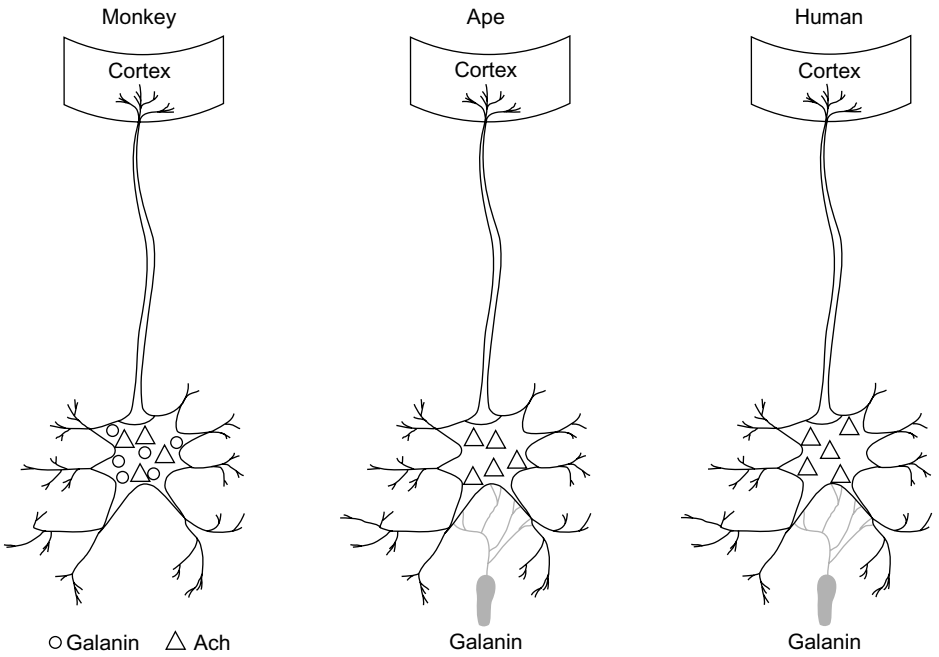

FIG. 19.23. Schematic diagram showing the relation between acetylcholine (Ach) and galanin within neurons of the primate cholinergic basal forebrain. Note that in the monkey, all cholinergic basal forebrain neurons colocalize galanin. In contrast, these neurons are galanin negative in apes and humans. In these species, galanin arises from a population of small interneurons (light gray) as well as in an extraforebrain fiber system (not shown), which terminates on the large cholinergic neurons of the substantia innominata. From Mufson *et al*. (1998), with permission.

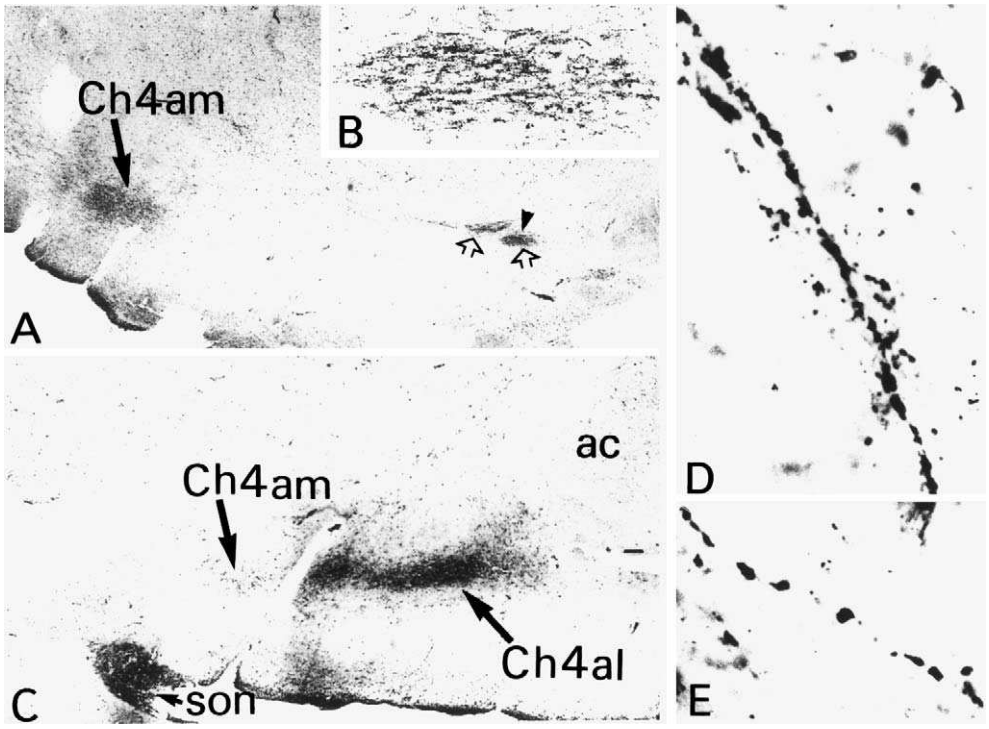

FIG. 19.24. (A) Fibers coursing through the anterior basal forebrain (open arrows) en route to Ch4am. X12. (B) High-power photomicrograph showing the fiber bundle depicted in A (black arrowhead). X94. (C) Dense GAL-immunoreactive ''woolly fiber.'' X350. (D) Thin-beaded fiber found within the fiber plexus seen in B. X131. From Mufson *et al*. (1993), with permission.

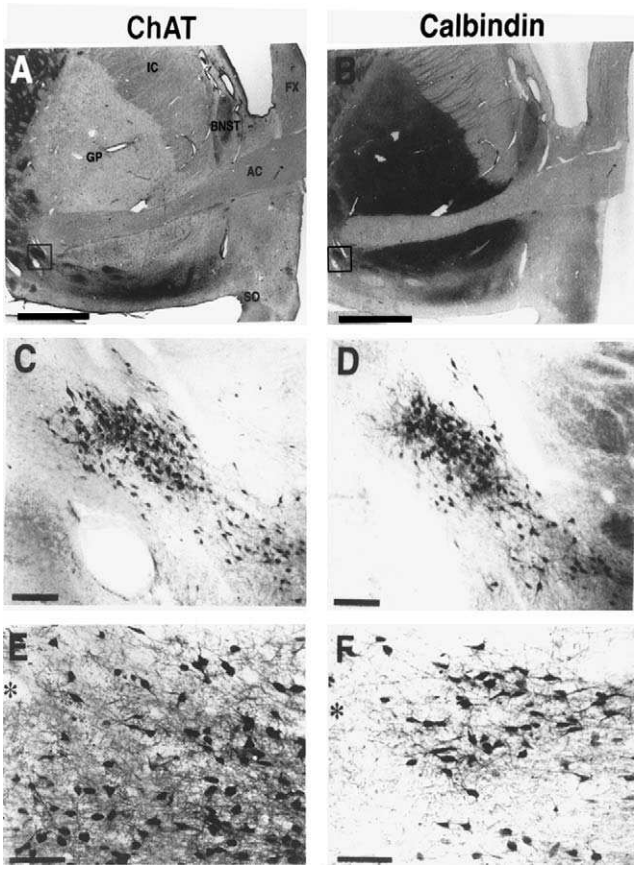

FIG. 19.25. A comparison of the immunocytochemical staining for ChAT and calbindin in the human basal forebrain. These adjacent sections show the overall regional colocalization of these two markers [colocalization at the cellular level was shown by Geula *et* al. (1993)]. Asterisks in E and F mark the same blood vessel. Scale bars: 0.5 cm (A and B) and 300 (E and F) μm. GP, globus pallidus; IC, internal capsule; BNST, bed nucleus of the stria terminalis; AC, anterior commissure; FX, fornix, SO, supraoptic nucleus. From de Lacalle and Saper (1997), with permission.

Quantitative analyses revealed a statistically significant increase in the density of intensely (type 1) and moderately (type 2) but not lightly (type 3) NADPH-d-containing neurons within the nucleus basalis in AD as compared to age-matched controls (Fig. 19.27; Benzing and Mufson, 1995). Increased numbers of NADPH-d-containing neurons suggest that excess nitric oxide production may be neurotoxic to surrounding cholinergic neurons within Ch4 in AD.

NADPH-d is also found in virtually all Ch5 and Ch6 neurons in macaques, baboons (Geula *et al.*, 1993), and humans (Mufson *et al*., 1988b), making it a reliable marker for ChAT neurons. In contrast to calbindin staining in Ch5 and Ch6 in rats, it is not seen in the mesopontine cholinergic system in macaques and baboons (Geula *et al*., 1993). Interestingly, the concurrent visualization of ChAT and NADPH-d displays an extensive overlap in Ch6 in humans (Mesulam *et al*., 1988). In patients with AD, NADPH-d/ChAT dual-stained neurons contain NFTs, suggesting a disconnection between these brain stem neurons and then projection sites in the thalamus (Mufson *et al*., 1988b). Whether the neurotoxic effects of NADPH-d play a role in the formation of NFTs remains to be clarified.

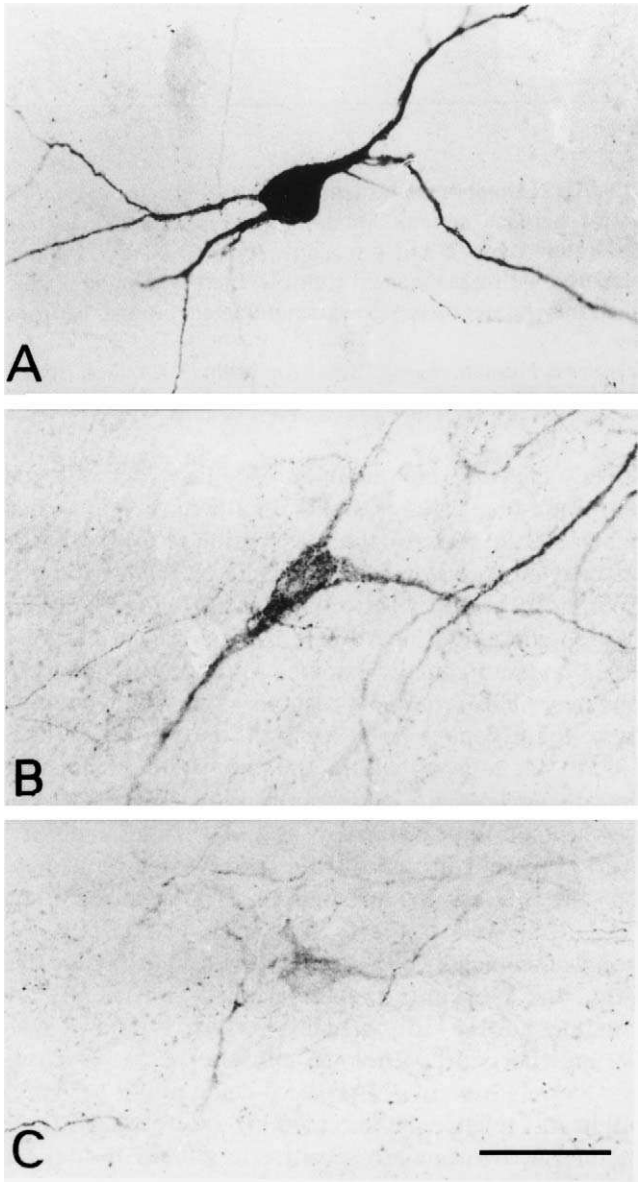

FIG. 19.26. Examples of three types of NADPH-d-positive neurons quantified in the present study: (A) intensely stained (type1) neuron, (B) moderately stained (type 2) neuron, and (C) lightly stained (type 3) NADPH-d neuron. Note Golgi-like appearance of neuron in A. Bar: 30 μm. From Benzing and Mufson (1995), with permission.

4. Estrogen Receptor and the Primate Cholinergic Basal Forebrain

Available data indicate a species difference in the localization of estrogen receptor α (ERα) containing nuclei within the cholinergic basal forebrain between rodents (Simerly *et al*., 1990; Shughrue *et al*., 1992; Gibbs and Pfaff, 1992; Mufson *et al*., 1999) and primates (Blurton-Jones *et al*., 1999; Sendera *et al*., 1999). The presence of estrogen receptors within cholinergic basal forebrain neurons in rodents suggests a potential mechanism by which estrogen could influence their biology and ultimately their behavioral sequela. Numerous

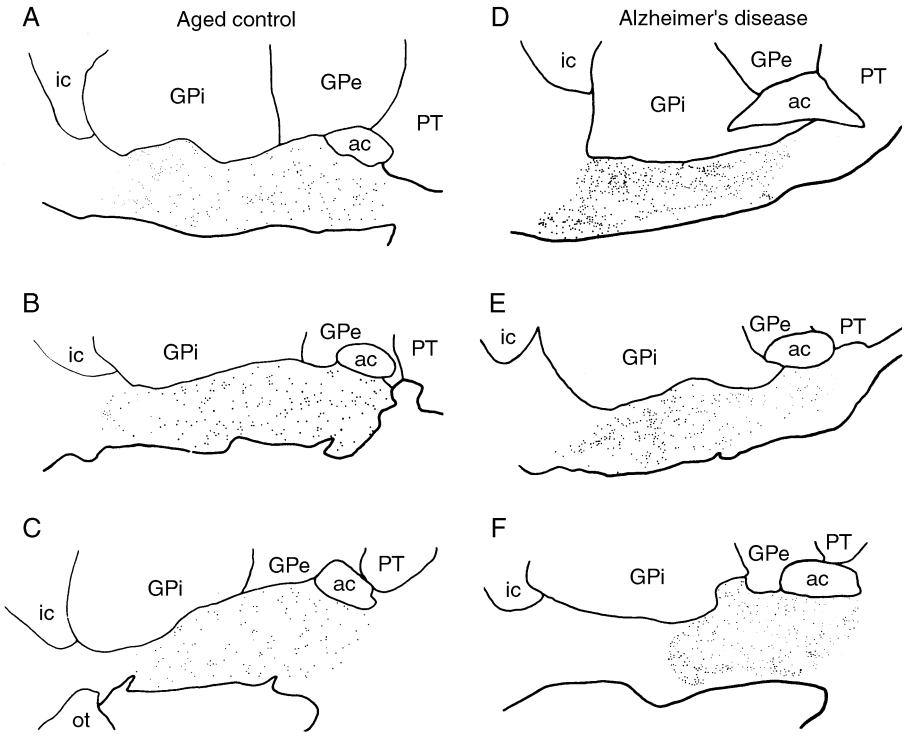

FIG. 19.27. (A–C) Chartings of dark (type 1) and medium (type 2) NADPH-d-positive neurons at three rostral to caudal levels (~1440 μm apart) of SI in an age-matched control (case 4). (D–F) Chartings drawn at similar levels in an AD patient (case 8). Light neurons (type 3) were omitted for clarity as their density did not differ between AD and controls. ac, anterior commissure; ic, internal capsule; GPe, globus pallidus pars external; GPi, globus pallidus pars internal; ot, optic tract; PT, putamen. From Benzing and Mufson (1995), with permission.

histochemical and gene expression studies in the rodent indicate that cholinergic basal forebrain neurons coexpress estrogen receptors (e.g., Toran-Allerand *et al*., 1992; Gibbs and Pfaff, 1992; Shughrue *et al*., 1997; Mufson *et al*., 1999). An evolving literature indicates that the cholinergic basal forebrain is involved in the regulation of cognitive and attentional processes in the normal, aged, and diseased brain (Gibbs, 1994). In fact, epidemiological studies indicate that treatment with the steroid hormone estrogen decreased the risk of AD in postmenopausal women (Tang *et al*., 1996) and enhanced the response to the cholinesterase inhibitor tacrine in these individuals (Schneider *et al*., 1996). Taken together, these biologic and clinical observations suggest that some of the pharmacological effects of estrogen may occur via its interaction with cholinergic basal forebrain neurons, which express the estrogen receptor, at least in the developing (Toran-Allerand *et al*., 1992) and adult (Gibbs and Pfaff, 1992; Gibbs, 1994; Mufson *et al*., 1999) rat. In contrast to the rodent, available immunohistochemical studies indicate that ERα does not colocalize with ChAT, p75, or trkA containing cholinergic basal forebrain neurons in the monkey (Figs. 19.27c and 19.27d; Blurton-Jones *et al*., 1999; Sendera *et al*., 1999) or human (E. J. Mufson *et al*., unpublished observations). By comparison, only a few scattered ERα-positive cholinergic immunonegative neurons were found within the anterior nucleus basalis (Ch4a) in the rhesus monkey (Blurton-Jones *et al*., 1999; Sendera *et al*., 1999). Blurton-Jones *et al*. (1999) reported only rare ERα-labeled nuclei in the horizontal and vertical limbs of the diagonal band. Initial studies in our laboratory in the rhesus monkey have not shown ERα-immunoreactive nuclei within ChAT, calbindin-, or parvalbumin-immunoreactive neurons within the nucleus basalis of the monkey as well (Sendera *et al*., 1999). However, ERα-immunostained nuclei as well as minor cytoplasmic staining were found within other forebrain areas, including the lateral septum (Fig. 19.28, see color insert), amygdala, the bed nucleus of the stria terminalis, subfornical organ, medial, and lateral hypothalamic nuclei, and the amygdalohippocampal transition zone in the rhesus monkey (Blurton-Jones *et al*., 1999; Sendera *et al*., 1999) and human (E. J. Mufson *et al*., unpublished observations; Fig. 19.28). The majority of ChAT-immunostained neurons in the lateral septum were also ERα immunopositive, whereas only about 29% of all ERα-immunoreactive nuclei in the lateral septum were also ChAT stained (Blurton-Jones *et al*., 1999). Further studies are needed to determine the chemical phenotype of ERα-containing neurons within the primate brain and whether these neurons are regulated via genomic or nongenomic mechanisms.

XI. Trajectory of Cholinergic Basal Forebrain Fiber Systems in Primates

A. Cholinergic Fiber Trajectories in the Monkey

Evidence has shown the presence of cholinergic innervation in the olfactory bulb, hippocampus, and amygdala, as well as

the neocortex and paralimbic cortex. These regions have been shown to contain numerous markers indicative of cholinergic innervation, including AChE, ChAT, acetylcholine, $p75^{NTR}$, and trkA receptors, as well as sites for cholinergic agonists (see de Lacalle and Saper, 1997, for review). This cholinergic innervation has been localized primarily to the subfields of the cholinergic basal forebrain, as lesions of this region lead to a considerable decrease in presynaptic cholinergic markers in the hippocampus, neocortex, amygdala, and olfactory bulb (Mufson *et al*., 1983). The trajectories of cholinergic fiber pathways emanating from the cholinergic basal forebrain groups are not well defined in the human, although much more is known in the monkey brain. Most of what is known about the pathways emanating from the cholinergic subfields of the nonhuman primate has been derived from axonal transport studies, enzyme assays, and immunohistochemistry (Mesulam *et al*., 1983b, 1986; Satoh and Fibiger, 1985; Kitt *et al*., 1987; Geula *et al*., 1993). Studies in the monkey reveal that the Ch1–2 system travels within the fornix to connect with the hippocampus. In the rhesus monkey, injections of the tritiated amino acids into the midportion of the Ch4 and surrounding structures were used to define basocortical projection pathways (Kitt *et al*., 1987). This study revealed a medial projection to the cingulate cortex that courses within the cingulum, a lateral projection to the frontal, parietal and insular cortices, and a ventral projection to the temporal cortex and the amygdala that travels within the uncinate fasciculus, although these investigators did not correlate their findings with specific histochemical or immunocytochemical markers to ascertain the cholinergic phenotype of those fiber systems examined.

B. Cholinergic Fiber Trajectories in Human

Human studies of the trajectory of cholinergic basal forebrain pathways have been derived from histochemical investigations obtained from autopsy material. The most obvious cholinergic fiber pathway emanates from Ch1–2 to the hippocampus and courses mainly within the fornix (Fig. 19.14; Green and Mesulam, 1988; de Lacalle *et al*., 1994; Alonso *et al*., 1996). Selden *et al*. (1998) provided unique information on the trajectories of the cholinergic fiber pathways that emanate from the Ch4 cell group to the cerebral cortex using whole hemisphere cryostat-cut human brain sections stained for AChE as well as ChAT and $p75^{NTR}$. These investigators described two well-defined and discrete cholinergic fiber bundles from Ch4 to the cerebral cortex and amygdala, which were designated as the medial and lateral cholinergic pathways. The medial bundle joined the white matter of the gyrus rectus and curved around the rostrum of the corpus callosum to enter the cingulum. These fibers joined with axons of the lateral pathway within the occipital lobe. The lateral fasiculus provided the paraolfactory, cingulate, pericingulate, and retrospinal cortices. Selden and co-workers (1998) divided the lateral pathway into a capsular portion that traveled in the white matter of the external capsule (Fig. 19.29; see also Mufson *et al*., 1989a) and the uncinate fasciculus and perisylvian sector coursing within the claustrum. Divisions of the perisylvian bundle provided the cholinergic innervation of the frontal, parietal, and temporal neocortices (Fig. 19.29). Cholinergic fiber pathways are greatly reduced in patients with AD (Fig. 19.30; Mufson *et al*., 1989b). Magnetic resonance imaging reconstruction of these anatomically defined cholinergic pathways was also observed in the human brain (Selden *et al*., 1998).

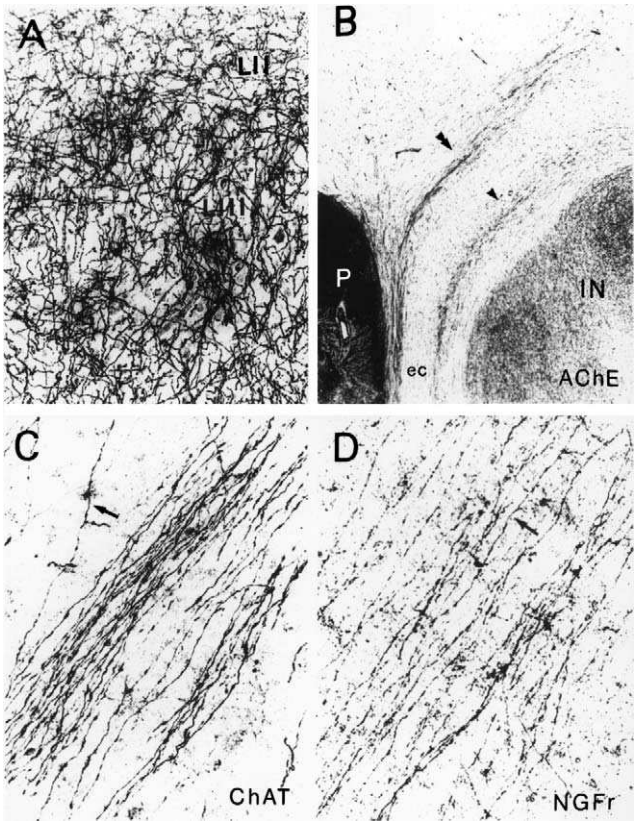

FIG. 19.29. (A) AChE histochemistry in the insula demonstrates a dense network of AChE-rich cholinergic axons. (B) Photomicrograph of the regions of the external capsule and claustrum revealing dense AChE reaction product over the putamen (P) and a thick network of AChE-rich fibers in the insular cortex (IN). The two components of the lateral cholinergic pathway are shown by the arrowhead (perisylvian division) and the double arrowhead (capsular division). The microscopic fields shown in C and D include the part of the capsular division located at the tip of the double arrowhead in B but immunostained in adjacent sections for ChAT (C) and $p75^{NTR}$ (D). The correspondence of the AChE-rich fiber bundle with ChAT- and $p75^{NTR}$-immunoreactive fibers demonstrates that the pathway is definitively cholinergic and that it originates in Ch1–4 cells of the nucleus basalsis. ec, external capsule; LII, LIII, cortical layers II and III; P, putamen. Medial is toward the left and dorsal toward the top. Magnifications: A, X186; B, X11.3; and C and D, X371. From Seiden *et al.* (1999), with permission.

XII. Connectivity of the Primate Cholinergic Basal Forebrain

A. Efferents of the Cholinergic Basal Forebrain

Combined horseradish peroxidase retrograde tracing and acetylcholinesterase (AChE) histochemistry to visualize double-labeled neurons (Mes-ulam and Van Hoesen, 1976; Mesu-

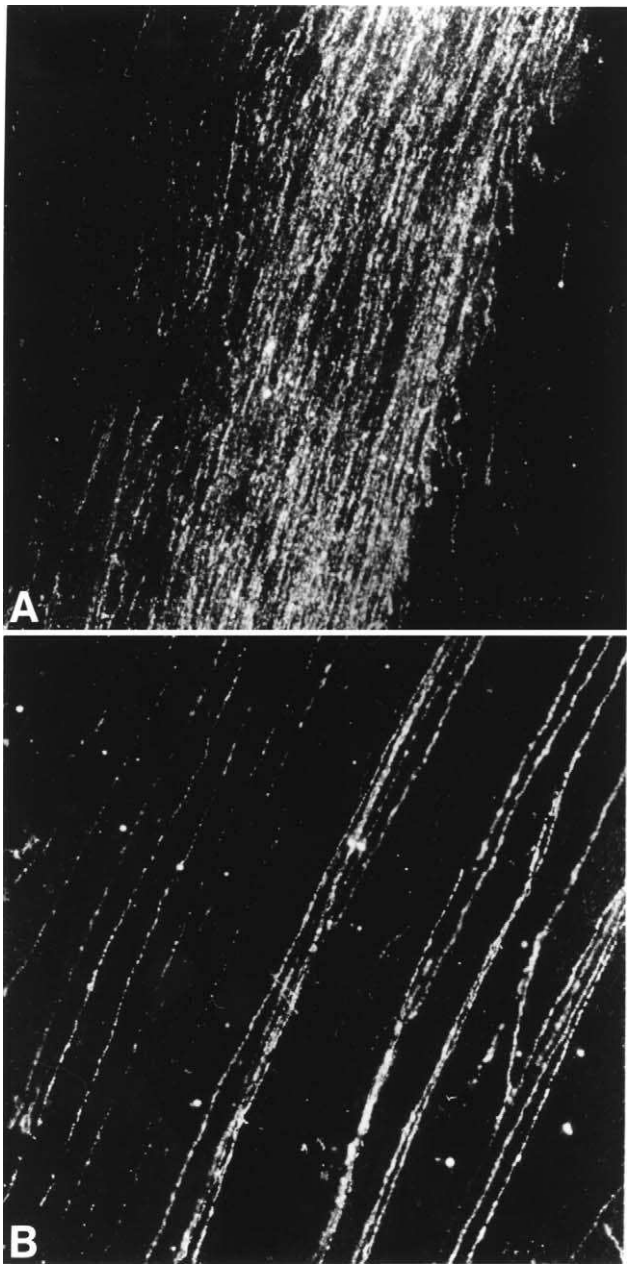

FIG. 19.30. Dark-field photomicrograph of $p75^{NTR}$ receptor-containing fibers coursing within the external capsule of a neurologically normal (A, 4X) and an AD (B, 11X) patient. Note the striking loss of $p75^{NTR}$-containing fibers between the control and the AD patient. B is presented at a magnification higher than that of A to better visualize the few remaining fibers seen in the AD case. From Mufson *et al*. (1989a), with permission.

lam *et al*., 1983b), demonstrated that the various cholinergic basal forebrain subfields project in a topographic fashion to different regions of the neocortical mantle, hippocampus, and amygdala and olfactory bulb of rhesus monkey. These findings have been substantiated in studies using concurrent labeling of neurons containing ChAT and HRP in the monkey (Mesulam *et al*., 1986).

1. Cholinergic Cell Group Medial Septum (Ch1) and Vertical Limb of the Diagonal Band Nucleus (Ch2)

Using the retrograde transport marker horseradish peroxidase, injections into the hippocampal formation with some spread to the parahippocampal area demonstrated cholinergic neurons within Ch1 and Ch2 in the rhesus monkey (Fig. 19.14). In contrast, only 1% were seen in Ch3 (horizontal limb of the diagonal band) and a small number within Ch4am. Others reported that neurons projecting to the hippocampus in the rhesus monkey were located mainly in the medial septum, the diagonal band, and the anteromedial aspect of Ch4. Approximately one-third of the retrogradely labeled neurons are ChAT immunoreactive (Koliatsos *et al*., 1990). Extensive cholinergic/$p75^{NTR}$ fiber labeling has been reported in the aged human hippocampus, presumably originating from cells, of the Ch1 and Ch2 (see also de Lacalle *et al*., 1994).

2. Cholinergic Cell Group Horizontal Limb of the Diagonal Band Nucleus (Ch3)

Projections from the Ch3 group (nucleus of horizontal limb of the diagonal band) are most prominent following injections into the olfactory bulb. Substantial projections of Ch3 were also seen within frontolateral area 12 and the amygdala.

3. Cholinergic Projections to the Cortex from the Nucleus Basalis (Ch4)

Projections of the Ch4 subregions are the most widespread and are directed mostly to the cortical mantle of the cerebral hemispheres. The extensive projections of the Ch4 group exhibit an internal topography. The Ch4am cell group projects mainly to the midprincipal, medial frontal pole, subcallosal gyrus, cingulate, dorsomedial motor, and parietal (area 7) cortices. Lesser but substantial projections of Ch4am are directed to the hypothalamus, hippocampal formation, ventral somatosensory cortex, amygdala, ventrolateral orbital, middle insula, periarcuate, peristriate cortices and parahippocampal region as well as the inferior parietal lobule.

The Ch4al subfield major source of innervation was to the lateral portion of area 12, frontal operculum, frontal operculum (M1), ventral S1, ventral posterior parietal cortex, and the amygdala. Additional retrograde tracer studies have reported a substantial projection from Ch4al to the amygdala (Kordower *et al*., 1989b). Other areas include the ventrolateral orbital cortex, medial, anterior, and posterior portions of the insula, as well as inferior temporal and parahippocampal regions.

These groups displayed many similarities in their projection patterns. Their major projections are to the ventrolateral orbital, insular, periarcuate, peristriate, inferotemporal, and parahippocampal areas, as well as the inferior lobule. Substantial contributions from these regions are also found in the medial frontal pole, dorsomedial motor cortex, and frontoparietal opercular areas, the amygdala, anterior auditory cortex, and the temporal pole.

The Ch4p region exhibits a very restricted distribution pattern. Its principal source of projections is to the auditory association cortex and the superior temporal gyrus, as well as the temporal pole. Additional major projections are also found in the adjacent inferotemporal and posterior insular regions.

4. Cholinergic Cell Groups 5 and 6 of the Brain Stem

The Ch5 and Ch6 sectors are located mostly within the pedunculopontine nucleus of the reticular formation (Ch5) and within the lateral dorsal tegmental nucleus (Ch6) and provide the major cholinergic innervation of the thalamus (Steriade *et al*., 1990; Hoover and Baisden, 1980). Rye *et al*., (1988) confirmed these connections using modern tract tracing procedures and the concurrent visualization of cholinergic markers. Although not confirmed in the primate, a minor projection from these cells to the cerebral cortex has been reported in the rodent (Vincent *et al*., 1983; Saper and Loewy, 1982). A significant descending projection from Ch5 to the brain stem reticular formation has also been described in rodents (Rye *et al*., 1988; Yasui *et al*., 1990), but has not been studied in primates. These connections have been suggested to play a role in coordinating switching from slow wave to rapid eye movement sleep (Rye *et al*., 1988).

5. Cholinergic Cell Groups 7 (Ch7, Medial Habenula) and 8 (Ch8, Parabigeminal Nucleus)

Ch7 projects mainly to the interpeduncular nucleus via the interpeduncluar tract (Fig. 19.14; Mesulam *et al*., 1986, Mufson *et al.*, 1987). The Ch8 region projects mainly to the superior colliculus (Fig. 19.14; Mufson *et al*., 1986). Following horseradish peroxidase–wheat germ agglutinin injections into the superior colliculus, the contralateral Ch8 contained six times as many labeled neurons as the ipsilateral nucleus. Some retrograde labeling was seen in the pedunculopontine (Ch5) and lateral dorsal tegmental nuclei (Ch6). Double labeling with ChAT and retrograde transport revealed that about 80% of retrogradely labeled neurons in the parabigeminal nucleus were also ChAT positive. Further support for the cholinergic nature of Ch8 neurons was derived from autoradiographic experiments that visualized intense labeling over the region corresponding to Ch8. In addition, tissue processed for a ChAT and AChE, as well as muscarine receptors, showed bands of these cholinergic markers in the superficial, intermediate, and deep layers of the superior colliculus (Fig. 19.15). These markers may correspond, at least in part, to the projection field of the cholinergic Ch8 neurons (Mufson *et al*., 1986).

B. Afferents to the Cholinergic Basal Forebrain Complex in Primates

1. Overview

Although it is well established in both the rodent and the monkey that the fornix carries cholinergic fibers from Ch1, very little is know about the neural inputs into the nucleus basalis (Ch4) in the monkey. This information is critical, as extensive pathological alterations have been reported in the Ch4 complex in a number of neuropsychiatric disorders, such as AD (Whitehouse *et al*., 1982; Arendt *et al*., 1985; Kordower *et al*., 1989a), schizophrenia (Stevens, 1982), Parkinson's disease (Whitehouse *et al*., 1983; Mufson *et al*., 1991), and Creutzfeldt–Jakob's disease (Arendt *et al*., 1984). Limited information on the behavioral specialization of the Ch4 complex in primates suggests that these neurons have response contingencies that go beyond simple sensory processing or motor control. In contrast to the neurons of the globus pallidus, which change their firing rate in temporal relationships to push–pull movements of the limbs, which most likely plays a significant role in motor control, the adjacent Ch4 neurons in the macaque respond to the delivery of juice reward (DeLong, 1971). These neurons also respond to the sight and taste of food. The size of the response is influenced by the interest of the food object and by the state of the animal's hunger (Burton *et al*., 1975). These findings indicate that the Ch4 perikarya are responsive to motivational states and that they must be receiving multiple sensory and limbic information that enables them to intergrate the external sensory events with the internal milieu.

C. Afferents of the Cholinergic Basal Forebrain

In 1984, Mesulam and Mufson placed tritiated amino acid injections within cortex and subcortical targets of the rhesus monkey. In contrast to their widespread projections to all parts of the cortex, these neurons receive reciprocal projections from only a few cortical areas. Most of the sensory, motor, and association areas in the frontal, parietal, occipital, and temporal lobes did not reveal projections back to the Ch4 complex. The Ch4 neurons receive their cortical input from the prepyriform cortex, orbital frontal (Fig. 19.31), the anterior insula, the temporal pole, entorhinal cortex (Fig. 19.32), and the medial temporal cortex. We found additional subcortical inputs from the septal nuclei, the nucleus accumbens–ventral pallidum complex, and the hypothalamus. Review of the organization of Ch4 input suggests that this region is in a position to act as a cholinergic relay for transmitting predominantly limbic and paralimbic information to the neocortical surface (Fig. 19.33). It would also appear that cortical areas that do not project into Ch4 may have not have a direct manner of controlling the cholinergic input that they receive. The findings supported and expanded earlier neuroanatomical experiments in the macaque, which demonstrated the presence of Ch4 projections to the amygdala, medial frontopolar cortex, ventromedial hypothalamic nucleus, the magnocellular portion of the dorsomedial nucleus of the thalamus, caudal orbitofrontal cortex, and the peripeduncular nucleus (see de Lacalle and Saper, 1997, for review). However, the apparent limited set of cortical areas that do project into Ch4 probably can control not only the cholinergic innervation that they receive but also cholinergic innervation into the cortex.

XIII. Pathology of Cholinergic Systems in Aging and Alzheimer's Disease

A. Overview

Over the last two decades, the relationship between cholinergic basal forebrain function and cognition, especially age-related cognitive decline, has engendered great scientific interest (e.g., Bartus *et al*., 1982; DeKosky *et al*., 1985). The cholinergic basal forebrain neurons located within the septal/diagonal band complex and the nucleus basalis provide the major cholinergic innervation to the cortex and hippocampus

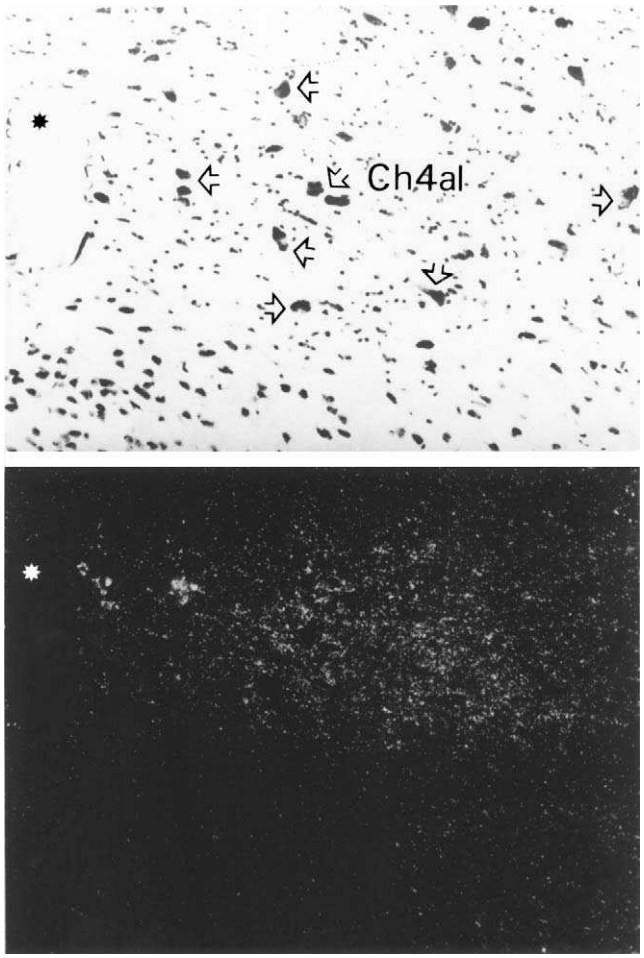

FIG. 19.31. Photomicrographs from orbitofrontal radioisotope-labeled injection. The upper photomicrograph was taken with bright-field illumination. It shows the part of Ch4al that overlies the most caudal portion of the olfactory tubercle. Open arrowheads point to some examples of the large hyperchromic Ch4 neurons. The smaller neurons at the bottom belong to the olfactory tubercle. The lower photomicrograph shows the same area but under dark-field illumination. This makes the silver label stand out as white dots. The label is clearly concentrated in Ch4al and forms dense homogeneous clusters. Because there are no linear streaks of silver grains, this label represents axonal terminals rather than passing fibers. For purposes of orientation, the arteriole in both photomicrographs has been marked with an asterisk. Magnification X266. From Mesulam and Mufson (1984), with permission.

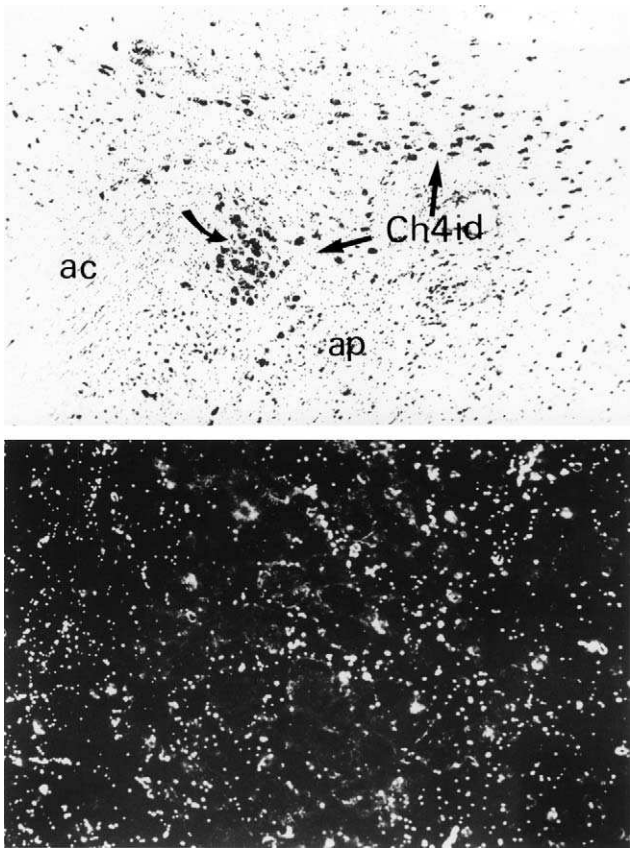

FIG. 19.32. Photomicrographs from an entorhinal radioisotope-labeled injection. (Top) Bright-field illumination shows two islands of Ch4id (straight arrows). The curved arrow points to neurons that have been magnified in the lower photomicrograph. Magnification X70. (Bottom) Dark-field illumination shows the silver grain label as white dots. This label surrounds the Ch4id perikarya indicated by the curved arrow in the top photomicrograph. Magnification X500. From Mesulam and Mufson (1984), with permission.

(Fig. 19.14; Mesulam *et al*., 1983b), respectively. Several reports demonstrated a significant reduction in both cholinergic basal forebrain neurons (Whitehouse *et al*., 1982) and cortical ChAT activity (DeKosky *et al*., 1998) in human aging. Price and co-workers (1991) noted that aged nondemented cases displayed remarkably few neurofibrillary tangles within the cholinergic neurons of the nucleus basalis, whereas mildly demented cases displayed increased tangle density. These data suggest that pathologic abnormalities co-occur with changes in cognition. However, the strongest evidence that the cholinergic basal forebrain system plays a pivotal role in human cognitive function is derived from clinicopathologic studies of this region in patients with AD (e.g., Whitehouse *et al*., 1983; Allen *et al*., 1988; Mufson *et al*., 1989a). Intensive study of the cholinergic basal forebrain system has been fueled by these findings and the observation that cholinergic deficits in AD were thought to occur early in the disorder (Wilcock *et al*., 1982) and correlate with severity (Wilcock *et al*., 1982) and duration (Mufson *et al*., 1989b) of the disease process. The extensive degeneration of cholinergic basal forebrain neurons in AD is accompanied by an increase in the number of NADPH-d neurons (Benzing and Mufson, 1995) and an overexpression of the neuropeptide galanin (Chan-Palay, 1988; Mufson *et al*., 1993), which may further enhance cholinergic basal forebrain dysfunction. However, the relationship of these plastic responses to the loss of cholinergic neurons is not well understood. Furthermore, there is also a vast animal literature linking cholinergic basal forebrain systems to normal and pathological memory function (see Bartus, 1983). Although it is clear that many brain regions degenerate in aging and AD, cholinergic hypofunction was originally thought to be central to its major symptomatology and to correlate with cognitive impairment (Bierer *et al*., 1995).

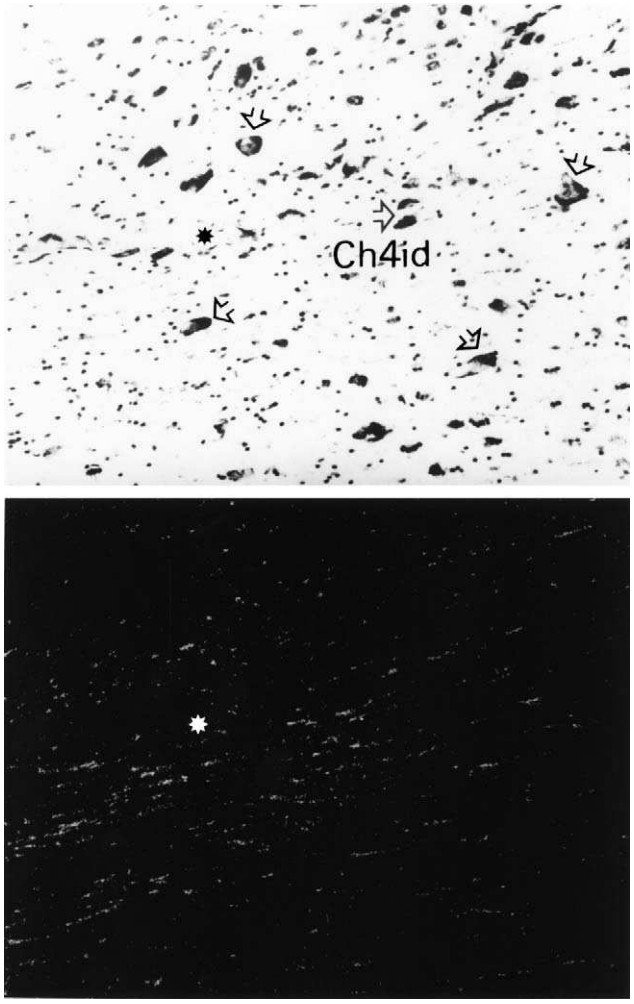

FIG. 19.33. Photomicrographs from Ch4id following a triated amino acid injection site in the midcaudal parahippocampal gyrus. The top photomicrograph was taken with bright-field illumination. Open arrowheads point to some examples of Ch4 neurons. The bottom photomicrograph shows the same area but under dark-field illumination. This makes the silver label stand out as white dots. The great majority of the label forms linear streaks and is undoubtedly within passing fibers. This was the only type of label seen in the Ch4 of this case that was therefore considered negative. In the vast majority of the other negative cases, even this linear labeling was absent from Ch4. For purposes of orientation, the arteriole in both photomicrographs has been marked with an asterisk. X266. From Mesulam and Mufson (1984), with permission.

B. Cholinergic Changes in Subjects with Early AD

1. Cortical ChAT Activity in Early AD

As mentioned previously, a major tenet of AD has been the loss of cholinergic markers, cholinergic basal forebrain cell degeneration, and the loss of ChAT and AChE activity within the cortex in postmortem AD brains (e.g., Davies and Maloney, 1976; Whitehouse *et al*., 1982). Most postmortem studies examining these changes in the basocortical cholinergic system in AD have been derived from end-stage patients with severe dementia. However, whether these deficits in cholinergic markers found in end-stage patients are also seen in subjects with much earlier disease has only been investigated recently. In fact, data derived from two different groups have provided information that changes in the cholinergic system are not severe in cases with mild cognitive impairment without dementia and early AD (Davis *et al*., 1999; Gilmore *et al*., 1999). The first data to demonstrate that cortical cholinergic levels were not altered in patients with early AD were characterized using the clinical dementia rating scale (CDR) (Davis *et al*., 1999). In this postmortem study, ChAT and AChE cortical levels did not differ significantly in subjects with CDR scores of 0 (no AD) to 2 (mild AD) in nine cortical areas. In contrast, significantly lower levels of these enzymes were found in cases with severe dementia (CDR 4–5), (Figs. 19.34 and 19.35). Similar findings have been reported from a cohort of retired Catholic clergy (DeKosky *et al*., 1998). CDR cognitive scores correlated when all groups were included in the analysis. However, when the CDR 5 scores were excluded from the analysis, the ChAT and AChE activity did not correlate significantly with cognitive scores in any cortical region examined. Interestingly, ChAT levels were significantly correlated with the severity of neuropathological lesions of AD [i.e., density of neuritic plaques and neurofibrillary tangles (NFTs)]. In summary, this study confirmed that severe reductions of cortical ChAT levels are found in end-stage but not mild AD patients.

C. Cholinergic Basal Forebrain Neuron Degeneration in Early AD

1. Overview

As indicated earlier, a second major tenet of AD is the loss of basal forebrain cholinergic neurons in end-stage patients (Whitehouse *et al*., 1982; Saper *et al*., 1985). Since the mid-1990s, numerous studies employing various markers have shown that alterations to the basocortical cholinergic system are more complex than originally proposed. For example, the vesicular acetylcholine transporter (VAChT), which is responsible for the accumulation of acetylcholine in synaptic vesicles in cholinergic axon terminals and coexpresses with ChAT within the human cholinergic basal forebrain (Fig. 19.36; Gilmore *et al*., 1999), is not severely altered in AD. In this regard, pharmacological studies of VAChT in postmortem AD tissue or *in vivo* imaging studies using vesamicol and its analogs suggest that VAChT levels remain steady or are minimally decreased coincident with a severe decline in ChAT activity in cortical areas (Ruberg *et al*., 1990). The discordance between ChAT and VAChT in the cortex is particularly surprising in light of the discovery that they are part of a single cholinergic gene locus with shared regulatory elements (for review, see Eiden, 1998). Moreover, evidence from experimental lesions in animals (Sofroniew *et al*., 1990) and from postmortem human studies (Pearson *et al*., 1983; Rinne *et al*., 1987) suggests that many cholinergic neurons shrink after injury or during the pathological process rather than degenerate. Taken together, these observations suggest that cholinergic basal forebrain neurons may be viable, albeit dysregulated, in AD.

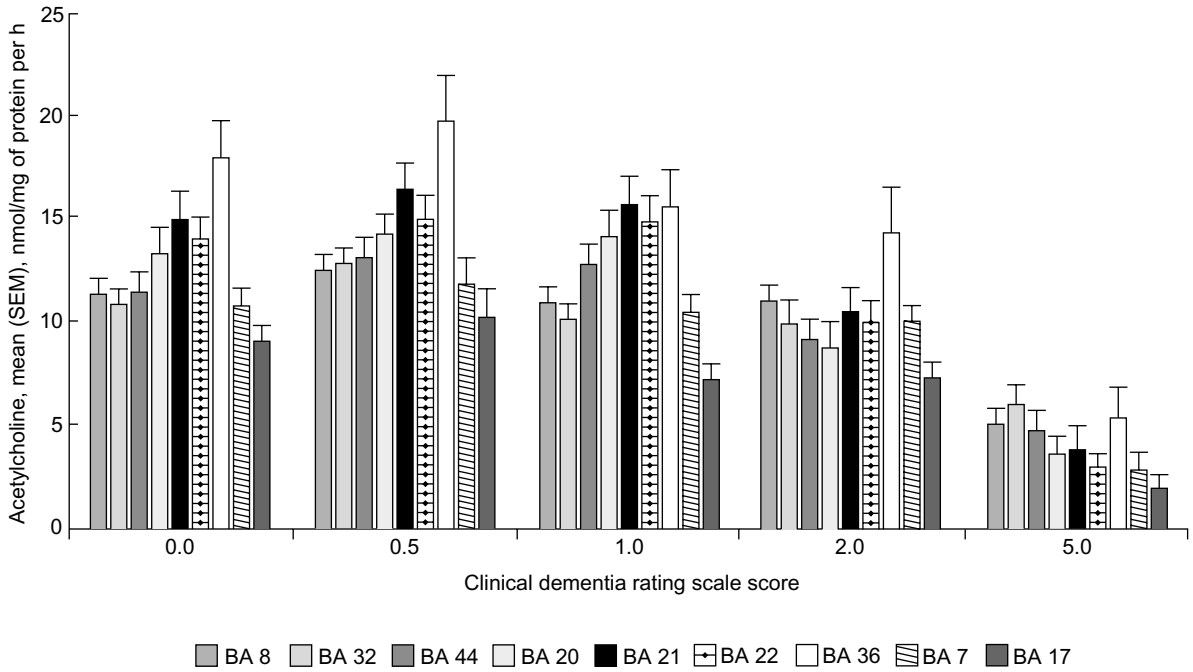

FIG. 19.34. Relative to the group without dementia [(clinical rating (CDR) scale score 0.0)], the activity of choline acetyltransferase (ChAT) was reduced significantly ($P < 0.001$ for all) in the CDR 5.0 group only. BA, Brodmann area. From Davis *et al*. (1999), with permission.

2. Cholinergic (Ch4) Cell Preservation in Early AD

To clarify the extent of cholinergic basal forebrain neuronal degeneration in the early stages of the disease process, we compared the total number of neurons containing ChAT and VAChT within the nucleus basalis of individuals classified clinically as displaying no cognitive impairment (NCI), mild cognitive impairment (MCI), or early AD. These cases are part of a longitudinal study of aging and AD of retired Catholic clergy (Gilmore *et al*., 1999; Mufson *et al*., 1999). Stereologic

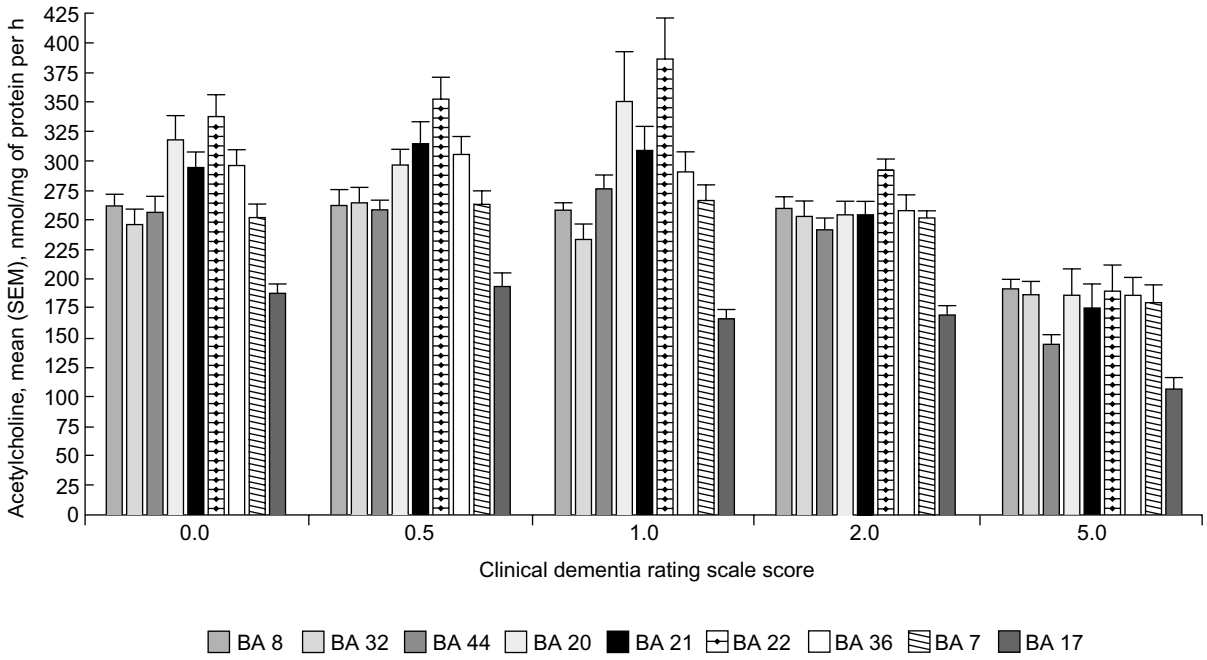

FIG. 19.35. Relative to the group without dementia [clinical rating (CDR) scale score 0.0], the activity of acetylcholinesterase (AChE) was reduced significantly ($P < 0.001$ for all) in the CDR 5.0 group only. BA, Brodmann area. From Davis *et al*. (1999), with permission.

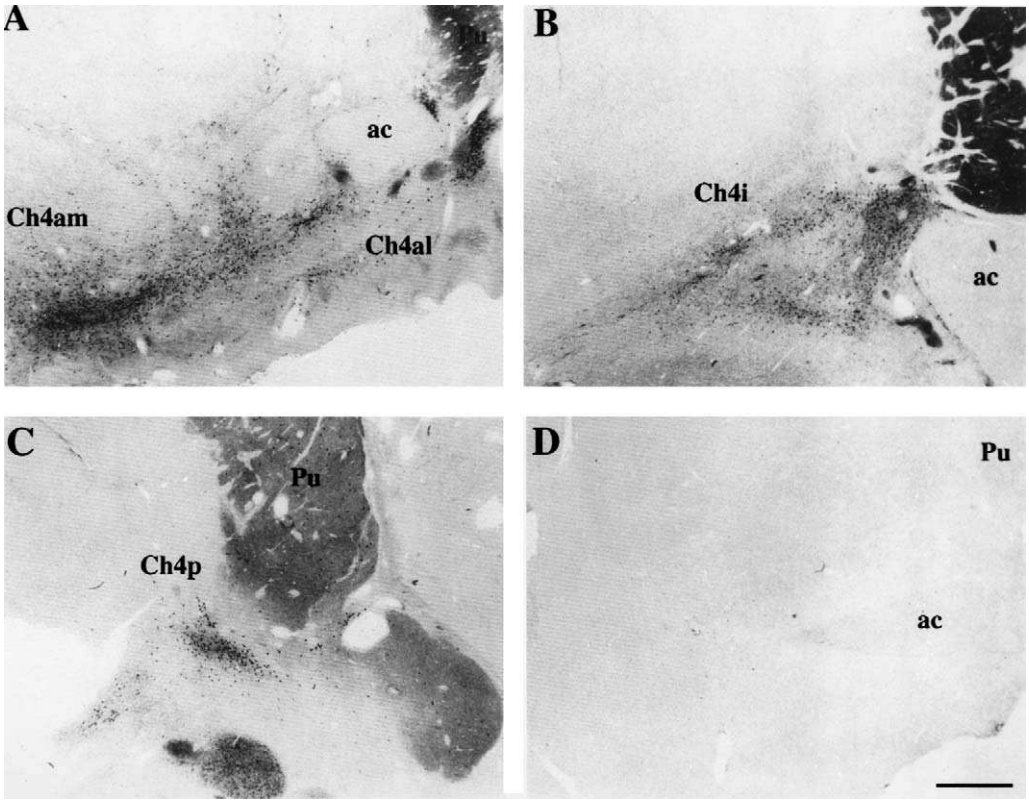

FIG. 19.36. Immunocytochemical localization of vesicular acetylcholine transporter-immunoreactive neuron immunoreactivity in the human nucleus basalis of Meynert. Shown are representative stained sections from rostral (A), intermediate (B), and caudal (C) levels of the basal forebrain from individuals with no cognitive impairment. The subdivisions of the nucleus correspond to those described previously using choline acetyltransferase-immunoreactive neurons to mark the cholinergic neurons (Mesulam and Geula, 1988). Also note the high density of immunoreactivity in the putamen and amygdala. (D) Staining was abolished when the primary antibody was omitted. ac, anterior commissure; Ch4am, anteromedial cell group; Ch4al, anterolateral cell group; Ch4i, intermediate cell group; Ch4p, posterior cell group; Pu, putamen. Scale bar: 2 mm. From Mufson *et al*. (1998), with permission.

counting methods were used to count the number of ChAT- and VAChT-immunoreactive neurons from tissue derived from these three groups (Gilmore *et al*., 1999). Both markers were expressed robustly in nucleus basalis neurons and across all three cognitive groups (Fig. 19.36). On average, there was no significant difference between the number of ChAT (210,000)- and VAChT (174,000)-immunopositive neurons in the nucleus basalis (Ch4) per hemisphere in NCI (see Table 19.1). There was a nonsignificant reduction in the number of cholinergic neurons in Ch4 in AD cases with no decline in MCI cases (Fig. 19.37; Table 19.1). The number of ChAT- and VAChT-immunopositive neurons was shown to significantly correlate with the severity of dementia determined by scores on the Mini-Mental State Examination, but showed no relationship to apolipoprotein E4 (ApoE) allele status, age, gender, education, or postmortem interval when all clinical groups were combined or evaluated separately. These data indicate that ChAT- and VAChT-immunoreactive neurons are relatively preserved in early stages of AD. These findings suggest that nucleus basalis neurons are capable of a compensatory increase in ChAT during the early stages of AD, which is reflected by no apparent loss of cortical ChAT activity early in the disease processes (Davis *et al*., 1999). An important therapeutic implication of these findings is that survival of the basal forebrain cholinergic neurons in early stages of the disease provides an opportunity for interventions aimed at restoring or maintaining function.

TABLE 19.1 ChAT- and VAChT-Immunoreactive Neurons in the Nucleus Basalis of Meynert[a]

Cases	ChAT (mean ± standard deviation)	VAChT (mean ± standard deviation)
NCI	210,540 ± 15,240 (n=6)	174,000 ± 12,773 (n=6)
MCI	167,879 ± 17,903 (n=7)	192,637 ± 34,737 (n=5)
AD	155,585 ± 17,949 (n=9)	149,423 ± 17,615 (n=9)

[a]ChAT, choline acetyltransferase-immunoreactive neurons; VAChT, vesicular acetylcholine transporter-immunoreactive neurons; NCI, no cognitive impairment; MCI, mild cognitive impairment; AD, Alzheimer's disease. From Mufson *et al*. (1998), with permission.

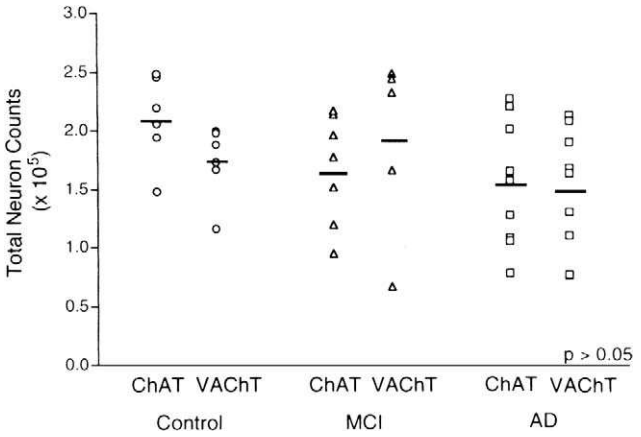

FIG. 19.37. Summary of choline acetyltransferase (ChAT) and vesicular acetylcholine transporter (VAChT) total neuron counts. No cognitive impairment (NCI) cases (O), mild cognitive impairment (MCI) cases (△), and Alzheimer's disease (AD) cases (□). Total neuron counts were estimated in the same NCI, MCI, and AD cases for ChAT and VAChT. There was no significant difference between the number of ChAT- and VAChT- immunopositive neurons for any of the three clinically diagnosed groups. Bars represent the mean. From Mufson *et al.* (1998), with permission.

XIV. Apolipoprotein E Genetics and Cholinergic Basal Forebrain Degeneration

Pathogenetic and biochemcial studies have shed new light on the involvement of the cholinergic basal forebrain system in neurobiology of AD. First, significantly lower levels of cortical and hippocampal ChAT activity and cholinergic basal forebrain neuron loss were found in AD patients with an ApoE ϵ4 genotype (Poirier *et al*., 1994; Poirier, 1994: Soininen *et al*., 1995; Allen *et al*., 1997). ApoE ϵ4 is a known genetic risk factor for AD (Saunders *et al*., 1993). These observations indicate that the ApoE ϵ4 genotype is associated with the function and integrity of the cholinergic basal forebrain system in the brain. Of particular importance are the findings that this genetic susceptibility results in subgroups of AD patients, which respond differentially to anticholinesterase agents such as tacrine (Schneider *et al*., 1996). This suggests that the ApoE ϵ4 carriers are at a greater risk for dysfunction of their ACh synthetic machinery and are therefore less likely to respond to anticholinesterease drugs. Second, it has been shown *in vivo*, that AChE, which is the degrading enzyme for ChAT, accelerates assembly of amyloid-β-peptides into AD-like fibrils (Inestrosa *et al*., 1996). Interestingly, it has been found that cortical ChAT but not AChE activity is reduced with normal aging (DeKosky *et al*., 1985). Taken together, these findings support the suggestions that (1) the presence of acetylcholine (ACh) in the neocortex plays an essential role in normal cognitive function, (2) genetic factors are associated with cholinergic basal forebrain dysfunction, and (3) defects in the cholinergic basal forebrain system potentially provide an important molecular link related to plaque formation.

XV. Cytoskeletal Abnormalities within the Cholinergic Basal Forebrain in AD

Degeneration of the cholinergic basal forebrain system has been a major tenent in the field of AD. In AD, cholinergic basal forebrain neurons display extensive NFT degeneration. These NFTs are formed by insoluble intracellular polymers of hyperphosporylated tau, the less phosporylated forms of which stabilize the microtubules of the axonal cytoskeleton (Trojanowski *et al*., 1993). To determine the extent to which cholinergic basal forebrain neurons exhibit cytoskeletal lesions, basal forebrain sections from AD and aged controls were dual stained with thioflavin-S or an antibody against paired helical filament (PHF) (Mufson *et al*., 1989b). A few senile plaques were seen scattered in the AD basal forebrain (Fig. 19.15B). Of particular interest is the observation derived from a fluorescence microscopic analysis, which revealed a large number of $p75^{NTR}$/cholinergic-immunoreactive neurons that are invested with differing degrees of neurofibrillary material (Fig. 19.38). Some neurons are thioflavin-S negative or only minimally fluorescent for this amyloid marker. Others are more heavily invested, suggesting a greater involvement in the disease process (Figs. 19.38D and 19.38E). In addition, many NFT-bearing profiles observed in the basal forebrain were ghost tangles, i.e., neurons appeared to be largely devoid of cytoplasm and organelles consisting of neurofibrillary remnants (Fig. 19.38D). This pattern of NFT expression is suggestive of a continuum of degenerative events related to the hyperphosphorylation of tau-like molecules. NFTs observed within the cholinergic basal forebrain neurons were globose in shape and fluoresced as a dense core mass (Fig. 19.38C). This is in contrast to the more wispy appearance of the NFTs observed within the cortex (Fig. 19.38A). These observations potentially represent differences in the pattern of amyloid aggregation between cortical and basal forebrain cell types. The overexpression of tau may play a crucial role in the selective degeneration of cholinergic basal forebrain neurons in AD over time.

XVI. NGF and the Cholinergic Basal Forebrain in Alzheimer's Disease

A. Overview

The trophic factor NGF specifically supports the viability and phenotypic expression of cholinergic basal forebrain neurons. The findings that NGF is synthesized mostly in cholinergic basal forebrain target regions, enhances cholinergic function both *in vitro* (Levi-Montalcini *et al*., 1954; Levi-Montalcini, 1987) and *in vivo* (Hefti *et al*., 1986; Koliatos *et al*., 1990), is specifically transported from the cortex and hippocampus to cholinergic basal forebrain consumer neurons (Connor and Varon, 1992), rescues medial septal cholinergic neurons that undergo degeneration following axotomy in rodents, young monkeys, and aged monkeys (for review, see Tuszynski *et al*., 1999), and prevents cholinergic basal forebrain atrophy and improves cognitive function in aged rats (see Fischer *et al*., 1987), support the contention that cholinergic basal forebrain neurons are exquisitely sensitive to the

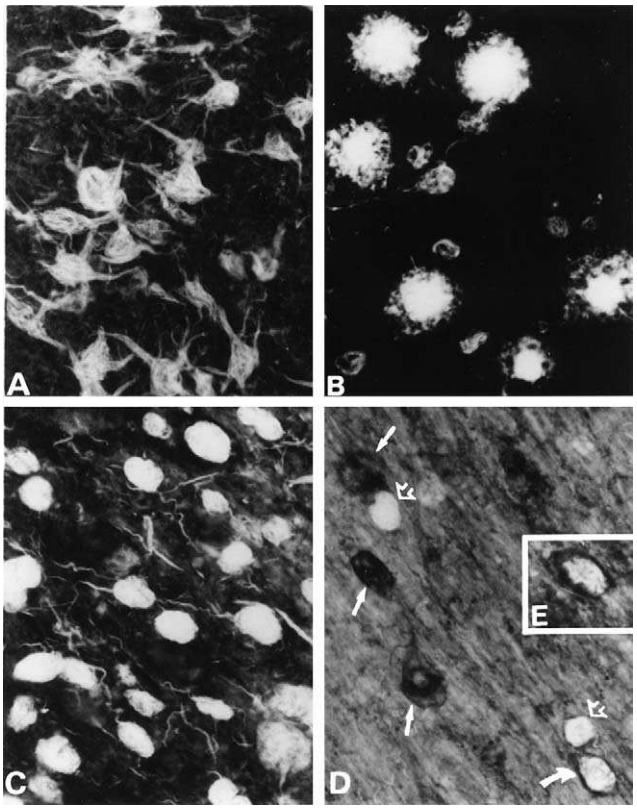

FIG. 19.38. Thioflavin-S-stained sections showing neurofibrillary tangles (NFTs) and neuritic plaques in AD patients. (A) NFTs within layer II of the entorhinal cortex. (B) Neuritic plaques located within the superior temporal cortex. (C) NFTs located within the nucleus basalis (Ch4am). (D) $p75^{NTR}$-immunoreactive nucleus basalis (Ch4al) neurons (DAB chromagen) counterstained with Thioflavin-S. Solid white arrows indicate NGF receptor-containing neurons, open arrows indicate ghost tangles (see text), and curved arrow denotes NFT-bearing NGF receptor-containing neuron. (E) Another example of a NFT-bearing NGF receptor-containing neuron. Note the dark rim of $p75^{NTR}$ immunoreactivity surrounding the fluorescent NFT staining (A–E, 380X; Ch4am, anteromedial division of the cholinergic cell group 4; Ch4al, anterolateral division of the cholinergic cell group 4). From Mufson *et al.* (1989a), with permission.

trophic influences of NGF. Furthermore, mRNA and protein for both high (trk)- and low-affinity (p75) NGF receptors are expressed by cholinergic basal forebrain neurons in monkeys and in young and aged humans (Kordower *et al*., 1988; Mufson *et al*., 1995, 1997b; Blurton-Jones *et al*., 1999) but not in the other cholinergic cell groups. These observations raise the possibility that neurotrophins and their receptors may be used therapeutically in the human to reverse the age-related cognitive decline associated with cholinergic basal forebrain degeneration in AD (Tuszynski *et al*., 1990a; Mufson *et al*., 1997b; Mufson and Kordower, 1999).

The extensive literature linking NGF processes to basal forebrain function led to the initial suggestion that impaired NGF trophic support underlies cholinergic basal forebrain degeneration in AD (Hefti and Weiner, 1983; Mufson *et al*., 1999). Subsequent studies primarily examining NGF message levels in cerebral cortex and the expression of protein and mRNA for the low-affinity $p75^{NTR}$ within the cholinergic basal forebrain failed to support this initial hypothesis (Mufson *et al*., 1997b; Mufson and Kordower, 1999) and enthusiasm for this concept diminished. While cortical NGF synthesis is not impaired in AD (Crutcher *et al*., 1993; Scott *et al*., 1995), it is important to keep in mind that NGF exerts trophic influences on cholinergic basal forebrain neurons via a series of complex events, including NGF synthesis, trkA receptor synthesis and anterograde transport, NGF binding to its high-affinity *trk* receptor, retrograde transport of the NGF/receptor complex from the cerebral cortex and hippocampus to cholinergic basal forebrain neurons, and autophosphorylation of the NGF signal. Failure at any of these steps could result in cholinergic basal forebrain degeneration secondary to impaired NGF trophic support. In this regard the low-affinity receptor may induce apoptotic events (Rabizadeh *et al*., 1998) by acting as a death receptor if the ratio of trkA to $p75^{NTR}$ levels is not in balance. Interestingly, even though there is not a change in cortical NGF levels (Crutcher *et al*., 1993; Scott *et al*., 1995), there is a reduction of retrogradely transported NGF by cholinergic basal forebrain consumer neurons in AD (Mufson *et al*., 1994). In aging, the two receptors most likely remain in equilibrium and continually transport NGF to cholinergic basal forebrain neurons, maintaining their survival and maintenance. By comparison to AD, there is a reduction in trkA protein and mRNA, a stability of $p75^{NTR}$ and cortical NGF. This led us to postulate that a discordance between trkA and $p75^{NTR}$ may result in a cascade of events leading to the degeneration of cholinergic basal forebrain neurons such as the induction of apoptosis (Rabizadeh *et al*., 1998) or the formation of NFTs.

XVII. Cholinergic Basal Forebrain and Experimental Therapeutics

A. Overview

Since the 1980s, numerous studies have established a link between the cholinergic basal forebrain and higher order cognitive function. The question has now arisen as to whether the cholinergic basal forebrain is involved specifically in attentional or memory processes (Voytko, 1996). However, it remains clear that an intact basal forebrain system is required for normal higher order informational processing.

Initial reports demonstrating reduced cholinergic markers within the cortex of AD patients (Davies and Maloney, 1976) led to intense interest in the source of this innervation in the cholinergic basal forebrain system. Cholinergic neurons within this region atrophy and degenerate in AD (Whitehouse *et al*., 1982; Doucette and Ball, 1987; Mufson *et al*., 1989b; Kordower *et al*., 1989a). There are a number of important concerns when considering which system to target with experimental therapeutic strategies. Is this system impaired when the symptoms first appear? Does this system continue to degenerate as the disease progresses? Does failure of this system in animal models mimic what is observed in humans? The answer to these types of questions furthers our interest in targeting the cholinergic basal forebrain with novel therapeutic interventions. The cortical cholinergic deficit has been reported to be present within 1 year of symptom onset and remains the neurotransmitter system that best correlates with the severity

and duration of this illness (Bierer *et al*., 1995). However, it should be noted that nondemented individuals with mild cognitive impairment do not display deficits in cortical cholinergic markers (Davis *et al*., 1999).

An extensive animal literature also indicates that an intact cholinergic basal forebrain is needed for normal cognition (Bartus *et al*., 1982; Dunnett and Fibiger, 1993, Wainer *et al*., 1993). Lesions of the cholinergic basal forebrain system in rodents and monkeys induce long-lasting impairments of trial-dependent and trial-independent memory tasks (Bartus *et al*., 1982; Dunnett and Fibiger, 1993). In addition to lesion studies, pharmacological experiments indicate that an intact cholinergic system is necessary for normal cognition. Administration of cholinergic antagonists to rodents, monkeys, and humans consistently impairs short-term memory (Bartus *et al*., 1982; Dunnett and Fibiger, 1993; Wainer *et al*., 1993). Conversely, administration of cholinergic agonists to aged rodents and aged nonhuman primates enhances cognitive performance (Bartus *et al*., 1982; Dunnett and Fibiger, 1992; Wainer *et al*., 1993). These data support the concept that the cholinergic deficit mediates, in part, the memory deficits seen in AD.

Because the loss of cholinergic neurons induces impairments on tasks of cognition and attention in rodents, monkeys, and humans, neuroprotective drug strategies and neural replacement strategies have been employed experimentally in an attempt to sustain and/or enhance cognitive function on the one hand (neuroprotection) or reverse cognitive deficits on the other (neural replacement). For neuroprotection, the molecule that has received the most empirical attention is the trophic factor NGF. We have already detailed the association of cholinergic basal forebrain neurons with NGF systems. The steroid estrogen has also received a great deal of attention as a possible treatment strategy for the slowing of the onset and risk of AD in women (Tang *et al*., 1996; Kawas *et al*., 1997).

B. Neuroprotection by NGF in Models of Cholinergic Degeneration

As an initial test of the hypothesis that NGF can prevent the degeneration of the cholinergic basal forebrain neurons, three groups virtually simultaneously demonstrated that intraventricular administration of NGF could completely prevent the death of cholinergic basal forebrain neurons that would normally result from transection of the fimbria fornix (Hefti, 1986; Gage *et al*., 1988). In the absence of NGF, control animals receiving fimbria-fornix transection demonstrate a 75% loss of ChAT neurons. In contrast, animals that received injections or continuous infusions of NGF into the ventricular system of the brain at the time of the lesion showed virtually no loss of ChAT labeling in cholinergic basal forebrain neurons. These dramatic findings provided direct evidence for the first time that a single molecule, NGF, was capable of preventing what had heretofore been thought to be an irreversible loss of neurons after injury. Subsequent experiments examined the effects of NGF on the entire population of cholinergic basal forebrain neurons. Like the fimbria-fornix model, studies consistently demonstrated that structural and functional changes seen following lesions of the nucleus basalis, which provides the entire neocortex with cholinergic input, could be reversed by NGF treatment (Liberini *et al*., 1993). NGF was found to influence the entire population of cholinergic basal forebrain neurons in the medial septal region (Ch1), vertical limb of the diagonal band (Ch2), and nucleus basalis (Ch4), cementing its role as an important neurotrophic factor for this neuronal population. In addition to the ability of NGF to reverse lesion-induced deficits, a critical study performed by Fischer and co-workers (1987) revealed that NGF could also prevent age-related *spontaneous* atrophy of cholinergic basal forebrain neurons and the associated cognitive deficits displayed by a subset of aged rats on the Morris water maze. Thus NGF neuronal protection was not merely evident following experimental trauma to neurons, but also exerted a protective effect in the natural biological process of neuronal loss with aging.

As this technology marched toward a clinical trial, follow-up studies virtually identical to the ones performed in rats were carried out in nonhuman primates (Tuszynski *et al*., 1990b, 1991; Koliatsos *et al*., 1990; Kordower *et al*., 1994b). Here too, transection of this projection to the hippocampus resulted in the retrograde degeneration of approximately 75% of cholinergic basal forebrain neurons. However, when monkeys received NGF infusions into the ventricular system, 80–100% of cholinergic neurons were rescued after fornix lesions.

Experiments described in the preceding section set the stage for trials of NGF therapy in AD. However, careful examination of noncognitive processes revealed that unacceptable adverse effects were directly related to the intraventricular NGF delivery. Intracerebral ventricular NGF delivery *floods* the cerebrospinal fluid pathways with NGF, delivering high concentrations into the lateral, third, and fourth ventricles and into the subarachnoid space overlying the cerebral hemispheres, brain stem, and spinal cord. Flooding of the ventricular system with this highly diffusable and potent trophic factor results in hypophagia, sprouting of sympathetic axons around the cerebral vasculature, migration and proliferation of Schwann cells in a thick pial layer that surrounds the brain stem and spinal cord, and dense sprouting of sensory axons into the proliferating Schwann cell layer (see Tuszynski *et al*., 1999, for review). Adult and aged monkeys received 75% of basal forebrain cholinergic neurons, whereas monkeys that received NGF-secreting, autologous genetically modified cell grafts showed an average loss of immunolabeling in only 28% of cholinergic neurons. The grafted monkey with the largest and most accurately targeted NGF-producing transplant showed a loss of only 8% of immunolabeled cholinergic neurons. Critically, monkeys receiving intraparenchymal NGF grafts do not experience weight loss, Schwann cell migration into the central nervous system, or abnormal sprouting of sympathetic and sensory systems. Transgene expression as determined by NGF ELISA protein measurements is maintained for at least 8 months *in vivo* in the primate brain. Thus, gene therapy is an effective means of delivering NGF to the large primate brain, rescuing cholinergic neurons without inducing adverse effects. Animals receiving intraventricular grafts of NGF-producing cells also displayed lethargy and an apparent pain syndrome (Emerich *et al*., 1994; Kordower *et al*., 1994b). Finally, two AD patients received intraventricular NGF infusions in Sweden several years ago, and although reports on these patients are limited (Olson *et al*., 1992), they apparently developed pain syndromes that were likely related to Schwann

cell and sensory axon growth. Some of the clinical and preclinical effects were reversed when NGF treatment was discontinued. However, it is likely that chronic NGF treatment would be needed for patients with AD, making this route of neurotrophin delivery untenable, requiring the development of other site-specific means of NGF delivery.

1. Gene Therapy

a. Overview. Relatively recent advances in molecular biology have introduced gene therapy as a potential means of delivering substances to the nervous system. Gene therapy offers the prospect of providing neurotrophic factors to the brain intraparenchymally in a well-targeted, regionally restricted, long term, and safe manner. Two approaches to gene therapy have been utilized to date in animal models and in human clinical trials: (1) *In vivo* gene therapy refers to the genetic alteration of host cells *in vivo* using viral-derived vectors that insert DNA directly into a targeted brain region, whereas (2) *ex vivo* gene therapy refers to the genetic alteration of dividing cells in the culture dish, followed by the transplantation of these genetically altered host cells into specific nervous system sites (see Tuszynski *et al.*, 1999, for review). In both approaches, the genetically modified cells act as biological "minipumps" that can deliver substances such as NGF to precise brain targets.

b. Ex Vivo Gene Therapy. Gage and co-workers (1988) pioneered gene delivery of NGF using *ex vivo* technology. In these studies, fibroblasts were modified genetically to produce human NGF at a rate of approximately 10 ng of human NGF/10^6 cells/day, whereas nonmodified fibroblasts produced no detectable NGF (Rosenberg *et al.*, 1988). This amount exceeds physiological levels of NGF production in the adult brain by approximately 500-fold. Thus, *in vitro* prior to transplantation to the brain, host cells can be modified genetically to produce large amounts of human NGF. To test the ability of NGF gene therapy to rescue degenerating basal forebrain cholinergic neurons, primary fibroblasts modified genetically to produce and secrete human NGF were transplanted to the rat septal nucleus after fimbria-fornix lesions (Rosenberg *et al.*, 1988). Control animals received identical lesions and grafts of fibroblasts transduced to produce a nonneurotrophic factor gene. Whereas the lesion induced the degeneration of approximately 75% of cholinergic neurons in control animals, rats that received grafts of NGF-producing fibroblasts showed a loss of only 5% of neurons. Thus, the transplantation of genetically modified cells to the brain was an effective means of preventing injury-induced cholinergic neuronal degeneration. In subsequent experiments, rats with spontaneous age-related atrophy of basal forebrain cholinergic neurons and associated deficits in learning and memory received NGF-producing fibroblasts to the nucleus basalis region of the cholinergic basal forebrain. Animals that received NGF-producing cells, but not control, nonmodified fibroblasts, showed reversal of mnemonic deficits and improvement of cholinergic neuronal morphology (Chen and Gage, 1995). Subsequently, other investigators replicated these findings using grafts of other genetically modified cell types (Martinez-Serrano *et al.*, 1995).

To determine whether gene therapy with human NGF was a practical means of protecting neurons in the larger primate brain, the degeneration of basal forebrain cholinergic neurons in adult monkeys was induced by performing unilateral fornix transections. Monkeys then received transplants of autologous fibroblasts modified genetically to produce human NGF (Tuszynski *et al.*, 1996). Control subjects received lesions and non-genetically modified fibroblast grafts. Transplants were placed intraparenchymally, directly into the septal region of degenerating cell bodies. The placement of cells intraparenchymally maximized neurotrophin delivery to degenerating neurons and shielded other potentially NGF-responsive neurons from the neurotrophin exposure encountered by delivery techniques such as intracerebroventricular infusions. One month after the lesion, control-lesioned monkeys showed degeneration of 75% of basal forebrain cholinergic neurons, whereas monkeys that received NGF-secreting, autologous, genetically modified cell grafts showed an average loss of immunolabeling in only 28% of cholinergic neurons. The grafted monkey with the largest and most accurately targeted NGF-producing transplant showed a loss of only 8% of immunolabeled cholinergic neurons. Critically, monkeys receiving intraparenchymal NGF grafts do not experience weight loss, Schwann cell migration into the central nervous system, or abnormal sprouting of sympathetic and sensory systems. Transgene expression as determined by NGF ELISA protein measurements is maintained for at least 8 months *in vivo* in the primate brain. Thus, gene therapy is an effective means of delivering NGF to the large primate brain, rescuing cholinergic neurons without inducing adverse effects.

2. Encapsulated Xenografts

An alternative method to *ex vivo* gene therapy using autologous host cells is *ex vivo* gene therapy using xenografted species different from a cells that are encapsulated in biopolymers to prevent graft rejection (Emerich *et al.*, 1994; Kordower *et al.*, 1994b). The advantage of the encapsulated gene therapy approach is that banks of cells can be maintained that would be available for transplantation on short notice. Further, these cells are retrievable should problems related to the growth factor be encountered by simply removing implanted capsules. To test the ability of encapsulated, genetically modified xenografts to rescue cholinergic neurons, baby hamster kidney (BHK) cells were modified genetically to secrete high levels of human NGF (hNGF); these cells were then implanted into rhesus monkeys to determine whether they would prevent lesion-induced degeneration of cholinergic basal forebrain neurons. Following polymer encapsulation, BHK cells were grafted into the lateral ventricle of four long-tailed macaque monkeys immediately following a unilateral transection/aspiration of the fornix. Three control monkeys received identical grafts, with the exception that BHK cells were not modified genetically to secrete human NGF, thus differing by only the single gene construct (Fig. 19.39). One monkey received a fornix transection only. All monkeys displayed complete transections of the fornix as revealed by a comprehensive loss of AChE-containing fibers within the hippocampus ipsilateral to the lesion. Control monkeys, which were either nonimplanted or received non-NGF-secreting BHK cell implants, did not differ from

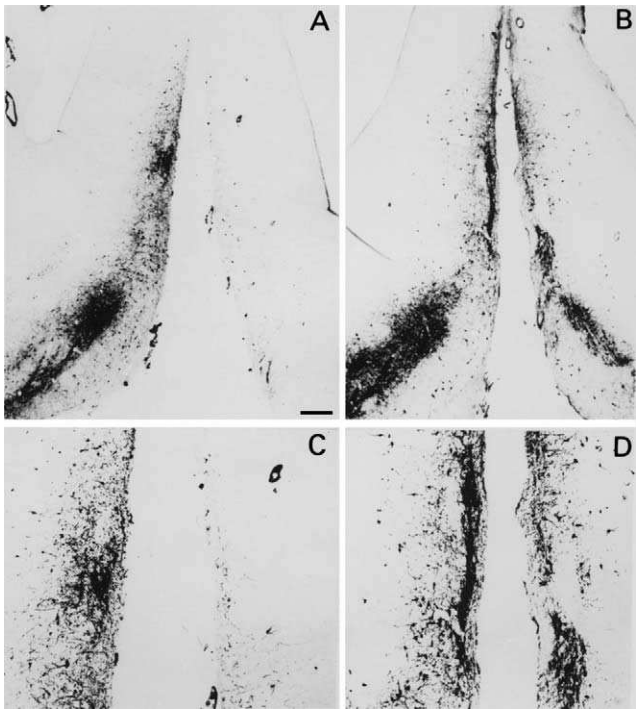

FIG. 19.39. Low (A and B)- and high (C and D)-power photomicrographs of $p75^{NTR}$-immunostained sections through the septal diagonal band complex of young adult long-tailed macaque monkeys that received unilateral fornix transections and intraventricular grafts of encapsulated BHK cells (A and C) or encapsulated BHK cells that had been modified genetically to secrete human NGF (B and D). (A and C) Note the loss of $p75^{NTR}$-immunoreactive neurons ipsilateral to the fornix transection in monkey receiving the fornix transection. (B and D). In contrast, the loss of $p75^{NTR}$-immunoreactive neurons was almost completely prevented in monkeys receiving the same lesion and the same graft with the single addition of the human NGF construct. From Emerich *et al*. (1994), with permission.

each other and displayed extensive losses of ChAT- and p75-immunoreactive neurons within the medial septum (53 and 54%) and vertical limb of the diagonal band (21 and 30%) ipsilateral to the lesion. In contrast, monkeys receiving implants of BHK–hNGF cells exhibited only a modest loss of cholinergic neurons within the septum (19 and 20%) and nucleus of the ventral limb of the diagonal band (7%). Furthermore, only grafts of human NGF-secreting cells induced a dense sprouting of cholinergic fibers within the septum that ramified against the ependymal lining of the ventricle adjacent to the transplant site. Examination of the capsules upon their removal from the animal prior to sacrifice revealed numerous healthy-appearing cells that produced detectable levels of human NGF sufficient to induced differentiation of PC12 cells in culture. These latter findings indicate that genetically modified fibroblasts can secrete biologically relevant levels of human NGF following transplantation into the primate brain.

3. Gene Therapy in Aged Primates

AD is a disease that afflicts the elderly. However, the vast majority of *in vivo* studies attempting to discover therapeutic strategies for AD patients employ young rodents or monkeys. Even rarer are studies employing aged nonhuman primates, a species that exhibits both behavioral and pathological sequelae similar to that seen in AD. To study the effects of cellular delivery of NGF upon degenerating basal forebrain neurons in an aged primate, rhesus monkeys between the ages of 24 and 29 years old received unilateral transections of the fornix. All of these monkeys displayed numerous diffuse plaques in the temporal and limbic cortices. Three aged monkeys received intraventricular transplants of polymer-encapsulated BHK fibroblasts that had been modified genetically to secrete hNGF. The additional three monkeys received identical grafts except that the cells were not modified to secrete hNGF. Monkeys receiving the fornix transection and control grafts displayed extensive reductions in the number of ChAT (57–75%)- and $p75^{NTR}$-immunoreactive (53%) medial septal neurons ipsilateral to the lesion/graft. In contrast, monkeys receiving grafts of encapsulated hNGF-secreting cells displayed only a modest loss of ChAT (0–36%)- and p75 (7–22%)-immunoreactive medial septal neurons (Fig. 19.40). Additionally, all monkeys receiving the hNGF-secreting implants, but none receiving control implants, displayed robust sprouting of cholinergic fibers within the septum ipsilateral to the graft. Just prior to sacrifice, the capsules were retrieved and determined to contain viable BHK cells releasing biologically relevant levels of hNGF. These data demonstrate that hNGF can provide trophic and tropic influences to degenerating cholinergic basal forebrain neurons in aged nonhuman primates, supporting the possibility that human NGF may prevent the degeneration of basal forebrain neurons in AD.

4. Replacement of Cholinergic Neurons by Fetal Grafts

The grafting of embryonic cholinergic neurons to replace those that are lost due to lesions or neural degeneration has been tested preclinically and provided a theoretical framework from which fetal grafting in humans with AD might be considered. Numerous studies have demonstrated that fetal cholinergic grafts survive, provide organotypic cholinergic innervation, and reverse the cognitive deficits seen in rats that are secondary to experimental lesions (fimbria-fornix transections or nucleus basalis lesions) or aging processes. The details of these studies have been reviewed extensively elsewhere (Kordower and Collier, 1999). Fetal cholinergic grafting studies have also been performed in nonhuman primates, and because this chapter focuses on the primate cholinergic basal forebrain system, they will be reviewed here.

Prior to the initiation of clinical trials, especially for behaviors as complex as cognition, we believe that experimental therapeutic strategies should be demonstrated to be safe and effective in nonhuman primate models of the disease. Unlike Parkinson's disease, where MPTP-treated monkeys represent an outstanding animal model, nonhuman primate models for AD are limited. For specifically modeling the cholinergic deficit in AD, lesion studies have been employed. Ridley and coworkers (1991, 1992, 1994) have pioneered the study of cholinergic basal forebrain transplants in monkeys with lesions of the basal forebrain system. Their initial investigations employed bilateral lesions of the fimbria fornix system (Ridley *et al*., 1991). If should be noted that, as detailed later, the

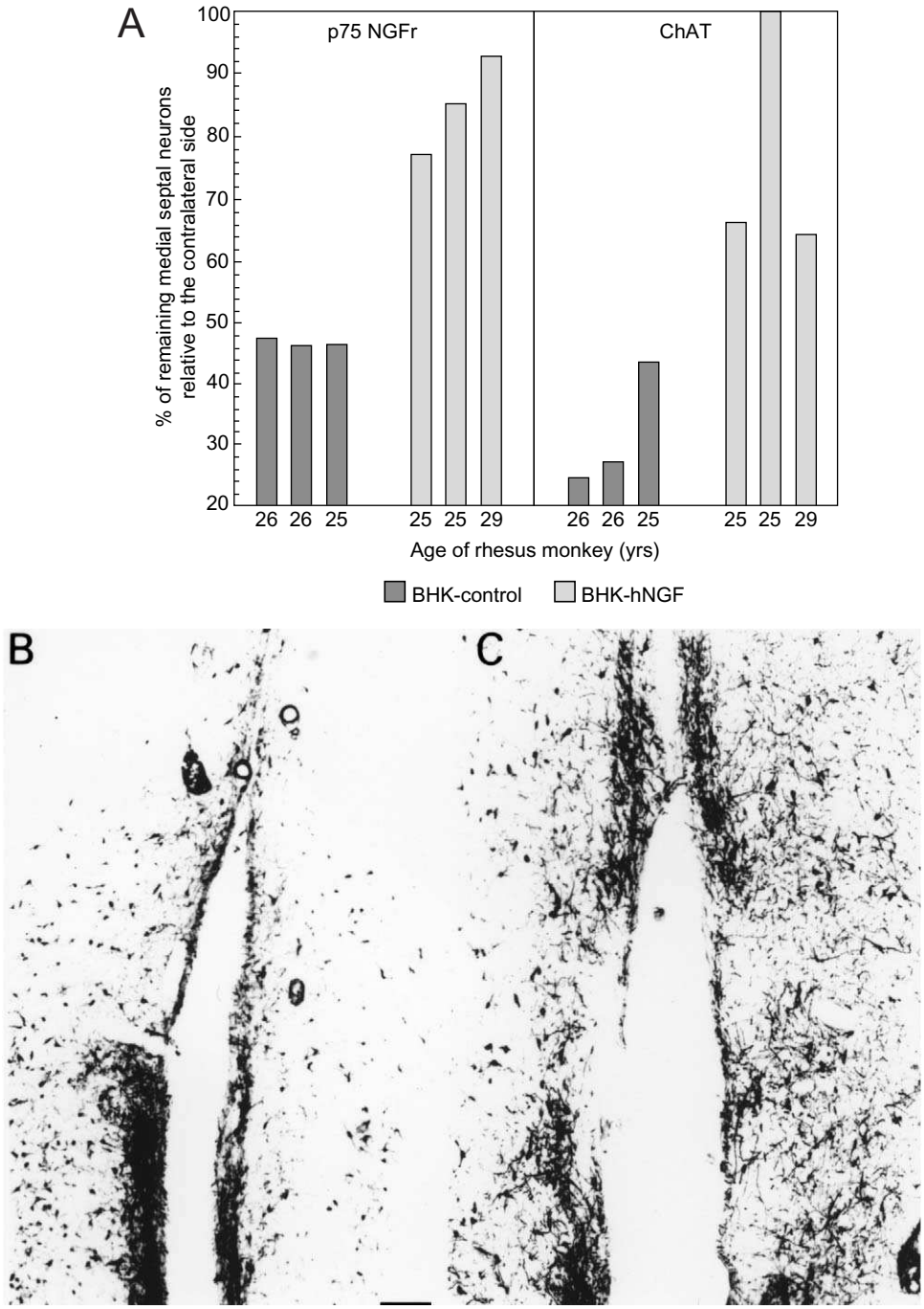

FIG. 19.40. (A) Quantification of the loss of $p75^{NTR}$- and ChAT-immunostained neurons in BHK controls and BHK–hNGF-grafted aged monkeys. (B) $p75^{NTR}$-immunostained section through the medial septum of a monkey receiving a BHK control graft. Note the extensive loss of neurons ipsilateral (right) to the lesion relative to the intact (left) side. (C) Monkeys receiving BHK–hNGF grafts displayed a symmetrical pattern of $p75^{NTR}$-immunoreactive staining within the medial septum with minimal neuronal loss (Bar: 100 μm). From Kordower *et al*. (1994b), with permission.

septohippocampal system is less affected in AD than the basocortical system and thus this lesion paradigm does not mimic the pattern of neural degeneration seen in AD. Still, given the more restricted target zone (hippocampus) and the numerous studies using this model system in rodents, this was a logical choice for initiating these investigations.

In their first study (Ridley *et al*., 1991), marmosets received bilateral transection of the fornix that resulted in impairment in function on a number of cognitive tasks, including visuospatial conditional discriminations, visual conditional discriminations, and nonconditional spatial-response tasks. These monkeys then received bilateral implants of cholinergic-rich fetal basal

forebrain tissue into the hippocampus. These grafts resulted in a significant improvement in function on these tasks 3–9 months posttransplantation. In contrast, lesioned animals receiving fetal hippocampal grafts remained impaired. These fornix-transected/control-grafted animals displayed a severe reduction in acetylcholinesterase staining throughout the dentate gyrus and hippocampus. Staining was largely restored to normal in the host hippocampus and dentate gyrus in monkeys with cholinergic transplants, whereas AChE staining was abnormal in those with noncholinergic grafts.

In a second study (Ridley *et al*., 1992), marmosets with bilateral transections of the fornix were severely impaired on learning visuospatial conditional tasks presented in a Wisconsin general test apparatus. Bilateral transplantation of cholinergic-rich embryonic basal forebrain tissue into the hippocampus led to on this task across a range of task difficulties. Administration of the direct cholinergic agonist pilocarpine to ungrafted animals immediately before testing also reduced this impairment, suggesting that the graft-associated recovery was mediated by acetylcholine release. Again, transection of the fornix produced a marked loss of AChE staining confined to hippocampus and entorhinal cortex relative to controls. In all transplanted animals, densely AChE-staining cellular masses were seen bilaterally in temporal lobe structures, with fiber outgrowth into the surrounding host tissue.

To investigate the potential benefits of fetal cholinergic basal forebrain grafts for AD, their latest study employed a model system that more closely mimics the pattern of degeneration seen in AD (Ridley *et al*., 1994). Rather than study graft effects in the septohippocampal system, these investigators examined the ability of implanted cholinergic basal forebrain neurons to improve memory function in marmosets receiving lesions of the nucleus basalis (Ch4). In this study, three groups of marmosets were trained to perform a series of visual discrimination tasks using a Wisconsin general test apparatus. Two groups then received bilateral lesions of the basal nucleus of Meynert using the excitotoxin *N*-methyl-D-aspartate. Monkeys receiving these lesions were severely impaired on relearning on a visual discrimination test. One lesioned group then received grafts of acetylcholine-rich tissue dissected from the basal forebrain of fetal marmosets. Three months later the marmosets with lesion alone remained impaired on a number of retention and reversal tasks, whereas the transplanted animals were no longer significantly impaired. Histological examination of the brains indicated that all lesioned animals had sustained substantial loss of Ch4 and that the lesion-alone animals showed marked loss of the cholinergic marker AChE in the dorsolateral and parietal cortices. All transplanted animals had surviving graft tissue as visualized by cresyl violet staining, dense AChE staining, and the presence of a limited number of nerve growth factor receptor-immunoreactive neurons in the neocortex. Five of the six cholinergic basal forebrain-transplanted marmosets showed near complete restitution of AChE staining in the frontal and parietal cortices. Examination of individual animal data showed that the one animal without a neuroanatomical recovery did not display a significant behavioral recovery. The performance of the remaining transplanted animals was significantly improved relative to animals with lesion alone. There was a significant positive correlation between the degree of AChE staining and performance on tasks sensitive to frontal lobe damage. These results demonstrate that acetylcholine-rich tissue transplanted into the neocortex of primates with damage to the cholinergic projections to the neocortex can produce substantial restitution of function, provided that an appropriate level of interaction between graft and host tissue is achieved.

For cholinergic basal forebrain grafts to be useful for AD patients, they would have to survive, innervate, and function for a long period of time. We carried out a series of fetal allografts in rhesus monkeys to assess the long-term survival in and innervation in a large primate brain (Kordower and Collier, 1999). Further, we examined whether graft viability was improved following immunosuppression with cyclosporin. Sixteen rhesus monkeys received unilateral lesions of the fornix to denervate the hippocampus of cholinergic afferents. Ten to 14 days later, these monkeys received implants of monkey fetal basal forebrain neurons into the hippocampus. All implants were between 35 and 50 days of gestation. This gestational age was chosen based on neurogenesis of the rhesus monkeys cholinergic basal forebrain (Kordower and Rakic, 1990). Monkeys were sacrificed 3, 9, 12, and 18 months following transplantation. Half of the monkeys received cyclosporin (15 mg/kg, im) daily beginning 1 day prior to the transplants and continuing until sacrifice. Viable grafts of cholinergic basal forebrain neurons were routinely observed. Importantly, large cholinergic basal forebrain grafts were observed in monkeys 12 and 18 months after transplantation (Fig. 19.1). Grafted cholinergic basal forebrain neurons appeared healthy with a magnocellular morphologically. Grafted cholinergic basal forebrain neurons displayed long multiplolar neuritic processes emanating from the cell soma.

Numerous ChAT-, trk-, $p75^{NTR}$-, and galanin-immunoreactive neurons, as well as AChE-containing perikaryon neurons, could be observed within the grafted hippocampus surviving in an organotypic fashion. Since most of these five proteins colocalize in cholinergic basal forebrain neurons, it was not surprising that similar numbers of neurons stained for each marker were seen within animals. Interestingly, an exception to this finding was one monkey with a graft surviving for 18 months, which displayed similar numbers of cholinergic basal forebrain neurons staining for all markers except for a marked diminution of ChAT-immunoreactive cells. Additionally, grafted cholinergic basal forebrain neurons sustained their expression of trk receptors, indicating that they would be responsive to NGF exposure. This is particularly important if fetal cholinergic basal forebrain grafts are supplemented by trophic factor treatment.

Fetal cholinergic basal forebrain neurons were capable of providing extensive innervation to the host hippocampus. Beginning 6–9 months after transplantation, grafts provided innervation in an organotypic pattern which by 12 months was similar in magnitude to the level seen in the normal hippocampus. These data indicate that fetal cholinergic basal forebrain neurons can survive long term following grafting into the primate brain. Qualitative observations indicate that immunosuppression did not enhance graft viability. Thus, fetal cholinergic basal forebrain neurons are capable of providing a long-term, relatively normal, cholinergic reinnervation in young adult lesioned monkeys. These data support the concept that fetal cholinergic basal forebrain grafts may be useful in treating the cholinergic deficit seen in AD.

XVIII. Estrogen as a Treatment for Cholinergic Basal Forebrain Changes in Aging and Alzheimer's Disease

Estrogen replacement in postmenopausal women decreases the risk of developing AD (Tang *et al*., 1996; Kawas *et al*., 1997). At present, very little is known where the putative neuronal action of estrogen occurs within the central nervous system. Based on studies in rodents, a possible site of action may be cholinergic basal forebrain neurons (Luine, 1985; Gibbs, 1994, 1998; Mufson *et al*., 1999), whose neurons consistently degenerate in AD (Whitehouse *et al*., 1982; Mufson *et al*., 1989a). Since the mid-1990s, a large body of data has accumulated, suggesting that estrogen may enhance cholinergic basal forebrain cell survival through interactions with the growth-promoting substance NGF and its high-affinity trkA receptor (Mufson *et al*., 1997a). In fact, several studies have shown that cholinergic basal forebrain neurons contain the trkA receptor in the rat (Sobreviela *et al*., 1994; Holtzman *et al*., 1995), monkey (Kordower *et al*., 1994b), and human (Mufson *et al*., 1997a) brain. In fact, trkA-containing neurons within the cholinergic basal forebrain coexpress ERα in the rat (McMillan *et al*., 1996). In AD, there is a downregulation in protein and message for trkA (Mufson *et al*., 1995, 1997a; Boissière *et al*., 1997) similar to that seen in ovariectomized rats (Gibbs, 1996). However, whether the cholinergic basal forebrain/NGF-responsive neurons within the primate brain also express ER is unclear. This is an important issue as studies have shown species differences in the chemoanatomical phenotype of the mammalian cholinergic basal forebrain among rodents, monkeys, and humans (Benzing *et al*., 1993; Kordower and Mufson, 1990; Kordower *et al*., 1992). Interestingly, available immunohistochemical studies indicate that ERα-positive nuclei do not correspond to ChAT-, $p75^{NTR}$- or trkA-containing cholinergic basal forebrain neurons in the monkey brain (Fig. 19.28; Blurton-Jones *et al*., 1999; Sendera *et al*., 1999). In this regard, preliminary studies in our laboratory have not revealed detectable ERα-immunopositive neurons within the human cholinergic basal forebrain (E. J. Mufson *et al*., unpublished observations). Studies from our laboratory have also failed to reveal ERα within neurons containing calbindin and parvalbumin (Sendera *et al*., 1999). These observations indicate the need for additional studies aimed at understanding the neural basis of estrogen's action within the mammalian forebrain and its role in the regulation of memory and attention. It has been shown that estrogen promotes axonal and dendritic plasticity in the limbic neurons of male and female brain (Woolley *et al*., 1996). In ovariectomized mice the impairment of reactive synaptogenesis within limbic structures is reversed by estrogen replacement (Stone *et al*., 1998). Thus, the age-related postmenopausal estrogen deficiency may suppress the potential for central nervous system neural plasticity (Mesulam, 1999), including cholinergic basal forebrain cortical projection neurons. Findings generated from further investigations of the role that estrogen plays in the central nervous system may lead to novel therapeutic treatments for the prevention of neurologic disorders such as AD.

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References

Allen, S. J., Dawbarn, D., and Wilcock, G. K. (1988). Morphometric immunochemical analysis of neurons in the nucleus basalis of Meynert in Alzheimer's disease. *Brain Res.* **454**, 275–281.

Allen, S. J., MacGowan, S. H., Tyler, S., Wilcock, G. K., Robertson, A. G., Holden, P. H., Smith, S. K., and Dawbarn, D. (1997). Reduced cholinergic function in normal and Alzheimer's disease brain is associated with apolipoprotein E genotype. *Neurosci Lett.* **239**, 33–36.

Alonso, J. R., HS, U., and Amaral, D. G. (1996). Cholinergic innervation of the primate hippocampal formation: II. Effects of fimbria/fornix transection. *J. Comp. Neurol.* **375**, 527–551.

Arendt, T., Bigl, V., and Arendt, A. (1984). Neuron loss in the nucleus basalis of Meynert in Creutzfeld-Jakob disease. *Acta Neuropathol.* **65**, 85–88.

Arendt, T., Bigl, V., Tennstedt, A., and Arendt, A. (1985). Neuronal loss in different parts of the nucleus basalis is related to neuritic plaque formation in cortical target areas in Alzheimer's disease. *Neuroscience* **14**, 1–14.

Bartus, R. T., Dean, R. L., Beer, B., and Lippa, A. S. (1982). The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217**, 408–417.

Bayer, S. (1985). Neurogenesis of the magnocellular basal telencephalic nuclei in the rat. *Int. J. Dev. Neurosci.* **3**, 229–243.

Beccari, N. (1911). La sostanza perforta anteriore e i suoi rapporti col rinencefalo dell'uomo. *Arch. Ital. Anat. Embriol.* **7**, 261–328.

Benzing, W. C., and Mufson, E. J. (1995). Increased number of NADPH-d positive neurons within the substantia innominata in Alzheimer's disease. *Brain Res.* **670**, 351–355.

Benzing, W. C., Kordower, J. H., and Mufson, E. J. (1993). Galanin immunoreactivity within the primate basal forebrain: Evolutionary change between monkeys and apes. *J. Comp. Neurol.* **336**, 31–39.

Bierer, L. M., Haroutunian, V., Gabreil, S., Knott, P. J., Carlin, L. S., Purohit, D. P., Perl, D. P., Schmeidler, J., Kanof, P., and Davis, K. L. (1995). Neurochemical correlates of dementia in Alzheimer's disease: Relative importance of cholinergic deficits. *J. Neurochem.* **64**, 749–760.

Blurton-Jones, M. M., Roberts, J. A., and Tuszynski, M. H. (1999). Estrogen receptor immunoreactivity in the adult primate brain: Neuronal distribution and association with p75, TrkA, and choline acetyltransferase. *J. Comp. Neurol.* **405**, 529–542.

Boissière, F., Faucheux, B., Ruberg, M., Agid, Y., and Hirsch, E. C. (1997). Decreased TrkA gene expression in cholinergic neurons of the striatum and basal forebrain of patients with Alzheimer's disease. *Exp. Neurol.* **145**, 245–252.

Bonner, T. I., Buckley, N. J., Young, A. C., and Brann, M. R. (1987). Identification of a family of muscarinic acetylcholine receptor genes appears in science. *Science* **237**, 527–532, (published erratum: *Ibid*., p. 237).

Bothwell, M. (1991). Keeping track of neurotrophin receptors. *Cell (Cambridge, Mass.)* **65**, 915–918.

Bowser, R., Kordower, J. H., and Mufson, E. J. (1997). A confocal microscopic analysis of galaninergic hyperinnervation of cholinergic basal forebrain neurons in Alzheimer's disease. *Brain Pathol.* **7**, 723–730.

Burton, M. J., Mora, F., and Rolls, E. T. (1975). Proceedings: Visual and taste neurones in the lateral hypothalamus and substantia innominata: Modulation of responsiveness by hunger. *J. Physiol. (London)* **252**, 50P–51P.

Candy, J. M., Perry, R. H., Thompson, J. E., Johnson, M., and Oakley, A. E. (1985). Neuropeptide localization in the substantia nigra innominata and adjacent regions of the human brain. *J. Anat.* **140**, 309–327.

Chan-Palay, V. (1988). Galanin hyperinnervates surviving neurons of the human basal nucleus of Meynert in dementias of Alzheimer's and Parkinson's disease: A hypothesis for the role of galanin in accentuating cholinergic dysfunction in dementia. *J. Comp. Neurol.* **273**, 543–557.

Chen, K. S., and Gage, F. H. (1995). Somatic gene transfer of NGF to the aged brain: Behavioral and morphological amelioration. *J. Neurosci.* **15**, 2819–2825.

Conner, J. M., and Varon, S. (1992). Distribution of nerve growth factor-like immunoreactive neurons in the adult rat brain following colchicine treatment. *J. Comp. Neurol.* **326**, 347–362.

Conner, J. M., Muir, D., Varon, S., Hagg, T., and Manthorpe, M. (1992). The localization of nerve growth factor-like immunoreactivity in the adult rat basal forebrain and hippocampal formation. *J. Comp. Neurol.* **319**, 454–462.

Côté, P. Y., and Parent, A. (1992). Calbindin D-28k and choline acetyltransferase are expressed by different neuronal populations in pedunculopontine nucleus but not in nucleus basalis in squirrel monkeys. *Brain Res.* **593**, 245–252.

Côté, P.-Y., Sadikot, A. F., and Parent, A. (1991). Complimentary distribution of calbindin D-28k and parvalbumin in the basal forebrain and midbrain of the squirrel monkey. *Eur. J. Neurosci.* **3**, 1316–1329.

Crutcher, K. A., Scott, S. A., Liang, S., Everson, W. V., and Weingarten, J. (1993). Detection of NGF-like activity in human brain tissue: Increased levels in Alzheimer's disease. *J. Neurosci.* **13**, 2540–2550.

Davies, P. and Maloney, A. J. (1976). Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet* **2**, 1403.

Davis, K. L., Mohs, R. C., Marin, D., Purohit, D. P., Perl, D. P., Lantz, M., Austin, G., and Haroutunian, V. (1999). Cholinergic markers in elderly patients with early signs of Alzheimer disease. *JAMA, J. Am. Med. Assoc.* **281**, 1401–1406.

Dawson, T. M., Bredt, D. S., Fotuhi, M., Hwang, P. M., and Snyder, S. H. (1991). Nitric oxide synthase and neuronal NADPH diaphorase are identical in brain and peripheral tissues. *Proc. Nat. Acad. Sci. U.S.A.* **88**, 7797–7801.

DeKosky, S. T., Scheff, S. W., and Markesbery, W. R. (1985). Laminar organization of cholinergic circuits in human frontal cortex in Alzheimer's disease and aging. *Neurology* **35**, 1425–1431.

DeKosky, S. T., Styren, S., Paulin, M., Cochran, E., Bennett D., and Mufson, E. J. (1998). Analysis of choline acetyltransferase activity in cortex of individuals with mild cognitive impairment and Alzheimer's disease. *Soc. Neurosci. Abstr.* **24**, 1215.

de Lacalle, S., and Saper, C. B. (1997). The cholinergic system in the primate brain: Basal forebrain and pontine-tegmental cell groups. *In* ''The Primate Nervous System'' (F. E. Bloom, A. Bjorklund, and T. Hokfelt, eds.), Vol. 13, Part I, pp. 217–262. Elsevier, Amsterdam.

de Lacalle, S., Lim, C., Sobreviela, T., Mufson, E. J., Hersh, L. B., and Saper, C. B. (1994). Cholinergic innervation in the human hippocampal formation including the entorhinal cortex. *J. Comp. Neurol.* **345**, 321–344.

DeLong, M. R. (1971). Activity of pallidal neurons during movement. *J. Neurophysiol.* **34**, 414–427.

Desjardins, C. and Parent, A. (1992). Distribution of somatostatin immunoreactivity in the forebrain of the squirrel monkey: Basal ganglia and amygdala. *Neuroscience* **47**, 115–133.

Doucette, R. and Ball, M. J. (1987). Left-right symmetry of neuronal cell counts in the nucleus basalis of Meynert of control and of Alzheimer-diseased brains. *Brain Res.* **422**, 357–360.

Dunnett, S. B., and Fibiger, H. C. (1993). Role of forebrain cholinergic systems in learning and memory: Relevance to the cognitive deficits of aging and Alzheimer's dementia. *Prog. Brain Res.* **98**, 413–420.

Ellison, D. W., Kowall, N. W., and Martin, J. B. (1987). Subset of neurons characterized by the presence of NADPH-diaphorase in human substantia innominata. *J. Comp. Neurol.* **260**, 233–245.

Emerich, D. F., Winn, S. R., Harper, J., Hammang, J. P., Baetge, E. E., and Kordower, J. H. (1994). Implants of polymer-encapsulated human NGF-secreting cells in the nonhuman-primate: Rescue and sprouting of degenerating cholinergic basal forebrain neurons. *J. Comp. Neurol.* **349**, 148–164.

Felten, D. L., and Sladek, J. R. J. (1983). Monoamine distribution in primate brain V. Monoaminergic nuclei: Anatomy, pathways and local organization. *Brain Res. Bull.* **10**, 171–284.

Fischer, W., Wictorin, K., Björklund, A., Williams, L. R., Varon, S., and Gage, F. H. (1987). Amelioration of cholinergic neuron atrophy and spatial memory impairment in aged rats by nerve growth factor. *Nature (London)* **329**, 65–68.

Fisone, G., Wu, C. F., Consolo, S., Nordstrom, O., Brynne, N., Bartfai, T., Melander, T., and Hökfelt, T. (1987). Galanin inhibits acetylcholine release in the ventral hippocampus of the rat: Histochemical, autoradiographic, in vivo, and in vitro studies. *Proc. Natl. Acad. Sci. U.S.A.* **84**, 7339–7343.

Flynn, D. D., Ferrari-DiLeo, G., Mash, D. C., and Levey, A. I. (1995). Differential regulation of molecular subtypes of muscarinic receptors in Alzheimer's disease. *J. Neurochem.* **64**, 1888–1891.

Gage, F. H., Armstrong, D. M., Williams, L. R., and Varon, S. (1988). Morphological response of axotomized septal neurons to nerve growth factor. *J. Comp. Neurol.* **269**, 147–155.

Gentleman, S. M., Falkai, P., Bogerts, B., Herrero, M. T., Polak, J. M., and Roberts, G. W. (1989). Distribution of galanin-like immunoreactivity in the human brain. *Brain Res.* **505**, 311–315.

Geula, C., Schatz, C. R., and Mesulam, M. M. (1993). Differential localization of NADPH-diaphorase and calbindin-D28k within the cholinergic neurons of the basal forebrain, striatum and brain stem in the rat, monkey, baboon and human. *Neuroscience* **54**, 461–476.

Gibbs, R. B. (1994). Estrogen and nerve growth factor-related systems in brain. Effects on basal forebrain cholinergic neurons and implications for learning and memory processes and aging. *Ann. N. Y. Acad. Sci.* **743**, 165–196.

Gibbs, R. B. (1996.) Expression of estrogen receptor-like immunoreactivity by different subgroups of basal forebrain cholinergic neurons in gonadectomized male and female rats. *Brain Res.* **720**, 61–68.

Gibbs, R. B. (1998). Impairment of basal forebrain cholinergic neurons associated with aging and long-term loss of ovarian function. *Exp. Neurol.* **151**, 289–302.

Gibbs, R. B., and Pfaff, D. W. (1992). Effects of estrogen and fimbria/fornix transection on p75NGFR and ChAT expression in the medial septum and diagonal band of Broca. *Exp. Neurol.* **116**, 23–39.

Gibbs, R. B., and Pfaff, D. W. (1994). In situ hybridization detection of trkA mRNA in brain: Distribution, colocalization with p75NGFR and up-regulation by nerve growth factor. *J. Comp. Neurol.* **341**, 324–339.

Gilmore, M. L., Erickson, J. D., Varoqui, H., Hersh, L. B., Bennett, D. A., Cochran, E. J., Mufson, E. J., and Levey, A. I. (1999). Preservation of nucleus basalis neurons containing choline acetyltransferase and the vesicular acetylcholine transporter in the elderly with mild cognitive impairment and early Alzheimer's disease. *J. Comp. Neurol.* **411**, 693–704.

Green, R. C., and Mesulam, M. M. (1988). Acetylcholinesterase fiber staining in the human hippocampus and parahippocampal gyrus. *J. Comp. Neurol.* **273**, 488–499.

Haber, S. and Elde, R. (1982). The distribution of enkephalin immunoreactive fibers and terminals in the monkey central nervous system: An immunohistochemical study. *Neuroscience* **7**, 1049–1095.

Haber, S. N., and Watson, S. J. (1985). The comparative distribution of enkephalin, dynorphin and substance P in the human globus pallidus and basal forebrain. *Neuroscience* **14**, 1011–1024.

Hayashi, M., Yamashita, A., and Shimizu, K. (1990). Nerve growth factor in the primate central nervous system: Regional distribution and ontogeny. *Neuroscience* **36**, 683–689.

Hayashi, M., Yamashita, A., Shimizu, K., Sogawa, K., and Fujii, Y. (1993). Expression of the gene for nerve growth factor (NGF) in the monkey central nervous system. *Brain Res.* **618**, 142–148.

Hefti, F. (1983). Is Alzheimer disease caused by lack of nerve growth factor? *Ann. Neurol.* **13**, 109–110.

Hefti, F. (1986). Nerve growth factor promotes survival of septal cholinergic neurons after fimbrial transections. *J. Neurosci.* **6**, 2155–2162.

Hefti, F., and Mash, D. C. (1989). Localization of nerve growth factor receptors in the normal human brain and in Alzheimer's disease. *Neurobiol. Aging* **10**, 75–87.

Hefti, F., Hartikka, J., Salviaterra, P., Weiner, W. J., and Mash, D. C. (1986). Localization of nerve growth factor receptors on cholinergic neurons of the human basal forebrain. *Neurosci. Lett.* **69**, 275–281.

Holtzman, D. M., Kilbridge, J., Li, Y., Cunningham, E. T., Jr., Lenn, N. J., Clary, D. O., Reichardt, L. F., and Mobley, W. C. (1995). TrkA expression in the CNS: Evidence for the existence of several novel NGF-responsive CNS neurons. *J. Neurosci.* **15**, 1567–1676.

Hoover, D. B., and Baisden, R. H. (1980). Localization of putative cholinergic neurons innervating the anteroventral thalamus. *Brain Res. Bull.* **5**, 519–524.

Hulme, E. C., Birdsall, N. J., and Buckley, N. J. (1990). Muscarinic receptor subtypes. *Annu. Rev. Pharmacol. Toxicol.* **30**, 633–673.

Inestrosa, N. C., Alvarez, A., Perez, C. A., Moreno, R. D., Vicente, M., Linker, C., Casanueva, O. I., Soto, C., and Garrido, J. (1996). Acetylcholinesterase accelerates assembly of amyloid-beta-peptides into Alzheimer's fibrils: Possible role of the peripheral site of the enzyme. *Neuron* **16**, 881–891.

Jing, S., Tapley, P., and Barbacid, M. (1993). Nerve growth factor mediates signal transduction through trk homodimer receptors. *Neuron* **9**, 1067–1979.

Kawas, C., Resnick, S., Morrison, A., Brookmeyer, R., Corrada, M., Zonderman, A., Bacal, C., Lingle, D. D., and Metter, E. (1997). A prospective study of estrogen replacement therapy and the risk of developing Alzheimer's disease: The Baltimore Longitudinal Study of Aging. *Neurology* **48**, 1517–1521.

Kitt, C. A., Mitchell, S. J., DeLong, M. R., Wainer, B. H., and Price, D. L. (1987). Fiber pathways of basal forebrain cholinergic neurons in monkeys. *Brain Res.* **406**, 192–206.

Koliatsos, V. E., Nauta, H. J., Clatterbuck, R. E., Holtzman, D. M., Mobley, W. C., and Price, D. L. (1990). Mouse nerve growth factor prevents degeneration of axotomized basal forebrain cholinergic neurons in the monkey. *J. Neurosci.* **10**, 3801–3813.

Kölliker, A. (1896). Handbuch der Gewebelehre des Menschen, ''Nervensystem,'' Vol. 2. Leipzig, Engelmann.

Kordower, J. H., and Collier, T. C. (1999). Cholinergic grafts in rodent and nonhuman primate models of Alzheimer's disease. *In* ''CNS Regeneration: Basic Science and Clinical Applications" (M. Tuszynski and J. H. Kordower, eds.), pp. 555–580. Academic Press, San Diego, CA.

Kordower, J. H., and Mufson, E. J. (1990). Galanin-like immunoreactivity within the primate basal forebrain: Differential staining patterns between humans and monkeys. *J. Comp. Neurol.* **294**, 281–292.

Kordower, J. H. and Rakic, P. (1990). Neurogenesis of the magnocellular basal forebrain nuclei in the rhesus monkey. *J. Comp. Neurol.* **291**, 637–653.

Kordower, J. H., Bartus, R. T., Bothwell, M., Schatteman, G., and Gash, D. M. (1988). Nerve growth factor receptor immunoreactivity in the nonhuman primate (*Cebus apella*): Distribution, morphology, and colocalization with cholinergic enzymes. *J. Comp. Neurol.* **277**, 465–486.

Kordower, J. H., Gash, D. M., Bothwell, M. A., Hersh, L. B., and Mufson, E. J. (1989a). Nerve growth factor receptor and choline acetyltransferase remain colocalized in the nucleus basalis (Ch4) of Alzheimer's patients. *Neurobiol. Aging* **10**, 67–74.

Kordower, J. H., Bartus, R. T., Marciano, F. F., and Gash, D. M. (1989b). Telencephalic cholinergic system of the New World non-human primate *(Cebus apella):* Morphological and cytoarchitectonic assessment and analysis of the projection to amygdala. *J. Comp. Neurol.* **279**, 528–545.

Kordower, J. H., Gash, D. M., Bothwell, M., Hersh, L., and Mufson, E. J. (1989c). Nerve growth factor receptor and choline acetyltransferase remain colocalized in the nucleus basalis (Ch4) of Alzheimer's patients. *Neurobiol. Aging* **10**, 287–294.

Kordower, J. H., Le, H. K., and Mufson, E. J. (1992). Galanin-immunoreactivity in the primate central nervous system. *J. Comp. Neurol.* **319**, 479–500.

Kordower, J. H., Chen, E.-Y., Sladek, J. R., Jr. and Mufson, E. J (1994a). Trk-immunoreactivity in the monkey central nervous system. *J. Comp. Neurol.* **349**, 20–35.

Kordower, J. H., Winn, S. R., Liu, Y. T., Mufson, E. J., Sladek, J. R. J., Hammang, J. P., Baetge, E. E., and Emerich, D. F. (1994b). The aged monkey basal forebrain: Rescue and sprouting of axotomized basal forebrain neurons after grafts of encapsulated cells secreting human nerve growth factor. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 10898–10902.

Kostovic, I. (1986). Prenatal development of nucleus basalis complex and related fiber systems in man: A histochemical study. *Neuroscience* **17**, 1047–1077.

Kowall, N. W., and Beal, M. F. (1989). Galanin-like immunoreactivity is present in human substantia innominata and in senile plaques in Alzheimer's disease. *Neurosci. Lett.* **98**, 118–123.

Kracun, I., and Rosner, H. (1986). Early cytoarchitectonic development of the anlage of the basal nucleus of Meynert in the human fetus. *Int. J. Dev. Neurosci.* **4**, 143–149.

Kromer, L. F. (1987). Nerve growth factor treatment after brain injury prevents neuronal death. *Science* **235**, 214–216.

Levey, A. I., Kitt, C. A., Simonds, W. F., Price, D. L., and Brann, M. R. (1991). Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. *J. Neurosci.* **11**, 3218–3226.

Levey, A. I., Edmunds, S. M., Hersch, S. M., Wiley, R. G., and Heilman, C. J. (1995). Light and electron microscopic study of m2 muscarinic acetylcholine receptor in the basal forebrain of the rat. *J. Comp. Neurol.* **351**, 339–356.

Levi-Montalcini, R. (1987). The nerve growth factor 35 years later. *Science* **237**, 1154–1162.

Levi-Montalcini, R., Meyer, H., and Hamburger, V. (1954). In vitro experiments on the effects of mouse sarcoma 180 and 37 on the spinal and sympathetic ganglia of the chick embryo. *Cancer Res.* **14**, 49–57.

Liberini, P., Pioro, E. P., Maysinger, D., Ervin, F. R., and Cuello, A. C. (1993). Long-term protective effects of human recombinant nerve growth factor and monosialoganglioside GM1 treatment on primate nucleus basalis cholinergic neurons after neocortical infarction. *Neuroscience* **53**, 625–637.

Luine, V. N. (1985). Estradiol increases choline acetyltransferase activity in specific basal forebrain nuclei and projection areas of female rats. *Exp. Neurol.* **89**, 484–490.

Martinez-Serrano, A., Fischer, W., and Björklund, A. (1995). Reversal of age-dependent cognitive impairments and cholinergic neuron atrophy by NGF-secreting neural progenitors grafted to the basal forebrain. *Neuron* **15**, 473–484.

Mash, D. C., and Potter, L. T. (1986). Autoradiographic localization of M1 and M2 muscarine receptors in the rat brain. *Neuroscience* **19**, 551–564.

Mash, D. C., Flynn, D. D., and Potter, L. T. (1985). Loss of M2 muscarine receptors in the cerebral cortex in Alzheimer's disease and experimental cholinergic denervation. *Science* **228**, 1115–1117.

McMillan, P. J., Singer, C. A., and Dorsa, D. M. (1996). The effects of ovariectomy and estrogen replacement on trkA and choline acetyltransferase mRNA expression in the basal forebrain of the adult female Sprague-Dawley rat. *J. Neurosci.* **16**, 1860–1865.

Melander, T., and Staines, W. A. (1986). A galanin-like peptide coexists in putative cholinergic somata of the septum-basal forebrain complex and

in acetylcholinesterase-containing fibers and varicosities within the hippocampus in the owl monkey *(Aotus trivirgatus). Neurosci. Lett.* **68**, 17–22.

Melander, T., Hökfelt, T., and Rokaeus, A. (1986). Distribution of galaninlike immunoreactivity in the rat central nervous system. *J. Comp. Neurol.* **248**, 475–517.

Mesulam, M. M. (1999). Neuroplasticity failure in Alzheimer's disease: Bridging the gap between plaques and tangles. *Neuron* **24**, 521–529.

Mesulam, M. M., and Geula, C. (1988). Nucleus basalis (Ch4) and cortical cholinergic innervation in the human brain: Observations based on the distribution of acetylcholinesterase and choline acetyltransferase. *J. Comp. Neurol.* **275**, 216–240.

Mesulam, M. M., and Mufson, E. J. (1984). Neural inputs into the nucleus basalis of the substantia innominata (Ch4) in the rhesus monkey. *Brain* **107**, 253–274.

Mesulam, M. M., and Van Hoesen, G. W. (1976). Acetylcholinesterase-rich projections from the basal forebrain of the rhesus monkey to neocortex. *Brain Res.* **109**, 152–157.

Mesulam, M. M., Mufson, E. J., Wainer, B. H., and Levey, A. I. (1983a). Central cholinergic pathways in the rat: An overview based on an alternative nomenclature (Ch1–Ch6). *Neuroscience* **10**, 1185–1201.

Mesulam, M. M., Mufson, E. J., Levey, A. I., and Wainer, B. H. (1983b). Cholinergic innervation of cortex by the basal forebrain: Cytochemistry and cortical connections of the septal area, diagonal band nuclei, nucleus basalis (substantia innominata), and hypothalamus in the rhesus monkey. *J. Comp. Neurol.* **214**, 170–197.

Mesulam, M. M., Mufson, E. J., Levey, A. I., and Wainer, B. H. (1984). Atlas of cholinergic neurons in the forebrain and upper brain stem of the macaque based on monoclonal choline acetyltransferase immunohistochemistry and acetylcholinesterase histochemistry. *Neuroscience* **12**, 669–686.

Mesulam, M. M., Mufson, E. J., and Wainer, B. (1986). Three-dimensional representation and cortical projection topography of the nucleus basalis (Ch4) in the macaque: Concurrent demonstration of choline acetyltransferase and retrograde trnasport with a stabilized tetramethylbenzidine method for horseradish peroxidase. *Brain Res.* **367**, 301–308.

Mesulam, M. M., Geula, C., Bothwell, M. A., and Hersh, L. B. (1989). Human reticular formation: Cholinergic neurons of the pedunculopontine and laterodorsal tegmental nuclei and some cytochemical comparisons to forebrain cholinergic neurons. *J. Comp. Neurol.* **283**, 611–633.

Meynert, T. (1872). Von Gehirn der Saugethiere. *In* "Handbuch der Lehre von der Geweben." (S. Stucher, ed.), pp. 694–808. Engelmann, Leipzig.

Mrzljak, L., Levey, A. I., and Goldman-Rakic, P. S. (1993). Association of m1 and m2 muscarinic receptor proteins with asymmetric synapses in the primate cerebral cortex: Morphological evidence for cholinergic modulation of excitatory neurotransmission. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 5194–5198.

Mrzljak, L., Levey, A. I., Belcher, S., and Goldman-Rakic, P. S. (1998). Localization of the m2 muscarinic acetylcholine receptor protein and mRNA in cortical neurons of the normal and cholinergically deafferented rhesus monkey. *J. Comp. Neurol.* **390**, 112–132.

Mufson, E. J., and Cunningham, M. G (1987). Observations on choline acetyltransferase containing structures in the CD-1 mouse brain. *Neurosci. Lett.* **84**, 7–12.

Mufson, E. J., and Kordower, J. H. (1997). Nerve growth factor and its receptors in the primate forebrain: Alterations in Alzheimer's disease and potential use in experimental therapeutics. *In* "Neuroprotective Signal Transduction" (M. P. Mattson, ed.), pp. 23–59. Humana Press, Clifton, NJ.

Mufson, E. J., and Kordower, J. H. (1999). Nerve growth factor in Alzheimer's disease. *In* "Cerebral Cortex" (A. A. Peters and J. H. Morrison, eds.), Vol. 14, pp. 681–731. Kluwer Academic/Plenum, New York.

Mufson, E. J., Martin, T. L., Mash, D. C., Wainer, B. H., and Mesulam, M. M. (1986). Cholinergic projections from the parabigeminal nucleus (Ch8) to the superior colliculus in the mouse: A combined analysis of horseradish peroxidase transport and choline acetyltransferase immunohistochemistry. *Brain Res.* **370**, 144–148.

Mufson, E. J., Benoit, R., and Mesulam, M. M. (1988a). Immunohistochemical evidence for a possible somatostatin containing amygdalostriatal pathway in normal and Alzheimer's Disease brain. *Brain Res.* **453**, 117–128.

Mufson, E. J., Mash, D. C., and Hersh, L. B. (1988b). Neurofibrillary tangles in cholinergic pedunculopontine neurons in Alzheimer's disease. *Ann. Neurol.* **24**, 623–629.

Mufson, E. J., Bothwell, M., Hersh, L. B., and Kordower, J. H. (1989a). Nerve growth factor receptor immunoreactive profiles in the normal aged human basal forebrain: Colocalization with cholinergic neurons. *J. Comp. Neurol.* **285**, 196–217.

Mufson, E. J., Bothwell, M., and Kordower, J. H. (1989b). Loss of nerve growth factor receptor-containing neurons in Alzheimer's disease: A quantitative analysis across subregions of the basal forebrain. *Exp. Neurol.* **105**, 221–232.

Mufson, E. J., Presley, L. N., and Kordower, J. H. (1991). Nerve growth factor receptor immunoreactivity within the nucleus basalis (Ch4) in Parkinson's disease: Reduced cell numbers and colocalization with cholinergic neurons. *Brain Res.* **531**, 19–30.

Mufson, E. J., Cochran, E., Benzing, W. C., and Kordower, J. H. (1993). Galaninergic innervation of the cholinergic vertical limb of the diagonal band (Ch2) and bed nucleus of the stria terminalis in aging, Alzheimer's disease and Down's syndrome. *Dementia* **4**, 237–250.

Mufson, E. J., Conner, J. M., Varon, S., and Kordower, J. H. (1994). Nerve growth factor-like immunoreactive profiles in the primate basal forebrain and hippocampal formation. *J. Comp. Neurol.* **341**, 507–519.

Mufson, E. J., Conner, J. M., and Kordower, J. H. (1995). Nerve growth factor in Alzheimer's disease: Defective retrograde transport to nucleus basalis. *NeuroReport* **6**, 1063–1066.

Mufson, E. J., Li, J.-M., Sobreviela, T., and Kordower, J. H. (1996). Decreased trkA gene expression within basal forebrain neurons in Alzheimer's disease. *NeuroReport* **8**, 25–29.

Mufson, E. J., Lavine, N., Jaffar, S., Kordower, J. H., Quirion, R., and Saragovi, H. U. (1997a). Reduction in p140-TrkA receptor protein within the nucleus basalis and cortex in Alzheimer's disease. *Exp. Neurol.* **146**, 91–103.

Mufson, E. J., Sobreviela, T., and Kordower, J. H. (1997b). Chemical neuroanatomy of the primate insula cortex: Relationship to cytoarchitectonics, connectivity, function and neurodegeneration. *In* "Handbook of Chemical Neuroanatomy: The Primate Nervous System" (F. E. Bloom, A. Björklund, and T. Hökfelt, eds.), Vol. 13, Part I, pp. 377–454. Elsevier, Amsterdam.

Mufson, E. J., Jaffar, S., and Levey, A. I. (1998). m2 muscarinic acetylcholine receptor immunoreactive neurons are not reduced within the nucleus basalis in Alzheimer's disease: Relationship with cholinergic and galaninergic perikarya. *J. Comp. Neurol.* **392**, 313–329.

Mufson, E. J., Chen, E.-Y., Cochran, E. J., Beckett, L. A., Bennett, D. A., and Kordower, J. H. (1999). Entorhinal cortex beta amyloid load in individuals with mild cognitive impairment. *Exp. Neurol.* **158**, 469–490.

Mufson, E. J., Cai, W. J., Jaffar, S., Chen, E.-Y., Stebbins, G., Sendera, T., and Kordower, J. H. (1999). Estrogen receptor immunoreactivity within subregions of the rat forebrain: Neuronal distribution and association with perikarya containing choline acetyltransferase. *Brain Res.* **849**, 253–274.

Olson, I., Norberg, A., von Holst, H., *et al*. (1992). Nerve growth factor affects 11-C nicotine hinding blood flow, EEG, and verbal episodic memory in an Alzheimer patient (case report). *J. Neural Transm.* **4**, 79–95.

Olszewski, J., and Baxter, D. (1982). "Cytoarchitecture of the Human Brain Stem." Basel.

Parada, L. F., Tsoulfas, P., Tessarollo, L., Blair, J., Reid, S. W., and Soppet, D. (1992). The trk family of tyrosine kinase-receptors for NGF-

related neurotrophins. *Cold Spring Harbor Symp. Quant. Biol.* **57**, 43–51.

Pearson, R. C., Sofroniew, M. V., Cuello, A. C., Powell, T. P., Eckenstein, F., Esiri, M. M., and Wilcock, G. K. (1983). Persistence of cholinergic neurons in the basal nucleus in a brain with senile dementia of the Alzheimer's type demonstrated by immunohistochemical staining for choline acetyltransferase. *Brain Res.* **289**, 375–379.

Pohorecki, R., Head, R., and Domino, E. F. (1988). Effects of selected muscarinic cholinergic antagonists on [^{3}H]acetylcholine release from rat hippocampal slices. *J. Pharmacol. Exp. Ther.* **244**, 213–217.

Poirier, J. (1994). Apolipoprotein E in animal models of CNS injury and in Alzheimer's disease. *Trends Neurosci.* **17**, 525–530.

Poirier J., Delisle, M.-C., Quirion, R., Aubert, I., Farlow, M., Lahiri, D., Hui, S., Bertrand, P., Nalbantoglu, J., and Gilfix, B. M. (1994). Apolipoprotein E4 allele as a predictor of cholinergic deficits a treatment outcome in Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 12260–12264.

Price, J. L., Davis, P. B., Morris, J. C., and White, D. L. (1991). The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. *Neurobiol. Aging* **12**, 295–312.

Rabizadeh, S., Oh, J., Zhong, L. T., Yang, J., Bitler, C. M., Butcher, L. L., and Bredesen, D. E. (1998). Induction of apoptosis by the low-affinity NGF receptor. *Science* **261**, 345–348.

Riccio, A., Pierchala, B. A., Ciarallo, C. L., and Ginty, D. D. (1997). An NGF-TrkA-mediated retrograde signal to transcription factor CREB in sympathetic neurons. *Science* **277**, 1097–1100.

Richardson, P. M., Verge Issa, V. M. K., and Riopelle, R. J. (1986). Distribution of neuronal receptor for nerve growth factor in the rat. *J. Neurosci.* **6**, 2312–2321.

Ridley, R. M., Thornley, H. D., Baker, H. F., and Fine, A. (1991). Cholinergic neural transplants into hippocampus restore learning ability in monkeys with fornix transections. *Exp. Brain Res.* **83**, 533–538.

Ridley, R. M., Gribble, S., Clark, B., Baker, H. F., and Fine, A. (1992). Restoration of learning ability in fornix-transected monkeys after fetal basal forebrain but not fetal hippocampal tissue transplantation. *Neuroscience* **48**, 779–792.

Ridley, R. M., Baker, J. A., Baker, H. F., and Maclean, C. J. (1994). Restoration of cognitive abilities by cholinergic grafts in cortex of monkeys with lesions of the basal nucleus of Meynert. *Neuroscience* **63**, 653–666.

Rinne, J. O., Paljarvi, L., and Rinne, U. K. (1987). Neuronal size and density in the nucleus basalis of Meynert in Alzheimer's disease. *J. Neurol. Sci.* **79**, 67–76.

Rosenberg, M. B., Friedmann, T., Robertson, R. C., Tuszynski, M., Wolff, J. A., Breakefield, X. O., and Gage, F. H. (1988). Grafting genetically modified cells to the damaged brain: Restorative effects of NGF expression. *Science* **242**, 1575–1578.

Ruberg, M., Mayo, W., Brice, A., Duyckaerts, C., Hauw, J. J., Simon, H., LeMoal, M., and Agid, Y. (1990). Choline acetyltransferase activity and [^{3}H]vesamicol binding in the temporal cortex of patients with Alzheimer's disease, Parkinson's disease, and rats with basal forebrain lesions. *Neuroscience* **35**, 327–333.

Rye, D. B., Wainer, B. H., Mesulam, M. M., Mufson, E. J., and Saper, C. B. (1984). Cortical projections arising from the basal forebrain. A study of cholinergic and non-cholinergic components employing retrograde tracing and immunohistochemical localization of choline acetyltransferase. *Neuroscience* **13**, 627–643.

Rye, D. B., Lee, H. J., Saper, C. B., and Wainer, B. H. (1988). Medullary and spinal efferents of the pedunculopontine tegmental nucleus and adjacent mesopontine tegmentum in the rat. *J. Comp. Neurol.* **269**, 315–341.

Saper, C. B., and Chelimsky, T. C. (1984). A cytoarchitectonic and histochemical study of nucleus basalis and associated cell groups in the normal human brain. *Neuroscience* **13**, 1023–1037.

Saper, C. B., and Loewy, A. D. (1982). Projections of the pedunculopontine tegmental nucleus in the rat, evidence for additional extrapyramidal circuitry. *Brain Res.* **252**, 367–372.

Saper, C. B., German, D. C., and White, C. L., III, (1985). Neuronal pathology in the nucleus basalis and associated cell groups in senile dementia of the Alzheimer's type: Possible role in cell loss. *Neurology* **35**, 1089–1095.

Satoh, K., and Fibiger, H. C. (1985). Distribution of central cholinergic neurons in the baboon *(Papio papio)*. II. A topographic atlas correlated with catecholamine neurons. *J. Comp. Neurol.* **236**, 215–233.

Saunders, A. M., Strittmatter, W. J., Schmechel, D., George-Hyslop, P. H., Pericak-Vance, M. A., Joo, S. H., Rosi, B. L., Gusella, J. F., Crapper-MacLachlan, D. R., Alberts, M. J., Hulette, C., Crain, B., Goldgaber, D., and Roses, A. D. (1993). Association of apolipoprotein E allele 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* **43**, 1467–1472.

Schambra, U. B., Sulik, K. K., Petrusz, P., and Lauder, J. M. (1989). Ontogeny of cholinergic neurons in the mouse forebrain. *J. Comp. Neurol.* **288**, 101–122.

Schatteman, G. C., Gibbs, L. Lanaham, A. A., Claude, P., and Bothwell, M. (1988). Expression of NGF receptor in the developing and adult primate central nervous system. *J. Neurosci.* **8**, 860–873.

Schneider, L. S., Farlow, M. R., Henderson, V. W., and Pogoda, J. M. (1996). Effects of estrogen replacement therapy on response to tacrine in patients with Alzheimer's disease. *Neurology* **46**, 1580–1584.

Schwaab, M. E., Otteb, U., Agid, Y., and Thoenen, H. (1979). Nerve growth factor (NGF) in the rat CNS. Absence of specific retrograde transport and tyrosine hydroxylase induction in locus coeruleus and substantia nigra. *Brain Res.* **168**, 473–483.

Scott, S. A., Mufson, E. J., Weingartner, J. A., Skau, K. A., and Crutcher, K. A. (1995). Nerve growth factor in Alzheimer's disease: Increased levels throughout the brain coupled with declines in nucleus basalis. *J. Neurosci.* **15**, 6213–6221.

Seiler, M., and Schwaab, M. E. (1984). Specific retrograde transport of nerve growth factor (NGF) from neocortex to nucleus basalis in the rat. *Brain Res.* **300**, 33–39.

Selden, N. R., Gitelman, D. R., Salamon-Murayama, N., Parrish, T. B., and Mesulam, M. M. (1998). Trajectories of cholinergic pathways within the cerebral hemispheres of the human brain. *Brain* **121**, 2249–2257.

Semba, K. and Fibiger, H. C. (1988). Time of origin of cholinergic neurons in the rat basal forebrain. *J. Comp. Neurol.* **269**, 87–95.

Sendera, T. J., Kordower, J. H, and Mufson, E. J. (1999). Estrogen receptor containing neurons in the primate brain: Association with choline acetyltransferase, GABAergic markers and substance. *Soc. Neurosci. Abstr.* **25**, 2119.

Sendera, T. J., Ma, S. Y., Jaffar, S., Kozlowski, P. B., Kordower, J. H., Mawal, Y., Saragovi, H. U., and Mufson, E. J. (2000). Reduction in trkA immunoreactive neurons is not associated with an overexpression of galaninergic fibers within the nucleus basalis in Down's syndrome. *J. Neurochem.* **74**, 1404–1412.

Shughrue, P. J., Lane, M. V., and Merchenthaler, I. (1997). Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system. *J. Comp. Neurol.* **388**, 507–525.

Simerly, R. B., Chang, C., Muramatsu, M., and Swanson, L. W. (1990). Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: An in situ hybridization study. *J. Comp. Neurol.* **294**, 76–95.

Smith, Y., Parent, A., Kerkerian, L., and Pelletier, G. (1985). Distribution of neuropeptide Y immunoreactivity in the basal forebrain and upper brain stem of the squirrel monkey (*Saimiri sciureus*). *J. Comp. Neurol.* **236**, 71–89.

Sobreviela, T., Clary, D. O., Reichardt, L. F., Brandabur, M. M., Kordower, J. H. and Mufson, E. J. (1994). TrkA immunoreactive profiles in the central nervous system: Colocalization with neurons containing p75 nerve growth factor receptor, choline acetyltransferase and serotonin. *J. Comp. Neurol.* **350**, 587–611.

Sofroniew, M. V, Galletly, N. P., Isacson, O., and Svendsen, C. N. (1990). Survival of adult basal forebrain cholinergic neurons after loss of target neurons. *Science* **247**, 338–342.

Soininen, H., Kosunen, O., Helisalmi, S., Mannermaa, A., Paljarvi, L., Talasniemi, S., Ryynanen, M., and Riekkinen, P. S. (1995). A severe loss of choline acetyltransferase in the frontal cortex of Alzheimer patients carrying apolipoprotein epsilon 4 allele. *Neurosci. Lett.* **187**, 79–82.

Steininger, T. L., Wainer, B. H. Klein, R., Barbacid, M., and Palfrey, H. C. (1993). High affinity nerve grwoth factor receptor (Trk) immunoreactivity is localized in cholinergic neurons of the basal forebrain and striatum in the adult rat brain. *Brain Res.* **612**, 330–335.

Steriade, M., Pare, D., Datta, S., Oakson, G., and Dossi, R. C. (1990). Different cellular types in mesopontine cholinergic nuclei related to ponto-geniculo-occipital waves. *J. Neurosci.* **10**, 2560–2579.

Stone, D. J., Rozovsky, I., Morgan, T. E., Anderson, C. P., and Finch, C. E. (1998). Increased synaptic sprouting in response to estrogen via an apolipoprotein E-dependent mechanism: Implications for Alzheimer's disease. *J. Neurosci.* **18**, 3180–3185.

Strada, O., Hirsch, E. C., Javoy-Agid, F., Lehericy, S., Ruberg, M., Hauw, J. J., and Agid, Y. (1992). Does loss of nerve growth factor receptors precede loss of cholinergic neurons in Alzheimer's disease? An autoradiographic study in the human striatum and basal forebrain. *J. Neurosci.* **12**, 4766–4774.

Tang, M. X., Jacobs, D., Stern, Y., Marder, K., Schofield, P., Gurland, B., Andrews, H., and Mayeux, R. (1996). Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease. *Lancet* **348**, 429–432.

Tatemoto, K., Rokaeus, A., Jornvall, H., McDonald, T. J. and Mutt, V. (1983). Galanin, a novel biologically active peptide from porcine intestine. *FEBS Lett.* **164**, 124–128.

Thoenen, H., and Barde, Y. A. (1980). Physiology of nerve growth factor. *Physiol. Rev.* **60**, 1284–1335.

Toran-Allerand, C. D., Miranda, R. C., Bentham, W. D., Sohrabji, F., Brown, T. J., Hochberg, R. B., and MacLusky, N. J. (1992). Estrogen receptors colocalize with low-affinity nerve growth factor receptors in cholinergic neurons of the basal forebrain, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 4668–4672.

Trojanowski, J. Q., Schmidt, M. L., Shin, R.-W., Bramblett, G. T., Goedert, M., and Lee, V. M.-Y. (1993). From pathological marker to potential mediator of neuronal dysfunction and degeneration in Alzheimer's disease. *Clin. Neurosci.* **1**, 184–191.

Tuszynski, M. H., HS, U., Amaral, D. G., and Gage, F. H. (1990a). Nerve growth factor infusion in the primate brain reduces lesion-induced cholinergic neuronal degeneration. *J. Neurosci.* **10**, 3604–3614.

Tuszynski, M. H., Buzsaki, G., and Gage, F. H. (1990b). Nerve growth factor infusions combined with fetal hippocampal grafts enhance reconstruction of the lesioned septohippocampal projection. *Neuroscience* **36**, 33–44.

Tuszynski, M. H., Sang, H., Yoshida, K., and Gage, F. H. (1991). Recombinant human nerve growth factor infusions prevent cholinergic neuronal degeneration in the primate brain. *Ann. Neurol.* **30**, 625–636.

Tuszynski, M. H., Roberts, J., Senut, M. C., HS, U., and Gage, F. H. (1996). Gene therapy in the adult primate brain: Intraparenchymal grafts of cells genetically modified to produce nerve growth factor prevent cholinergic neuronal degeneration. *Gene Ther.* **3**, 305–314.

Tuszynski, M. H., Gage, F. H., Mufson, E. J., and Kordower, J. H. (1999). Neurotrophic factors, gene therapy, and Alzheimer's Disease. *In* "CNS Regeneration" (M. H. Tuszynski and J. H. Kordower, eds.), Vol. 1, pp. 505–528. Academic Press, San Diego, CA.

Vincent, S. R., Satoh, K., Armstrong, D. M., and Fibiger, H. C. (1983). Substance P in the ascending cholinergic reticular system. *Nature (London)*, **306**, 688–691.

Vogels, O. J., Renkawek, K., Broere, C. A., ter Laak, H. J., and van Workum, F. (1989). Galanin-like immunoreactivity within Ch2 neurons in the vertical limb of the diagonal band of Broca in aging and Alzheimer's disease. *Acta Neuropathol.* **78**, 90–95.

Voytko, M. L. (1996). Cognitive functions of the basal forebrain cholinergic system in monkeys: Memory or attention? *Behav. Brain Res.* **75**, 13–25.

Wainer, B. H., Steininger, T. L., Roback, J. D., Burke-Watson, M. A., Mufson, E. J., and Kordower, J. (1993). Ascending cholinergic pathways: Functional organization and implications for disease models. *Prog. Brain Res.* **98**, 9–30.

Wall, S. J., Wolfe, B. B., and Kromer, L. F. (1994). Cholinergic deafferentation of dorsal hippocampus by fimbria-fornix lesioning differentially regulates subtypes (m1–m5) of muscarinic receptors. *J. Neurochem.* **62**, 1345–1351.

Whitehouse, P. J., Price, D. L., Struble, R. G., Clarke, A. W., Coyle, J. T., and Delong, M. R. (1982). Alzheimer's disease and senile dementia: Loss of neurons in the basal forebrain. *Science* **215**, 1237–1239.

Whitehouse, P. J., Hedreen, J. C., White, C. L., III, and Price, D. L. (1983). Basal forebrain neurons in the dementia of Parkinson disease. *Ann. Neurol.* **13**, 243–248.

Wilcock, G. K., Esiri, M. M., Bowen, D. M., and Smith, C. C. (1982). Alzheimer's disease. Correlation of cortical choline acetyltransferase activity with the severity of dementia and histological abnormalities. *J. Neurosci.* **57**, 407–417.

Woolley, C. S., Wenzel, H. J., and Schwartzkroin, P. A. (1996). Estradiol increases the frequency of multiple synapse boutons in the hippocampal CA1 region of the adult female rat. *J. Comp. Neurol.* **373**, 108–117.

Wynick, D., Small, C. J., Bacon, A., Holmes, F. E., Norman, M., Ormandy, C. J., Kilic, E., Kerr, N. C., Ghatei, M., Talamantes, F., Bloom, S. R., and Pachnis, V. (1998). Galanin regulates prolactin release and lactotroph proliferation. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 12671–12676.

Yasui, Y., Cechetto, D. F., and Saper, C. B. (1990). Evidence for a cholinergic projection from the pedunculopontine tegmental nucleus to the rostral ventrolateral medulla in the rat. *Brain Res.* **517**, 19–24.

Zaborszky, L., Pang, K., Somogyi, J., Nadasdy, Z., and Kallo, I. (1999). Basal forebrain corticopetal system revisted. *Ann. N. Y. Acad. Sci.* **877**, 339–367.

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20

Glutamate Receptors in Aging and Alzheimer's Disease

The discovery that the stimulation of ionotropic glutamate receptors mediates not only the physiological action of glutamate but also a neurotoxic response provided the impetus for numerous investigations that sought to determine the role of glutamate in the pathophysiology of a number of neurodegenerative diseases, including Alzheimer's disease (AD). To date, nearly 200 articles have been published that specifically focus on the integrity and/or functional properties of the glutamate receptors in aging and AD. This chapter attempts to synthesize the results of these many studies and present them in as unbiased manner as possible. The chapter is divided into four main sections: (i) overview of the glutamate receptors, (ii) glutamate receptors in the aging (rodent) brain, (iii) glutamate receptors in the AD brain, and (iv) current topics of glutamate toxicity in AD. What will become obvious following the reading of this chapter is that data are at times inconsistent, with some investigators reporting that glutamate receptors are highly vulnerable in the aged and AD brain, whereas others state that these receptors are intact or even hyperfunctional. Contributing to these inconsistencies are a host of technical issues. To address this matter, the details of a number of studies (i.e., strain, species, age, brain region investigated, postmortem interval, when applicable) are presented in tabular form (Tables 20.1–20.14). By providing this information, it is our goal that the reader will be able to make some personal assessment of the extent to which technical details, may or may not, confound the biological relevance of the findings. It is also important to bear in mind that much of our knowledge of glutamate receptors in aging and AD has come about through studies employing autoradiographic techniques to investigate the anatomical distribution and density of various ligand-specific binding sites. Although these studies have been of considerable value in defining the role of glutamate in the aging brain and AD, it is clear from our knowledge of the molecular biology of the glutamate receptor that specific techniques are required allowing for the identification of individual glutamate receptor subtypes. Studies employing these latter techniques are currently underway in several laboratories and while much of the data remain unpublished it is clear that these works will play a critical role in the future in defining glutamates participation as an excitotoxic agent in the aging brain and AD.

I. Introduction

For many years it has been known that in Alzheimer's disease (AD), and during normal aging, specific populations of neurons are more susceptible to pathologic insult than others. With this knowledge, investigators have sought to determine those molecular determinants that distinguish vulnerable neurons from those more resistant. While investigating potential mechanisms underlying neuronal vulnerability, it is important to consider that glutamate is likely the most abundant excitatory neurotransmitter in the brain. Importantly, the main physiological role of glutamate is to act at excitatory synapses. However, the response of a cell to glutamate depends on a host of variables, including the abundance and molecular composition of the various glutamate receptors expressed by the cell. In this same context, it is important to bear in mind that the neurotoxic action of glutamate is triggered by the stimulation of many of the same ionotropic receptors that mediate the physiological action of this neurotransmitter (Choi, 1988). Nevertheless, neurons within the same or even different brain region are not equally susceptible to glutamate toxicity. It appears that excitotoxicity follows the abusive stimulation of *N*-methyl-D-aspartate (NMDA) and non-NMDA ionotropic glutamate receptors that are permeable to Ca^{2+}. Although traditionally NMDA receptors have been considered to play the dominant role in the mediation of excitotoxic injury, a growing body of evidence affirms the important contribution of α-amino-3-hydroxy-5-methyl-4-isoaxolepropionate (AMPA)/kainate receptors with high permeability to calcium.

As a result of the putative role of glutamate in the pathophysiology of a number of neurodegenerative diseases, a wealth of studies have emerged in an attempt to define the integrity and/or functional properties of these receptor subtypes during normal aging and in disease. Data that have emerged from these

studies are, at times, highly inconsistent, thus contributing to a number of controversies concerning the role of glutamate during disease and normal aging. To our knowledge, few, if any, comprehensive reviews on this subject exists and as a result we have attempted to compile and present the results of the majority of those studies investigating the role of glutamate, particularly the glutamate receptor, in aging and AD. This work is divided into four parts: (i) overview of the glutamate receptor, (ii) glutamate receptors in the aging brain, (iii) glutamate receptors in the Alzheimer's brain, and (iv) current topics of glutamate toxicity in Alzheimer's disease. This chapter is limited to ionotropic glutamate receptors, and while every effort has been made to include all relevant publications, it is inevitable that some works are overlooked and to those authors whose work was excluded an apology is extended.

II. Overview of the Glutamate Receptors

Glutamate is a dicarboxylic acid that is stored in synaptic vesicles and is released from the nerve terminal following neuronal stimulation. Glutamate fulfills all criteria of a chemical neurotransmitter in the vertebrate central nervous system (CNS) (Fonnum, 1991) and is the most abundant excitatory neurotransmitter present in the mammalian brain. The release of glutamate and its interactions with specific membrane receptors is responsible for many important neurologic functions, including cognition and memory. While glutamate is traditionally considered to function as a rapidly acting neuronal transmitter, it too possesses the potential of producing long-lasting changes in neuronal excitability, such as the appearance of long-term potentiation (LTP) following the stimulation of hippocampal neurons. Under physiological conditions the interaction of glutamate with its receptor is relatively brief, and within a millisecond or less specific uptake mechanisms and/or degradative enzymes begin to clear glutamate from the synaptic cleft. However, if the natural order of these processes is overwhelmed, e.g., by the excessive release of glutamate due to uncontrolled transmitter release following neuronal damage, glutamate then accumulates in persistently high concentrations at the synaptic cleft, resulting in the continuous and protracted binding of glutamate to its receptor, leading to neuronal damage or death. This process, also termed ''excitotoxicity'' (Olney, 1990), is believed to be operative in several acute and chronic neurodegenerative diseases, including AD.

Notably, the responsiveness of a neuron to glutamate depends to a large extent on the repertoire of glutamate receptors expressed by the cell. Historically, the pharmacological profile of glutamate receptors has been delineated based on their ability to be activated selectively and specifically by synthetic agonists. In brief, ionotropic glutamate receptors are divided into a class of NMDA-selective and another class of non-NMDA-selective receptors (for review, see Seeburg, 1993). The latter class is subdivided into kainate and AMPA-sensitive receptors. In addition, in mammalian brain a family of metabotropic receptors exists that differs functionally and pharmacologically from ionotropic receptors. For example, these latter receptor subtypes often are coupled to the G-protein/inositol phosphate (IP)/Ca^{2+} signaling pathway (Nakanishi, 1992). Although the study of metabotropic receptors and their role in neurotoxicity is an emerging area of investigation (Lipartiti *et al*., 1993), this chapter focuses largely on the ionotropic glutamate receptor because functionally they account for the majority of the excitotoxic responses to glutamate.

cDNAs encoding subunits of various ionotropic glutamate receptor subunits have been cloned (Seeburg, 1993). These studies have revealed that ionotropic glutamate receptors are formed from five main families of genes, with each gene encoding an individual subunit of heterooligomeric complexes acting as ligand-gated conveyors of ion fluxes for the receiving cells (reviewed in Seeburg, 1993). One family of genes encodes subunits that form AMPA-selective receptors (GluR1-4 or, alternatively, GluR-A, -B, -C, -D), two families encode kainate-selective subunits (GluR5-7, KA1, and KA2), and two encode NMDA-selective subunits (NMDAR1 and NMDAR2A-D). In addition, all four AMPA receptor subunits occur in two alternatively spliced variants (designated flip and flop) that are encoded by exons 14 and 15 (GluR2) positioned just before the M4 domain (see later). Whereas both spliced variants are expressed in the adult brain, the flip variant predominates in pre- and neonatal rat brains (Sommer *et al*., 1991). Functionally, the flip forms of most subunits densensitize more slowly and to a less extent than the flop forms. GluR2, GluR4, and the kainate receptor subunits all express carboxy-terminal splice variants. In the rat, GluR5 displays four different carboxy-terminals (designated GluR5-1, GluR5-2, GluR5a, and GluR5b), whereas GluR6 and GluR7 each display two splice variants that differ in the carboxy-terminal (GluR6-1, GluR6-2; GluR7a, GluR7b). To date, relatively little is known about the functional differences among the various splice variants of the kainate receptor except for the finding that the GluR7a subunit, when expressed as homomeric receptors in HEK 293 cells, gives rise to significantly larger currents than receptors assembled from the GluR7b subunit (Schiffer *et al*., 1997). The NR1 subunit exists as eight splice variants (designated NR1-1a,b; NR1-2a,b; NR1-3a,b; NR1-4a,b), which assemble to form receptors of distinct functional properties. Information in the cassettes that are spliced into or out of NR1 appears to be vital to the regulation (e.g., phosphorylation) and localization (e.g., synaptic vs nonsynaptic) of the assembled receptor (Durand *et al*., 1993; Ehlers *et al*., 1995). For example, Ehlers *et al*. (1995) have demonstrated that NR1 subunits expressed in transiently transfected cells are localized predominantly either in the cytoplasm or on the cell surface, depending on which splice variant is used. Thus, variants that contain the C1 exon cassette, a 37 amino acid insert in the carboxy-terminal region of NR1, are targeted to the plasma membrane, whereas splice variants that do not contain this exon cassette are found mainly in the cytoplasm. In addition, it is suggested that the presence or absence of specific exon cassettes of NR1 directs whether this receptor subunit will be phosphorylated, a process that appears to be required for clustering of the receptor (Ehlers *et al*., 1995).

Studies on the structure of the glutamate receptor have yielded different results over time with earlier studies favoring a pentamer [similar to nicotinic acetylcholine receptors (nAChRs)] and more recent investigations suggesting a tetrameric structure similar to that of potassium channels (Mano

and Teichburg, 1998). In addition, relatively little is known about the rules that determine which subunits coassemble, although it is generally accepted that a subunit will assemble only with other subunits within its own family. Coexpression studies of selected combinations of these receptor subunits demonstrate that not only do all the GluR subunits participate in the formation of ion channels when coexpressed with another related subunit, but many subunits are capable of forming functional homomeric channels. The latter is particularly true for the AMPA/kainate receptor subunits. In contrast, functional NMDA receptors require coexpression of NMDAR1 and one of four NMDAR2 subunits. Moreover, a number of important channel properties depend to a large extent on the specific NMDAR2 subunit expressed by the cell and its ability to assembly with NMDAR1. For example, studies of transfected HEK293 cells demonstrate that heterodimeric NMDAR1a/NR2A and NMDA1a/NR2B but not NMDAR1a/NR2C result in glutamate-mediated cell death (Anegawa *et al*., 1995). In addition, recombination studies in cell cultures demonstrate that NMDA receptors assembled from NR1 and NR2D subunits show a prolonged decay rate of glutamate-induced ion currents and a lowered threshold for voltage-dependent Mg^{2+} blockade compared to properties of NMDA receptors assembled from NR1 and NR2A or NR2B subunits (Monyer *et al*., 1994). These kinetic properties of NMDA receptors containing the NR2D subunit are thought to result in a hyperexcitable type of NMDA receptor.

The finding that nAChRs are characterized by four transmembrane-spanning domains with both amino-terminal and the carboxy-terminal located extracellularly led investigators to speculate that glutamate receptors would have a similar transmembrane topology. However, contrary to these initial predictions, it has been determined that glutamate receptors have only three transmembrane domains (M1, M3, and M4) plus a cytoplasm-facing reentrant pore loop segment (M2) that contributes to the opening of the ion channel. Thus, GluRs have a large extracellular amino-terminal and an intracellular carboxy-terminal that vary considerably in length between different subunits.

Glutamate receptor mRNAs were the first neurotransmitter receptor RNAs found to be modified by posttranscriptional RNA editing. To date, editing has not been demonstrated for any NMDA subunit RNA, but is recognized to occur at multiple positions for AMPA and kainate receptor RNAs. For example, in primary transcripts of GluR2, GluR5, and GluR6, a glutamine codon in the p loop (i.e., M2 domain) can be edited to arginine at the Q/R site. Nearly 99% of all GluR2 transcripts are edited, whereas the other AMPA subunits (i.e., GluR1,3,4) are not edited. Functionally, homomeric GluR2 subunit channels and heterooligomeric assemblies containing this subunit exhibit different current–voltage relationships than receptors composed without it. Furthermore, the presence of the GluR2 subunit substantially reduces Ca^{2+} conductance through AMPA receptors in response to ligand binding (Hollmann *et al*., 1991). Thus, it would appear that the presence or absence of the GluR2 subunit in non-NMDA receptor assemblies may have a profound influence on the ability of a cell to gate extracellular Ca^{2+} and to maintain intracellular calcium homeostasis. As discussed later, it is hypothesized that the absence of the GluR2 receptor subunit in the AMPA receptor allows Ca^{2+} to enter the cell more readily, resulting in an increase in intracellular calcium and hence progressive neuronal damage as a consequence of ionotropic glutamate receptor stimulation. Editing of the Q/R site in GluR6 transcripts also controls anion permeability (Burnashev *et al*., 1996). In addition, the AMPA receptor subunits GluR2–4 are edited at the R/G site found at the end of the exon that precedes the alternatively spliced flop and flip exons. The extent to which the R/G site is edited is developmentally regulated and depends, in part, on the specific subunit and the flip/flop variant.

III. Glutamate Receptors in the Aging Rodent Brain

The following section is devoted to a review of glutamate receptors in the aging rodent (mice and rats). As one will quickly discern, data are at times discrepant with some authors reporting a decrease in glutamate receptor function, whereas others report that these receptors are unchanged or even hyperexcitable. Contributing to these various findings are a number of factors, including the age and strain of the rodent, the method employed to investigate the glutamate receptor, and the subtype of glutamate receptor, examined (including specific binding sites within a receptor). The various works discussed in this chapter are categorized first according to glutamate subtype (i.e., NMDA, AMPA, kainate receptors) and then to the ligand-specific binding site associated with the receptor. Admittedly, the vast majority of these studies employ ligand-binding techniques and thus are limited in their ability to address important issues related to the molecular composition of the glutamate receptor. Molecular studies, however, are emerging, and the results of these investigations are included immediately following the ligand-binding studies. Whereas ligand binding and molecular studies are highly useful in evaluating the density and to some extent the integrity of the glutamate receptor, they fail to reveal much about the functional status of the receptor. In order to assess various functional properties of the glutamate receptor, most investigations have employed *in vivo* electrophysiological recording techniques. These latter investigations provide valuable information with respect to the functional integrity of the glutamate receptor during normal aging and are presented at the end of this section.

A. NMDA Receptor-Binding Sites

1. Glutamate

Thus far, many studies have investigated the glutamate-binding site in the NMDA receptor in aged rodents with the majority of these investigations reporting a decrease in [^{3}H]glutamate-binding sites (Tamaru *et al*., 1991; Wenk *et al*., 1991; Clark *et al*., 1992; Ingram *et al*., 1992) (Table 20.1). For example, Tamaru *et al.* (1991) observed significant decreases in [^{3}H]glutamate binding in membrane preparations of the hippocampus and neocortex of Fischer 344 rats 7 and 29 months of age compared to 3-month-old rats. Similarly, Wenk *et al.* (1991) observed a significant decrease in glutamate binding in the sensory motor neocortex, parietal-occipital neo-

TABLE 20.1 Glutamate Binding in Aged Rodents

Strain/species	Sex	Age (months)	Region	Change	Reference
Fisher 344 rat	Male	3, 12, 24	Hippocampus	Increase[a]	Baudry *et al.* (1981)
Fisher 344 rat	Male	3, 24	Hippocampus	30% decrease	Bonhaus *et al.* (1990)
Fisher 344 rat	Male	2, 7, 29	Neocortex	45% decrease by 29 months (M)[a]	Tamaru *et al.* (1991)
			Hippocampus	30% decrease	
Fisher 344 rat	Male	5, 24	Neocortex	Decrease	Wenk *et al.* (1991)
			Hippocampus	Decrease	
			Cerebellum	ns[b]	
			Caudate nucleus	Decrease	
Fisher 344 rat	Male	3, 28	Hippocampus	Decrease[c]	Clark *et al.* (1992)
Fisher 344 rat	Male	3–4, 24–25	Hippocampus	63% decrease	Ingram *et al*. (1992)
Long-Evans rat		4, 24–25	Hippocampus	No change	Nicolle *et al*. (1996)
			Striatum	Decrease	
C57BL mice		3, 10, 30	Horizontal sections	55% decrease by 30 M[a]	Peterson and Cotman (1989)
C57BL mice		3, 10, 30	Neocortical regions	Decrease by 30 M[a]	Magnusson and Cotman (1993)
			Hippocampus	Decrease by 30 M[a,c]	
			Subcortical regions	Decrease by 30 M[a]	
			Cerebellum	No change	
C57BL mice		3, 10, 28–30	Neocortical regions	Decrease by 30 M[a,c]	Magnusson (1995)
			Hippocampus	Decrease by 30 M[a,c]	
			Subcortical regions	Decrease by 30 M[a]	
			Cerebellum	No change	
C57BL mice		3, 10, 26	Neocortex	Decrease by 26 M	Magnusson (1997)
			Hippocampus	Decrease by 26 M[c]	
			Subcortical regions	Decrease by 26 M[c]	
BALB/c mice		3, 10, 30	Horizontal sections	16% decrease by 30 M	Peterson and Cotman (1989)
BALB/c mice		3, 10, 30	Neocortical regions	Decrease by 30 M[a,c]	Magnusson and Cotman (1993b)
			Hippocampus	Decrease by 30 M[a,c]	
			Subcortical regions	Decrease by 30 M	
			Cerebellum	No change	

[a]Significant change at intermediate age.
[b]Not significant.
[c]Changes noted only in certain subregion(s).

cortex, hippocampus, and the caudate nucleus of 24-month-old Fisher 344 rats compared to 5-month-old animals. In interpreting their data, Tamaru *et al.* (1991) speculated that NMDA-bearing neurons in the neocortex and hippocampus of old animals may be vulnerable to excitotoxic injury as the result of excessive increases in extracellular glutamate. That extracellular glutamate may be elevated in the aged rat is supported by the work of Freeman and Gibson (1987), who observed in BALB/c mice a 77% increase in the basal release of glutamate in the striatum and a 94% increase in the hippocampus of 30-month-old mice compared to those 3 months of age. In addition, Matsumoto *et al.* (1982) and Price *et al.* (1981) demonstrated age-related impairments in glutamate uptake mechanisms, which may also contribute to increases in extracellular glutamate. Collectively, these data support the notion that extracellular glutamate levels may increase with age, thereby leading Tamaru and colleagues (1991) to speculate that the age-related reduction in NMDA receptors potentially represents a compensatory response to protect NMDA-bearing neurons from the pathologic consequences of elevated extracellular levels of this excitatory amino acid. However, in consideration of the work of Tamaru *et al*. (1991), it is important to note that while differences in levels of glutamate binding were observed in 7- and 29-month-old rats compared to 3-month-old rats, no decreases in binding were observed in rats 7 and 29 months of age. These data led Nicolle *et al*. (1996) to suggest that the observed differences between groups could, in fact, represent a developmental change rather than an effect of aging.

The hypothesis that deficits in glutamatergic neurotransmission may produce deficits in cognitive performance provided the impetus for a number of investigators to examine the integrity of the NMDA receptor in behaviorally characterized rodents. For example, Clark *et al*. (1992) tested young and aged Fischer 344 on the Morris water maze and divided aged animals into two groups (i.e., achievers and nonachievers) on the basis of their ability to meet selected criterian. Whereas *in vitro* autoradiographic studies demonstrated significantly

higher levels of NMDA binding in nearly every hippocampal subdivision of young rats compared to aged rats, no differences were observed between aged achievers and nonachievers. The failure to detect a correlation between levels of glutamate binding and performance on the water maze may, in part, be explained by the fact that this study focused exclusively on the relationship between hippocampal glutamate binding and spatial memory and did not take into account other neocortical and subcortical brain regions, within which age-related decreases in glutamate binding have also been reported (Clark *et al*., 1992). As stated by Clark and colleagues (1992), additional insights into the brain systems involved in age-related decline in memory must await detailed analyses of the relationship between glutamate binding and spatial memory within these other brain regions. In support of the notion that specific behaviors involve the complex interactions between NMDA receptors and other neuronal systems comes from the work of Ingram *et al*. (1992). Specifically, Ingram and colleagues (1992), similar to Clark *et al*. (1992), observed a marked reduction in NMDA binding in the hippocampus of aged rats (i.e., 23–24 months-old) compared to young animals (i.e., 3–4 months-old) (Ingram *et al*., 1992). However, when Ingram *et al*. (1992) correlated glutamate binding with performance in a shock-motivated 14 unit T maze, they found a positive correlation between errors in the maze and hippocampal [^{3}H]glutamate binding (i.e., rats that made the most errors had the highest level of NMDA receptor binding). In interpreting these data, Ingram *et al*. (1992) suggested that the observed learning impairment may not be specific to hippocampal function. Alternatively, they suggest that brain regions with higher glutamate receptor densities might be more vulnerable to the neurotoxicity of glutamate via a mechanism involving the activation of NMDA receptors and subsequent increases in nitric oxide production. Ingram *et al*. (1992) also pointed out that because cholinergic systems are likely involved in radial maze performance, it is reasonable to consider that NMDA-bearing neurons are, in fact, cholinergic and thus the behavioral deficits may reflect a loss of cholinergic neurons due to glutamate neurotoxicity.

Davis and colleagues (1993) also reported an age-related decline in [^{3}H]glutamate binding in the frontal neocortex of old rats (24 months). Notably, this decrease was ameliorated following a 10-month administration of acetyl-L-carnitine, the ester of the natural compound carnitine. Comparable results with L-carnitine were observed by Castorina *et al*. (1994), who, following treatment for 6 months, demonstrated a significant impediment in the decline in the number of NMDA receptors within the hippocampus, the frontal neocortex, and striatum compared to untreated animals.

In contrast to those studies reporting a decline in glutamate binding, Baudry *et al*. (1981) demonstrated an increase in Na-independent glutamate binding in hippocampal membranes of male Fischer rats 12 and 24 months of age compared to 3-month-old rats. Moreover, binding levels in 24-month-old rats were significantly greater compared to 12-month-old rats. The authors attributed this elevation to an increase in the maximum number of binding sites rather than a change in the affinity of the receptor for glutamate. However, when [^{3}H]glutamate binding was measured with a saturating concentration of calcium, the density of glutamate-binding sites appeared to remain constant throughout all ages examined. The finding that glutamate binding in the absence of calcium increases with age whereas in the presence of calcium it does not suggests that the extent to which calcium stimulates glutamate binding must decrease with age. Functionally, the decreased ability of calcium ions to stimulate [^{3}H]glutamate receptor binding may account for age-related deficits in, for example, LTP (Baudry *et al*., 1981).

Glutamate binding has also been examined in aged mice. For example, Peterson and Cotman (1989) studied [^{3}H]-glutamate binding in two strains of aged mice, C57B1 and BALB/c (3, 10, and 30 months of age). In brief, the authors observed an age-related decrease in binding of glutmate to the NMDA receptor, although the magnitude of the decline was greater in the BALB/c strain than in the C57B1 strain. In explaining their data, they, like Tamaru *et al*. (1991), suggested that an age-related increase in basal glutamate release and a corresponding reduction in glutamate uptake mechanisms (Freeman and Gibson, 1987; Aprikyan and Gekchyan, 1988) may lead to a prolonged elevation of extracellular glutamate and, in turn, the downregulation of the NMDA receptor. Other studies of these same mice strains have likewise shown decreases in glutamate binding (Magnusson and Cotman, 1993b; Magnusson, 1995, 1997).

2. CPP

Despite abundant evidence for a decrease in glutamate binding, investigations of CPP binding (i.e., a potent and selective antagonist of the L-glutamate-binding site) appear somewhat more controversial (Table 20.2). For example, using *in situ* autoradiographic techniques in aged rats (i.e., 21 months old), Miyoshi and colleagues (1991b; Kito *et al*., 1990) found a marked preservation of [^{3}H]CPP-binding sites in the hippocampus and neocortex, regions of the brain where the highest density of binding sites were found. In contrast, both studies reported significant declines in [^{3}H]glycine-binding sites. At the molecular level, the NMDA receptor complex has been shown to consist of an L-glutamate binding site, a strychine-insensitive glycine modulatory site, and a voltage-dependent cation channel. In the neocortex and hippocampus, [^{3}H]CPP- and [^{3}H]glycine-binding sites codistribute and are present at high levels. The finding that [^{3}H]glycine but not [^{3}H]CPP-binding sites are affected with increasing age suggests that the NMDA receptor in the hippocampus and neocortex itself remains unchanged, while the glycine modulatory site is affected.

Additional investigations of [^{3}H]CPP binding demonstrate a 30% decrease in the density of CPP-binding sites in hippocampal membranes of 24-month-old rats compared to those 3 months of age (Bonhaus *et al*., 1990). In contrast, these same investigators observed no significant age-related reductions in the total number of [^{3}H]CPP-binding sites or in the affinity of these binding sites (Bonhaus *et al*., 1990). Notably, a significant increase in protein content was observed in membranes prepared from old rats (3.6 ± 0.2 mg protein/pair hippocampal formations) compared to young rats (2.7 ± 0.2 mg protein/pair hippocampal formations), thus providing a reasonable explanation for the reduction in receptor density (Bonhaus *et al*., 1990). Although it is unclear, the precise mechanism

TABLE 20.2 CPP Binding in Aged Rats

Strain/species	Sex	Age (months)	Region	Change	Reference
Fisher 344 rat	Male	3, 28	Hippocampus	Decrease[a]	Clark *et al.* (1992)
Fisher 344 rat	Male	2, 5, 13, 21	Hippocampus	No change	Miyoshi *et al.* (1991b)
			Neocortex	Decrease by 21 months (M)[a,b]	
			Caudate-putamen	Decrease by 21 M[b]	
			Nucleus accumbens	Decrease by 21 M[b]	
			Septal nucleus	Decrease by 21 M	
			Olfactory tubercle	Decrease by 21 M[b]	
			Cerebellum	No change	
Fisher 344 rat	Male	2, 5, 13, 21	Neocortex	Decrease by 21 M[a,b]	Kito *et al.* (1990)
			Striatum	Decrease by 21 M[b]	
			Nucleus accumbens	Decrease by 21 M[b]	
			Septal nucleus	Decrease by 21 M	
			Hippocampus	No change	
			Cerebellum	No change	
Fisher 344 rat	Male	3, 24	Hippocampus	30% decrease	Bonhaus *et al.* (1990)
Long–Evans rat		5, 24–25	Hippocampus	45% decrease	Pelleymounter *et al.* (1990)
C57BL mice		3, 10, 28–30	Neocortical regions	Decrease by 30 M[a,b]	Magnusson (1995)
			Hippocampus	Decrease by 30 M[a]	
			Subcortical regions	Decrease by 30 M[a,b]	
			Cerebellum	No change	

[a]Changes noted only in certain subregion(s).
[b]Significant change at intermediate age.

underlying the increase in hippocampal membrane protein, Bonhaus *et al*. (1990) suggested that an increase in nonneuronal elements (i.e., the hypertrophy of hippocampal astrocytes) could be a contributing factor. Hippocampal [^{3}H]CPP binding was also investigated in behaviorally characterized Long–Evans rats (Pelleymounter *et al*., 1990). In brief, hippocampal [^{3}H]CPP binding was reduced approximately 45% in aged animals regardless of whether they were naive or trained in the Morris water maze, thus suggesting that training itself does not affect [^{3}H]CPP binding (Pellymounter *et al*., 1990). However, [^{3}H]CPP did corrrelate significantly with the number of trials to criterion for the aged subjects and was significantly lower in impaired aged rats compared to unimpaired aged coherts and young rats. Collectively, these data led Pellymounter and colleagues (1990) to conclude that a reduction in hippocampal-binding sites, as measured by [^{3}H]CPP binding, may contribute to age-associated cognitive deficits. Consistent with the previous findings, Magnusson (1995) observed a significant decrease in [^{3}H]CPP binding in the majority of neocortical and hippocampal regions of aged C57B1 mice.

[^{3}H]CPP binding was also examined in rats with an inborn high (HP) or low (LP) learning capacity to perform in a shuttle box (Keller *et al*., 1992). The inborn ability of HP and LP rats has been related to the plasticity of hippocampal circuits. Keller *et al*. (1992) reported that HP rats show an increased number of NMDA receptor-binding sites in the hippocampus compared to control and LP rats. Although the current work does not provide a direct link with the aging brain, it nevertheless provides compelling evidence to suggest that the putative differences in hippocampal synaptic plasticity observed in HP and LP rats may be related to variations in the density of NMDA receptors.

3. MK-801

MK-801 is a noncompetitive NMDA antagonist used to label the activated state of the cation channel of the NMDA receptor complex. To date, considerable evidence indicates that the density of MK-801 binding is decreased in the aged rodent (Tamaru *et al*., 1991; Mitchell and Anderson, 1998; Serra *et al*., 1994) (Table 20.3). For example, Tamaru *et al*. (1991) observed significant reductions in [^{3}H]MK-801 binding in the neocortex and hippocampus of rats 7 and 29 months of age compared to 2-month-old rats. Moreover, Scatchard analysis revealed that the reduction in [^{3}H]MK-801 binding was due to a significant decrease in binding sites with aging, with their affinities being unaltered. However, as stated previously, in the absence of any significant differences in binding between 7- and 29-month-old rats, one must consider whether these differences in [^{3}H]MK-801 binding are more representative of a developmental/maturational change rather than an age-related event.

Age-related decreases in [^{3}H]MK-801 binding have also been reported by a number of additional investigators. For example, while failing to observe any decreases in [^{3}H]MK-801 binding in middle aged (12-month-old) rats compared to young rats (3-month-old), Wardas *et al*. (1997) found significant reductions in binding in old (36-month-old) rats compared to both middle-aged and young rats. Mitchell and Anderson (1998) also observed a significant age-related decrease in ^{3}H-binding in the inner frontal neocortex, entorhinal neocortex, and lateral striatum of 24-month-old rats compared to 6-month-old rats. No differences in binding were observed between 24- and 12-month-old rats or between 12- and 6-month-old animals. Moreover, a number of brain regions did

TABLE 20.3 MK-801 Binding in Aged Rodents

Strain/species	Sex	Age (months)	Region	Change	Reference
Fisher 344 rat	Male	2, 7, 29	Neocortex	57% decrease by 29 months (M)[a]	Tamaru *et al.* (1991)
			Hippocampus	20% decrease by 29 M[a]	
Fisher 344 rat	Male	6, 12, 24	Inner frontal cortex	Decrease by 24 M[b]	Mitchell and Anderson (1998)
Sprague Dawley			Entorhinal cortex	Decrease by 24 M[b]	
			Hippocampus	No change	
			Lateral striatum	Decrease by 24 M[b]	
Wistar Kyoto rat	Male	3, 18, 24	Hippocampus	Decrease	Serra *et al.* (1994)
			Striatum	Decrease	
			Neocortex	Decrease	
Wistar rat	Female	3, 12, 36	Caudate-putamen	26–41% decrease by 36 M[c]	Wardas *et al.* (1997)
			Nucleus accumbens	32–34% decrease by 36 M[c]	
			Hippocampus	27% decrease by 36 M[c,d]	
			Neocortex	19–39% decrease by 36 M[c,d]	
Long–Evans rat		6, 24–25	Neocortex	Decrease in aged cognitively unimpaired rats[d]	Le Jeune *et al.* (1996)
			Hippocampus	Decrease[d]	
NMRI mice		3, 12, 23	Hippocampus		Scheuer *et al.* (1996)
			Frontal cortex	22% decrease[e]	
NMRI mice		3, 20	Forebrain	35% decrease	Cohen and Müller (1992)
C57BL mice		3, 10, 28–30	Neocortical regions	Decrease[d,e]	Magnusson (1995)
			Hippocampus	Decrease[d]	
			Subcortical regions	Decrease[d,e]	
			Cerebellum	No change	
Senescence-acc		2, 14	Cortical membranes	Decrease	Kitamura *et al.* (1992)

[a] In the presence of glycine/glutamate.
[b] Changes at intermediate age not significant.
[c] No change at intermediate age.
[d] Changes noted only in certain subregion(s).
[e] Significant change at intermediate age.

not undergo any significant age-related change in [^{3}H]MK-801 binding, including the dentate gyrus and CA1 region of the hippocampus.

Le Jeune *et al*. (1996) studied aged Long–Evans rats and grouped them based on their performance in the Morris water maze. In brief, they observed a decrease in [^{125}I]MK-801 binding in 4 of 35 neocortical areas of aged-unimpaired rats (24–25 months old) compared to young rats (6 months old). No changes in binding were observed in aged-impaired rats. Collectively, these data led Le Jeune *et al*. (1996) to conclude that aging itself has little, if any, influence on the expression of the NMDA receptor in the brains of Long–Evans rats.

A number of investigators have also examined MK-801 binding in aged mice with the vast majority reporting age-related decreases in binding (see Muller *et al*., 1994a,b, for reviews). For example, Scheuer *et al*. (1996) examined young (3 months), middle-aged (12 months), and aged (23 months) female Naval Medical Research Institute (NMRI) mice and observed a 19 and 22% decrease in MK801 binding in the forebrain of middle-aged and aged rats, respectively. Scheuer *et al*. (1996) also reported a significant age-related correlation between NMDA receptor density and performance in a passive avoidance acquisition task. In an attempt to provide a biochemical basis by which aging affects NMDA receptor number and cognitive function, Scheuer *et al*. (1996) measured membrane fluidity and found that changes in the fluidity of forebrain membranes correlates inversely with NMDA receptor density. While the authors concluded that their findings do not prove a causal relationship, they are nevertheless compatible with the hypothesis that changes in membrane fluidity, by decreasing the number of NMDA receptors, affects performance in a passive-avoidance task. In an additional study of NMRI mice, Cohen and Müller (1992) observed a 35% reduction in MK-801 binding of mice 20 months of age compared to 3-month old-rats.

Likewise, Magnusson (1995) examined C57B1 mice and observed a decrease in binding in 6/10 neocortical regions, 3/6 hippocampal regions, and the caudate nucleus by 28–30 months of age. Senescence-accelerated mice also showed reduced MK-801 binding in neocortical membranes (Kitamura *et al*., 1992) and in the hippocampus (Nomura *et al*., 1997) compared to normal strain mice.

4. TCP

TCP, like MK-801, binds to the cation channel in the NMDA receptor complex. Both compounds bind to the activated state of the channel, as evidenced by the ability of

TABLE 20.4 TCP Binding in Aged Rodents

Strain/species	Sex	Age (months)	Region	Change	Reference
Fisher 344 rat	male	3, 24	Hippocampus	30% decrease	Bonhaus *et al.* (1990)
C57BL mice		3, 10, 28–30	Neocortical regions	Decrease[a,b]	Magnusson (1995)
			Hippocampus	Decrease[b]	
			Subcortical regions	Decrease[a,b]	
			Cerebellum	No change	

[a]Significant change at intermediate age.
[b]Changes noted only in certain subregion(s).

glutamate to increase the rate of binding of both TCP and MK-801. Similar to findings with MK-801, Magnusson (1995) observed a significant decline in [^{3}H]TCP binding in six neocortical, one hippocampal, and one subcortical region in C57B1 mice between 3 and 28–30 months of age. Bonhaus *et al*. (1990) also observed a significant decrease (i.e., 30%) in the density of TCP-binding sites in the hippocampus of Fischer 344 rats (Table 20.4). However, as stated previously, the reduction in receptor density may not be due to a decrease in the number of receptors, but rather to an age-related increase in protein content.

5. Glycine

To date, a general agreement exists in the literature regarding a decrease in glycine binding in aged rodent brains. For example, Fischer 344 rats show severe decreases in selective telencephalic regions by 21 months of age (Kito *et al*., 1990; Pelleymounter *et al*., 1990; Miyoshi *et al*., 1991b). Tamaru *et al*. (1991) also observed a 40% decrease in the neocortex and a 27% reduction in the hippocampus of Fisher 344 rats between 2 months of age and 29 months of age. In addition to decreases in [^{3}H]CPP and [^{3}H]TCP binding, Bonhaus *et al*. (1990) observed significant decreases in the density of the glycine-binding site. C57B1 mice also exhibited a significant decrease in [^{3}H]glycine binding, although this binding site appeared to be less affected by aging compared to [^{3}H]MK-801 and [^{3}H]TCP binding both in percentage decline and the number of brain regions affected (Magnusson, 1995) (Table 20.5).

B. AMPA Receptor-Binding Sites

While a substantial number of studies report an age-related reduction in the NMDA receptor, the vast majority of works support the preservation of the AMPA receptor with advanced age (Table 20.6). For example, in aged Fischer 344 rats (i.e., 21 months), Miyoshi *et al*. (1991a) found no significant changes in [^{3}H]AMPA-binding sites in the hippocampus or neocortex compared to young rats (i.e., 2 months of age). These data led the authors to conclude that AMPA-binding sites are not involved in the age-related decline of neuronal functions, especially impairment of learning and memory. Tamaru *et al*. (1991) also stated that AMPA receptors are relatively unaltered by the aging process, despite the finding that [^{3}H]AMPA binding, determined in the presence of KSCN, is decreased in the aged rat. Cimino *et al*. (1992) also reported relatively modest age-related alterations in AMPA binding with decreases

TABLE 20.5 Glycine Binding in Aged Rodents

Strain/species	Sex	Age (months)	Region	Change	Reference
Fisher 344 rat	Male	2, 5, 13, 21	Neocortex	Decrease by 21 months (M)[a]	Kito *et al.* (1990)
			Striatum	Decrease by 21 M[a]	
			Septal nucleus	Decrease by 21 M[a]	
			Nucleus accumbens	Decrease by 21 M[a]	
			Olfactory tubercle	Decrease by 21 M[a]	
			Hippocampus	Decrease by 21 M[a]	
			Amygdala	Decrease by 21 M[a]	
			Thalamus	Decrease by 21 M[a,b]	
			Cerebellum	No change	
Fisher 344 rat	Male	2, 7, 29	Neocortex	40% decrease by 29 M[c]	Tamaru *et al.* (1991)
			Hippocampus	27% decrease by 29 M[c]	
Fisher 344 rat	Male	3, 24	Hippocampus	30% decrease	Bonhaus *et al.* (1990)
C57BL mice		3, 10, 28–30	Neocortical regions	Decrease[b]	Magnusson (1995)
			Hippocampus	No change	
			Subcortical regions	Decrease[a,b]	

[a]Significant change at intermediate age.
[b]Changes noted only in certain subregion(s).
[c]Changes at intermediate age not significant.

TABLE 20.6 AMPA Binding in Aged Rodents

Strain/species	Sex	Age (months)	Region	Change	Reference
Fisher 344 rat	Male	2, 7, 29	Neocortex	Decrease by 29 M[a,b]	Tamaru *et al.* (1991)
			Hippocampus	Decrease by 29 M[b]	
Fisher 344 rat	Male	2, 5, 13, 21	Neocortex	Decrease by 21 M[a,c]	Miyoshi *et al.* (1991a)
			Caudate nucleus	Decrease by 21 M[a]	
			Nucleus accumbens	Decrease by 21 M[a]	
			Olfactory tubercle	Decrease by 21 M	
			Hippocampus	No change	
			Amygdala	No change	
			Cerebellum	No change	
Fisher 344 rat	Male	3, 28	Hippocampus	Decrease[c]	Clark *et al.* (1992)
Long–Evans rat		6, 24–25	Neocortex	Decrease in AI[c,d]	
			Hippocampus	Decrease in AI[c]	
Long–Evans rat	Male	4, 24–25	Hippocampus	Decrease[c]	Nicolle *et al.* (1996)
			Striatum	Not significant	
			Lateral septum	Not significant	
Long–Evans rat	Male	6, 24–25	Neocortex	Increase in AI[c]	Le Jeune *et al.* (1996)
			Hippocampus	Increase in AI[c]	
Wistar Kyoto rat	Male	2, 6, 12, 18, 24	Neocortex	No change by 24 M	Cimino *et al.* (1992)
			Hippocampus	Decrease by 24 M[a,c]	
			Striatum	No change by 24 M	
			Septum	No change by 24 M	
Wistar rat	Female	3, 12, 36	Hippocampus	17–29% decrease by 36 M[e]	Wardas *et al.* (1997)
			Caudate-putamen	Non–significant decrease	
			Nucleus accumbens	No change	
			Neocortex	Decrease by 36 M[c]	
C57BL mice		3, 10, 30	Neocortical regions	Decrease by 30 M[c]	Magnusson and Cotman (1993a)
			Hippocampus	Decrease by 30 M[c]	
			Subcortical regions	No change	
			Cerebellum	No change	
C57BL mice		3, 10, 30	Neocortical regions	Decrease by 30 M[c]	Magnusson (1995)
			Hippocampus	Decrease by 30 M[c]	
			Subcortical regions	No change	
			Cerebellum	No change	
BALB/c mice		3, 25	Telencephalon	Decrease	Bahr *et al.* (1992)
			Brain stem-cerebellum	No change	
BALB/c mice		3, 10, 30	Neocortical regions	Decrease by 30 M[c]	Magnusson (1995)
			Hippocampus	Decrease by 30 M[c]	
			Subcortical regions	No change	
			Cerebellum	No change	

[a]Significant change at intermediate age.
[b]In presence of KSCN.
[c]Changes noted only in certain subregions(s).
[d]AI, aged cognitively impaired rats.
[e]No change at intermediate age.

restricted to sites localized in the CA3 region of the hippocampus and in the molecular layer of the dentate gyrus. In contrast, NMDA binding was decreased in many more regions, thus suggesting that NMDA and non-NMDA recognition sites are affected differentially by the aging process (Cimino *et al*., 1992). This latter view is supported by the work of Wardas *et al*. (1997), who observed age-related declines in AMPA binding within only limited regions of the striatum and hippocampus, whereas decreases in NMDA binding were observed throughout most of these regions. The latter data are supportive of the concept that NMDA receptors are more vulnerable to aging than AMPA receptors.

Using *in situ* hybridization methods, Pagliusi *et al*. (1994) observed significant declines in message levels for the AMPA receptor subunits GluR1 and GluR2 in all hippocampal subfields of aged rats (24 months) compared to young controls (2 months). In an attempt to evaluate the potential changes in calcium permeability of the AMPA receptors, Pagliusi *et al*. (1994) determined the ratio of mRNA levels of GluR1, which forms calcium-permeable channels, and of GluR2, which

blocks calcium from passing through the ion channel when part of the assembled receptor. In all regions of the hippocampus, the ratio of GluR1/GluR2 was increased modestly in the aged rat, thus suggesting that glutamate receptors in aged hippocampus may exhibit greater calcium permeability and thus are rendered more vulnerable to cell death as a consequence of altered calcium homeostasis. In contrast, Nicoletti *et al*. (1995) studied Sprague–Dawley rats at 4, 12, and 24 months and found no significant decrease in levels of GluR1, GluR2, GluR3, or GluR4 mRNA. Likewise, protein levels remained unaltered for all subunits except GluR1 for which significant decreases were observed. These data led Nicoletti *et al*. (1995) to conclude that AMPA receptors are influenced only slightly by the aging process. Moreover, the observed decrease in GluR1 protein may be explained by a decrease in the translational efficiency or an increase in protein degradation for the GluR1 subunit.

A number of investigators have also examined the integrity of the AMPA receptor in behaviorally characterized rodents with rather mixed findings. For example, Clark *et al*. (1992) observed significant declines in [^{3}H]AMPA within 8 out of 10 hippocampal subfields of aged rats. On the one hand, no relationship was observed between the density or the distribution of AMPA receptors and performance on the water maze. On the other hand, Nicolle *et al*. (1996) found [^{3}H]AMPA binding to be preserved in the striatum of aged rats whereas regionally selective effects of age were detected in the hippocampus, although limited to the CA1 field. Consistent with the results of Clark *et al*. (1992), this reduction in AMPA binding was not correlated with the cognitive status (Nicolle *et al*., 1996). In contrast to the previous two studies, Le Jeune *et al*. (1996) observed a significant increase in the density of [^{3}H]AMPA binding within most hippocampal subfields of cognitively impaired rats versus young adult rats. No changes in [^{3}H]AMPA bindings were observed in the unimpaired cohort. Although the mechanism underlying the alterations in AMPA binding in the aged impaired rats is unclear, it is hypothesized that this increase may be an attempt to reverse the cognitive deficits, as heightened AMPA receptor signaling is associated with improved cognitive performance (Le Jeune *et al*., 1996).

[^{3}H]AMPA binding has also been studied in mice. For example, using an antibody against the GluR-A (GluR1) glutamate subunit, Bahr *et al*. (1992) observed a significant decrease in immunolabeling in the telencephalon of 25-month-old BALB/c mice compared to 3-month-old mice. Notably, the magnitude of the decrease (i.e., 30%) was comparable to the decrease in [^{3}H] AMPA binding. Scatchard analysis revealed that this latter reduction was due to a decrease in receptor density and not to a change in binding affinity. Moreover, binding to the GABA, dopamine, or serotonin receptor was not reduced significantly in these mice nor were reductions observed in the nerve terminal markers, synaptophysin, and SV2 glycoprotein. Collectively, these data suggest that AMPA receptors in the telecephalon of BALB/c mice are selectively altered by the aging process (Bahr *et al*., 1992). Additional support for age-related alterations in AMPA binding (albeit modest) within the forebrain of mice comes from the work of Magnusson and colleagues. They observed significant reductions in [^{3}H]AMPA binding within 4 of 21 brain regions (Magnusson and Cotman, 1993a) and in a subsequent study within 1 of 21 brain regions (Magnusson, 1997). In contrast, NMDA receptors were altered to a much greater extent, thus underscoring the possibility that NMDA receptors are selectively vulnerable to the aging process.

C. Kainate Receptor-Binding Sites

In an attempt to provide a comprehensive view of the ionotropic glutamate receptor, a number of investigators have examined the kainate receptor in addition to the NMDA and AMPA receptor (Table 20.7). Similar to the findings for NMDA and AMPA receptors, a number of inconsistencies exist with respect to the integrity of the kainate receptor during aging.

TABLE 20.7 Kainate Binding in Aged Rodents

Strain/species	Sex	Age (months)	Region	Change	Reference
Fisher 344 rat	Male	3, 28	Hippocampus	Decrease[a]	Clark *et al.* (1992)
Fisher 344 rat	Male	2, 7, 29	Neocortex Hippocampus	No change No change	Tamaru *et al.* (1991)
Long–Evans rat	Male	7–8, 27–29	Hippocampus	Decrease[a]	Nagahara *et al.* (1993)
Long–Evans rat	Male	4, 24–25	Hippocampus Striatum Lateral septum	Decrease[a] Decrease[a] Not significant	Nicolle *et al.* (1996)
Long–Evans rat	Male	6, 24–25	Neocortex Hippocampus Caudate putamen Septum	Decrease[a] Decrease[a] Decrease Decrease	Le Jeune *et al.* (1996)
C57BL mice		3, 10, 30	Cortical regions Hippocampus Subcortical regions Cerebellum	Decrease by 30 M[a,b] Decrease by 30 M[a] Decrease by 30 M[a,b] No change	Magnusson and Cotman (1993a)

[a]Significant change at intermediate age.
[b]Changes noted only in certain subregion(s).

For example, Tamaru *et al*. (1991) found no significant changes in kainate binding in the hippocampus and neocortex of aged rats. These same investigators observed AMPA receptors to remain relatively unaltered during the aging process while observing marked reductions in the NMDA-sensitive receptor complex. However, Clark *et al*. (1992) reported age-related reductions in NMDA, AMPA, and kainate binding, although the extent to which [^{3}H]kainate binding is reduced is far less than for NMDA or AMPA receptors. Notably, in no instance did binding levels for any of the three glutamate receptor subtypes correlate with the behavioral status of the rat (Clark *et al*., 1992). Nagahara *et al*. (1993) was also unable to demonstrate a strong correlation between [^{3}H]kainate binding and the spatial learning performance of the animal in a Morris water maze, even though the density of [^{3}H]-kainate binding was reduced in several regions of the hippocampus (i.e., CA3, CA1, hilus) and within related cortical areas (i.e., subicular complex, entorhinal cortex, perirhinal cortex).

Le Jeune *et al*. (1996) also observed significant decreases in [^{3}H]kainate binding in 21 of 37 brain regions of aged Fisher 344 rats. However, similar to the results of Clark *et al*. (1992) and Nagahara *et al*. (1993), no correlations were observed between levels of kainate binding and the cognitive status of the rat (i.e., reductions were observed in both impaired and unimpaired groups). In contrast, binding to the NMDA receptor was reduced significantly in the cognitively unimpaired group whereas binding to the AMPA receptor was increased in the impaired cohert (Nagahara *et al*., 1993). Collectively, these data suggest that the three classes of ionotropic glutamate receptors are regulated differentially in impaired and unimpaired rats. Changes in the NMDA and AMPA receptor subtypes may play a more critical role than alterations in kainate-binding sites for the emergence of the behavioral deficits observed in some aged impaired rats. In a more recent investigation, Nicolle *et al*. (1996) observed a significant decrease in [^{3}H]kainate binding in 1 of 7 hippocampal subfields (i.e., CA3) in aged (24–25 months) Long–Evans rats. Moreover, [^{3}H]kainate binding in the CA3 region revealed a significant correlation with spatial learning performance in the aged group. Specifically, rats with the most preserved spatial learning ability had lower values for [^{3}H]kainate binding, thus suggesting a compensatory reduction in receptor binding in a subpopulation of aged rats (Nicolle *et al*., 1996). In contrast, reductions in AMPA binding, although also observed in 1 of 7 hippocampal subfields (i.e., CA1), were not strongly correlated with spatial learning (Nicolle *et al*., 1996). These data reaffirm the concept that age-related alterations in ionotropic receptors differ with respect to the receptor subtype and anatomical region examined.

Magnusson and Cotman (1993b) studied kainate binding in C57Bl and BALB/c mice and observed significant age-related reductions (30 months of age) in the frontal and parietal cortices, caudate nucleus, and stratum lacunosum/moleculare of CA1, as well as the outer two-thirds of the dorsal blade of the dentate molecular layer in both strains of mice. The authors also reported reductions in AMPA and NMDA binding in these same regions, thus suggesting that these brain areas are particularly vulnerable to the effects of aging (Magnusson and Cotman, 1996).

D. Glutamate Receptor Function

In addition to studies that focus primarily on levels of binding of the various ionotropic glutamate receptor subtypes, a number of investigations utilize a host of approaches to assess the functional status of these receptor subtypes in the aged rat. For example, Dawson *et al*. (1989) assessed the functional integrity of intrinsic and extrinsic glutamate-utilizing pathways innervating the frontal neocortex of 6-month-old adult and 24-month-old aged Fisher 344 rats by examining the release, uptake, and content of glutamate in brain slices of frontal cortex. In brief, the stimulated release and high-affinity uptake of glutamate were not altered appreciably in aged rats. These data suggest that glutamate-utilizing neurons in the frontal cortex are functionally intact and are not affected significantly by the aging process. In contrast, glutamate content is decreased signficantly in the frontal cortex, reflecting either an age-related loss of neurons or a metabolic defect in the biochemical pathways that normally produce or utilize glutamate (Dawson *et al*., 1989).

Baskys *et al*. (1990) also utilized brain slices and compared NMDA-induced depolarizations in neocortical neurons of young (4–6 months) and old (24–29 months) Fischer 344 rats. Whereas increasing amounts of NMDA produced membrane depolarizations in cells obtained from both groups, regression analysis revealed significantly reduced sensitivity to NMDA in old neurons compared to young neurons. Moreover, tetanic stimulation induced LTP in the young neocortex but failed to do so in the neocortex of aged rats. Although the authors failed to establish a direct link between decreased responsiveness to NMDA and the failure to establish LTP, such an association is highly tenable. In explaining their data, Baskys *et al*. (1990) stated that the age-related decrease in sensitivity to NMDA is consistent with a reduced number of agonist receptor sites (Peterson and Cotman, 1989; Bonhaus *et al*., 1990; Tamaru *et al*., 1991; Clark *et al*., 1992; Ingram *et al*., 1992).

Gonzales *et al*. (1991) also examined brain slices to determine the functional status of the NMDA receptor in young (3–5 months), middle (12–14 months), and old (24–28 months) Fischer 344 rats. The functional status of NMDA receptors was determined by examining the NMDA-induced inhibition of muscarinic-stimulated phosphoinositide hydrolysis in the hippocampus and the NMDA-stimulated release of [^{3}H]norepinephrine or [^{3}H]dopamine. Briefly, these investigators observed a marked attenuation, in an age-dependent manner, of NMDA-induced inhibition of muscarinic-stimulated phosphoinositide hydrolysis in the hippocampus. Moreover, using hippocampal slices, Gonzales *et al*. (1991) demonstrated that the maximal NMDA-stimulated release of [^{3}H]norepinephrine declines by 30% as the rat matures from an age of 3–5 months to 12–14 months with no additional decrements in the hippocampus of old rats (28 months). In the neocortex, [^{3}H]norepinephrine release was also decreased, although the largest decline occurred between middle and old age. NMDA-stimulated [^{3}H]dopamine release from striatal slices also displayed an age-dependent reduction with maximum declines again observed between middle and old age. Collectively, these data support the notion that NMDA-mediated responses in the neocortex, hippocampus, and striatum are attenuated with increasing age (Gonzales *et al*., 1991).

Additional evidence for an age-related decline in the function of the NMDA receptor comes from the work of Pittaluga *et al*. (1993), who sought to determine whether aging affected the NMDA receptor-mediated enhancement of noradenaline release from rat hippocampal synaptosomes. Briefly, the authors observed that the maximal effects of NMDA on the release of [^{3}H]noradrenaline decreased with age, whereas the apparent affinity did not seem to change significantly. In addition, the authors investigated the age-related changes of the strychnine-insensitive glycine allosteric site, in part, because the NMDA-binding site requires glycine for its activation and glycine has been demonstrated to potentiate the NMDA-induced release of [^{3}H]noradrenalin. Notably, the effects of adding 1 μM glycine increased the effect of 100 μM NMDA by nearly 100% at 3 months and 270% at 24 months. In explaining these data, the authors suggested that the apparent ''superresponsiveness'' of the glycine-binding site may be due to a more efficient removal and/or to impaired release of glycine, as the uptake of the amino acid was increased by 350% in 24-month-old rats versus 3-month-old rats, whereas the K^+-evoked tritium release from synaptosomes prelabeled with [^{3}H]glycine was decreased. Although these latter findings appear to be in contradiction to studies that show glycine binding to be decreased with age (Kito *et al*., 1990; Pelleymounter *et al*., 1990; Bonhaus *et al*., 1990; Miyoshi *et al*., 1991b; Tamaru *et al*., 1991; Magnusson, 1995), it should be considered that these latter studies were limited in their ability to determine whether the glycine-binding site may have undergone a functional ''upregulation'' with age. These latter data are also consistent with the work of Serra *et al*. (1994), who demonstrated an age-dependent increased sensitivity of [^{3}H]MK-801 binding to the stimulatory action of glycine, despite reduced numbers of NMDA-binding sites. This increase in sensitivity of ^{3}H binding to glycine is hypothesized to reflect an increase in NMDA receptor activity to compensate for the decrease in receptor number (Serra *et al*., 1994).

Using *in vitro* slice preparations, Cepeda *et al*. (1996) investigated the age-induced alterations in the interaction of glutamate and dopamine in the neostriatum of rats and cats. They determined that populations of aged neurons from both species displayed qualitative and quantitative alterations in response to an iontophoretic application of NMDA and glutamate. These alterations included a lack of response, unusual responses consisting of depolarizations without action potentials or combinations of prepotentials, and full amplitude action potentials. In addition, the ability of dopamine to modulate responses mediated through the activation of glutamate receptors was reduced in aged animals. Specifically, subpopulations of neurons were either unresponsive to dopamine or required higher iontophoretic current intensities to modulate the glutamate-induced responses. Collectively, these data support the view that with increasing age, the interactions between glutamate and dopamine appear to be compromised in the striatum. Moreover, this compromise may be largely attributed to decreases in NMDA receptor function during aging.

Electrophysiological techniques were similarly employed by Barnes *et al*. (1997) to determine the effects of age on the amplitude of NMDA receptor-mediated synaptic responses in the CA1 region across the life span of the animal. All animals were behaviorally characterized using a Morris water maze to ensure that old animals were in fact memory impaired. Throughout these studies, Barnes *et al*. (1997) measured the presynaptic fiber potential, as well as the NMDA component of synaptic responses at the Schaffer collateral-CA1 synapse. In brief, the authors observed an equivalent age-related decline in the NMDA and non-NMDA receptor-mediated excitatory postsynaptic potentials for a given fiber potential amplitude in the CA1 region (Barnes *et al*., 1997). In interpreting these data, Barnes *et al*. (1997) suggested that these findings are consistent with the hypothesis that there are fewer NMDA receptors per Schaffer collateral during aging (possibly because of a reduction in the number of Schaffer collateral synapses per presynaptic axon). Of importance, in the aged brain those NMDA receptors that remain appear to be functionally normal.

Further support for a functionally intact NMDA receptor in the aged hippocampus comes from the studies of Billard *et al*. (1997). Specifically, *ex vivo* extra- and intracellular electrophysiological recording techniques were used to investigate the effects of aging on the activation of NMDA receptors in the CA1 field of hippocampal slices of Sprague–Dawley rats (Billard *et al*., 1997). In summary, only modest alterations in NMDA activation were observed in the CA1 region of aged rats. Moreover, NMDA receptor-induced synaptic plasticity was not altered in aged animals. While these data appear to be in contradiction to the several reports indicating a decrease in NMDA binding in the aged hippocampus (Bonhaus *et al*., 1990; Pelleymounter *et al*., 1990; Tamaru *et al*., 1991; Wenk *et al*., 1991; Peterson and Cotman, 1989; Cohen and Müller, 1992), it can be argued that the current study focused solely on the CA1 region, whereas the majority of binding studies used homogenates of relatively gross regions of the hippocampus. This raises the possibility that the previously reported decreases in NMDA binding may reflect decrements within hippocampal subfields other than the CA1 region.

Jasek and Griffith (1998) pharmacologically characterized ionotropic excitatory amino acid receptors in an acutely dissociated medial septum/nucleus of diagonal band neurons from young (1–4 months) and aged (24–26 months) male Fischer 344 rats. The authors found that while NMDA-induced currents were relatively maintained during aging, AMPA-induced current densities were increased significantly. Although the increase in AMPA-induced current density provides a possible mechanism for changes in calcium entry during aging, the authors did not investigate if the subunit composition of these receptors, particularly those involved in the gating of calcium (i.e., GluR2), was changed.

Jouvenceau *et al*. (1998) also used *ex vivo* extracellular recording techniques to examine the synaptic responses mediated by NMDA receptor and non-NMDA receptors in the CA1 hippocampal region of 3- to 4-month-old and 25- to 33-month-old Sprague–Dawley rats. In brief, Jouvenceau *et al*. (1998) found that the functional properties of these glutamate receptor subtypes are affected differentially by aging in the CA1 region. Specifically, they observed an age-dependent decrease in the magnitude of non-NMDA receptor-mediated field excitatory postsynaptic potentials (fEPSP) in aged rats, whereas increases were observed in the duration, but not amplitude, of NMDA receptor-mediated fEPSPs. As demonstrated previously, data are supportive of the concept that NMDA and non-NMDA receptors are affected differentially by aging.

IV. Glutamate Receptors in Alzheimer's Disease

The finding that glutamate is a potent excitotoxin provided the impetus for a host of studies investigating the role of this exictatory amino acid in a variety of neurodegenerative diseases, including AD. Similar to work in the aged rodent, the results of studies of AD brains are variable, thus contributing to the many controversies concerning the role of glutamate in the pathogenesis of AD. The following section presents the vast majority of work related to the integrity and/or dysfunction of the glutamate receptor in AD. Like the previous section, the works are subdivided broadly according to receptor subtype (i.e., NMDA, AMPA, kainate) and, when appropriate, according to specific ligand-binding sites.

A. NMDA Receptors

1. Glutamate and Aspartate Binding

Thus far, a number of receptor autoradiographic studies have investigated the levels of glutamate and aspartate binding in the brains of patients with AD, with the majority reporting disease-related decreases (Table 20.8). For example, Greenamyre *et al*. (1985) examined the binding of sodium-independent [^{3}H]glutamate in patients with senile dementia of the Alzheimer's type and observed significant reductions in layers I and II (i.e., 45%) and layers V and VI (i.e., 35%) in the superior temporal neocortex of AD patients compared to controls. Binding was not reduced in the caudate, putamen, claustrum, or nucleus basalis of Meynert. Notably, cortical binding for the $GABA_A$ or muscarinic cholinergic receptors was not reduced, thus supporting the notion that glutamate receptors are uniquely affected in AD. In a subsequent study, Greenamyre *et al*. (1987) found marked reductions in NMDA-sensitive binding sites in all areas of the hippocampus and adjacent parahippocampal cortex in subjects with dementia of the AD type compared to controls. Most profound were the reductions observed in the stratum pyramidale (84%) and stratum moleculare (87%) of the CA1 subfield of the hippocampus of brains. In these latter studies, $GABA_A$ receptors were also reduced, although to a lesser extent than glutamate receptors (i.e., 21–46%), and muscarinic receptors were unchanged. Although these earlier studies provided important evidence for the selective loss of NMDA receptors in AD, they nevertheless were conducted without the advantage of highly specific autoradiographic assays for the NMDA receptor. Moreover, in these latter studies, the majority of, if not all, cases were affected with severe AD pathology, thus making it difficult to discern whether receptor loss contributed to the pathology or simply reflected the loss of glutamate receptor-bearing neurons. Accordingly, in a later study, these same investigators sought to reexamine the status of the glutamate receptor in the hippocampus of AD brains using an assay more specific for the NMDA receptor (Penney *et al*., 1990). In addition, binding was examined in 10 AD brains (ranging from early to late disease), 9 controls, and 6 demented non-AD brains. Overall, binding to the NMDA receptor was reduced 50% in the stratum pyramidale of CA1 and 40% in the CA3 fields of AD patients compared to controls and demented non-AD brains. Binding was not correlated with the number of neurofibrillary tangles, age, postmortem delay, or storage time. Although modest decreases were also observed for kainate, muscarinic, and benzodiazepine receptors, the extent of loss for NMDA receptors was much greater than for the other receptors. Notably, AMPA (i.e., quisqualate) receptors were unchanged. The finding that NMDA receptors were decreased to a greater extent than other receptors was taken as evidence by Penney *et al*. (1990) to be consistent with the hypothesis that these receptors are located on the distal portions of hippocampal neurons where they are more vulnerable to the pathologic consequences of AD.

In support of the findings of Greenamyre and colleagues, Represa *et al*. (1988) investigated the binding density of [^{3}H]glutamate and reported significant reductions in NMDA receptor binding ($>40\%$) in the hippocampus of nondemented elderly compared to younger controls. This reduction was significantly greater in AD brains. Moreover, in AD brains the extent to which NMDA receptor binding was reduced in the CA1 subfield was significantly correlated with the density of neurofibrillary tangles and senile plaques. The authors suggested that this decrease in binding was due to cellular degeneration as well as age and AD-related loss of pyramidal neuron dendritic spines.

In agreement with the preceding studies, Simpson *et al*. (1988) examined the binding of D-[^{3}H]aspartate in AD and control brains. D-[^{3}H]aspartate was employed as a marker of glutamate-releasing nerve terminals. Reductions in D-[^{3}H]aspartate-binding density was observed in the temporal cortex and caudate nucleus in AD subjects. The authors stated that the reduction observed in D-[^{3}H]aspartate binding is consistent with a loss of glutamate-releasing terminals in these regions.

Chalmers *et al*. (1990) also studied the distribution and density of sodium-dependent glutamate uptake sites and receptor subtypes in the frontal cortex of patients with senile dementia of AD and age-matched controls. In agreement with Represa *et al*. (1988), D-[^{3}H]asparate binding to sodium-dependent uptake sites was reduced markedly in SAT brains throughout the frontal cortex compared to controls. In addition, [^{3}H]glutamate binding to NMDA receptors was reduced in layers I and II of the frontal cortex of AD brains compared to controls, although no relationship was observed in the AD brain between numbers of senile plaques and levels of glutamate binding. In considering their data, the authors stated that the loss of [^{3}H]asparate binding is consistent with a marked reduction of glutamatergic terminals in the AD frontal cortex and may reflect the degeneration of excitatory corticocortical association fibers (Represa *et al*., 1988).

Reductions in [^{3}H]glutamate binding to NMDA receptors in the AD hippocampus were also observed by Jansen *et al*. (1990). Specifically, they observed significant reductions in binding to the glutamate site of the NMDA receptor in the CA1 region (52%) and entorhinal neocortex (37%) of the AD brain compared to controls. Significant parallel losses were also observed for the quisqualate, serotonin$_2$, and adenosine A1 binding sites (CA1 region) and benzodiazepine, serotonin$_2$, neurotensin, and opioid receptors (entorhinal neocortex). In contrast, the dentate gyrus molecular layer and CA3 regions showed no significant changes in any NMDA-binding site, although reductions were observed for serotonin$_2$

TABLE 20.8 Glutamate Binding in Aged Human

Age	Subjects	Postmortem (hr)	Ligand	Region	Change in AD	Reference
63 ± 4	Control	16 ± 3.2	[^{3}H]Glutamate	Cortex	Decrease	Greenamyre *et al.* (1985)
71 ± 4	AD	11.4 ± 2.9		Caudate	Not Significant (ns)	
				Putamen	ns	
				Nucleus basalis of Meynert	ns	
55–74	Control	5–18	L-[^{3}H]Glutamate	Hippocampus	ns	Geddes *et al.* (1986)
62–85	AD	4–29		Parahippocampal region	ns	
55–73	Control	1.5–30	L-[^{3}H]Glutamate	Hippocampus	ns	Geddes and Cotman (1986)
61–84	AD	1.5–30				
62 ± 3	Control	16 ± 3	[^{3}H]Glutamate	Hippocampus	Decrease	Greenamyre *et al.* (1987)
67 ± 3	AD	19 ± 4		Parahippocampal region	Decrease	
78 ± 5	Control	8 ± 4	[^{3}H]Glutamate	Hippocampus	ns	Cowburn *et al.* (1988)
78 ± 10	AD	10 ± 7		Frontal cortex	ns	
				Temporal cortex	ns	
				Parietal cortex	ns	
				Caudate nucleus	ns	
82–97	Old non-demented		L-[^{3}H]Glutamate	Hippocampus	Decrease[a,b]	Represa *et al.* (1988)
82–97	AD					
84 ± 2	Control	11–23	[^{3}H]Glutamate	Frontal cortex	Decrease[a]	Chalmers *et al.* (1990)
89 ± 2	AD	3–15				
64.4 ± 5.8	Control	15.3 ± 2.4	[^{3}H]Glutamate	Hippocampus	Decrease[a]	Penney *et al.* (1990)
68.3 ± 1.3	Non-AD demented	12 ± 1.5				
70.9 ± 2.7	AD	16 ± 3.4				
70 ± 5	Control	13 ± 5	[^{3}H]Glutamate	Hippocampus	Decrease[a]	Jansen *et al.* (1990)
77 ± 8	AD	17 ± 7				
79 ± 3	Control AH[c]	11 ± 2	[^{3}H]Glutamate	Hippocampus	ns except decrease in CA1	Ulas *et al.* (1992)
81 ± 3	AD AH[c]	8 ± 2				
78 ± 4	Control PH[c]	11 ± 2				
80 ± 3	AD PH[c]	9 ± 3				
72 ± 6	Control	16 ± 4	[^{3}H]Glutamate	Primary visual cortex	Decrease	Carlson *et al.* (1993)
73 ± 9	AD	14 ± 6		Visual association cortex	Decrease[a]	
				Higher-order visual cortex	Decrease	
67 ± 3	Control	10 ± 2	[^{3}H]Glutamate	Caudate, putamen	Increase	Ulas *et al.* (1994)
72 ± 3	Parkinson's disease	7 ± 3		Nucleus accumbens		
72 ± 4	AD	14 ± 3				
74 ± 4	PD/AD	12 ± 3				

[a]Significant change only in certain layers or subfields.
[b]Compared to young controls and old nondemented.
[c]AH: anterior hippocampus; PH: posterior hippocampus.

receptors and binding to opioid and adenosine A1 receptors. Although these studies support a role for the NMDA receptor in AD, they also raise the possibility that changes in multiple receptor subtypes contribute to the symptomatology of AD.

While the vast majority of studies focus on the hippocampus or other brain regions highly vulnerable to the pathologic consequences of AD, Carlson *et al*. (1993) examined the laminar distribution of NMDA-binding sites in the visual cortex. Notably, the number of neurofibrillary tangles increases progressively across primary (area 17), secondary (area 18), and higher-order association visual cortex (areas 19, 20, and 21). These latter observations are consistent with the findings that the dementia in AD results from the loss of the structural and functional integrity of the long corticocortical projection systems. The NMDA receptor complex was detected using [^{3}H]glycine and [^{3}H]glutamate. In area 17 (i.e., primary visual

neocortex), binding to the NMDA receptor was not altered significantly in the AD brain. In contrast, in areas 18 and 19 (i.e., secondary and tertiary association visual cortices), binding to the NMDA receptor was reduced compared to controls. The demonstration that the reduction in NMDA receptor binding parallels the hierarchical pattern of increasing complexity of association visual cortices and with increasing numbers of neurofibrillary tangles in these latter regions is consistent with a role of NMDA receptor-bearing neurons in the pathology of AD (Carlson *et al*., 1993).

D'Aniello *et al*. (1998) examined the levels of free D-aspartate in human brains from AD and control patients. Levels of D-aspartate were found to be reduced in the frontal (43%), parietal (38%), and temporal (35%) cortices, hippocampus (47%), and amygdala (41%). D'Aniello *et al*. (1988) interpreted these data to suggest that decreased levels of D-aspartate could contribute to a lower NMDA receptor function and consequently to the memory deficits seen in AD.

Although a number of studies report reductions of NMDA receptors in AD, still others fail to support a disease-related loss of this receptor subtype. For example, Geddes and Cotman (1986) employed [^{3}H]glutamate to investigate NMDA receptors in the hippocampus of control and AD brains. While no significant changes in the density or distribution of NMDA receptors were observed when comparing AD and control brains, the distribution of NMDA receptors did, however, correlate with the density of AD pathology. These latter data were taken as evidence to suggest that excitotoxic mechanisms may be involved in the pathogenesis of AD. In a second study, Geddes *et al*. (1986) provided further evidence supporting the lack of any change in hippocampal NMDA receptor density in AD patients compared to controls. They do note, however, that in cases displaying major hippocampal neuron loss, reductions in receptor density were observed.

Cowburn *et al*. (1988) also employed [^{3}H]glutamate binding to examine the integrity of NMDA receptors in the caudate, hippocampus, and frontal, temporal, and parietal cortices from control and AD brains and observed no change in binding within any of the regions examined. In contrast, reductions in [^{3}H]asparate binding (40%) were observed in the AD hippocampus compared to controls, thus suggesting a loss of glutamate terminals within this region of the brain. Collectively, these findings led Cowburn and colleagues (1988) to conclude that in AD there is a general integrity of NMDA receptors, at least in the early phases of the disease. However, reductions in [^{3}H]asparate suggest that these receptors may not be appropriately innervated, thus contributing to the learning and memory deficits of AD.

Ulas *et al*. (1992) also employed L-[^{3}H]glutamate and observed a 35% reduction in binding in the CA1 region of AD cases compared to controls. Notably, Ulas *et al*. (1992) demonstrated marked intersubject variability in NMDA receptor binding, with some AD patients showing significant decreases in receptor levels, whereas others displayed no changes or even increased binding. This variability could not be attributed to age, sex, postmortem interval, or even a loss of neurons, thereby leading Ulas and colleagues (1992) to hypothesize that the variability may result from differences in genetic background and/or an accumulation of life experiences that result in distinctive receptor characteristics. Although this hypothesis remains largely untested, it does suggest that the analysis of individual AD cases may prove valuable in determining the role of glutamate in AD. These data also provide a reasonable explanation for discrepant results among the various investigations discussed in this chapter.

Ulas *et al*. (1994) also examined NMDA receptors by *in vitro* quantitative autoradiography in Parkinson's disease and AD patients. Binding to the NMDA receptor by [^{3}H]glutamate was increased in the caudate, the putamen, and the nucleus accumbens in Parkinson's disease, AD, and Parkinson's/AD brains compared to controls. The authors suggest that the increase in NMDA receptors is in response to an insult or insults within the striatothalamocortical circuits, which may contribute to the clinical similarities in AD and PD patients.

2. CPP Binding

As an additional means of assessing the status of the NMDA receptor in AD, several investigators have employed the ligand [^{3}H]CPP, a potent and selective antagonist (Table 20.9). For example, Porter *et al*. (1992) studied the binding of [^{3}H]CPP in synaptic membranes of human brain and found the presence of two distinct binding components for CPP, one of high ($K_d \sim 70$ nM) and the other of low ($K_d \sim 5$ μm) affinity.

TABLE 20.9 CPP Binding in Aged Humans

Age	Subjects	Postmortem (hr)	Ligand	Region	Change in AD	Reference
77 ± 2	Control	20 ± 3	[^{3}H]CPP	Medial frontal cortex	Not significant (ns)	Porter *et al.* (1992)
80 ± 3	AD			Medial temporal cortex	ns	
79 ± 3	Control AH[b]	11 ± 2	[^{3}H]CPP	Hippocampus	ns	Ulas *et al.* (1992)
81 ± 3	AD AH[b]	8 ± 2		Parahippocampal region	Decrease anterior[a]	
78 ± 4	Control PH[b]	11 ± 2		Subiculum	ns	
80 ± 3	AD PH[b]	9 ± 3				
77–80	Control	~ 20	[^{3}H]CPP	Frontal cortex	ns	Porter *et al.* (1993)
77–80	AD	~ 20		Temporal cortex	ns	

[a] Significant change only in certain layers or subfields.
[b] AH: anterior hippocampus; PH: posterior hippocampus.

Examination of the medial frontal and medial temporal cortices of AD and control brains revealed that neither of the two components of [^{3}H]CPP binding were changed in either the AD or the control group. Ulas *et al*. (1992) also examined [^{3}H]CPP-binding levels in the hippocampus using autoradiographic techniques. On average, binding levels were unchanged in the AD brain compared to control brains except in the outer layer of the parahippocampal gyrus (anterior portion). In this latter region, [^{3}H]CPP-binding levels in AD brains were below the levels observed in control brains. Notably, the studies of Ulas *et al*. (1992) and Porter *et al*. (1992) reported marked intersubject variability, as discussed in the preceding section. In addition, Porter *et al*. (1993) reinvestigated the binding of [^{3}H]CPP in cortical synaptic membranes of AD and control brains and again found no significant changes in binding in the temporal and frontal cortices in either group. Consistent with their 1992 study, considerable intersubject variation in binding parameters was detected (Porter *et al*., 1992, 1993). The authors speculate that while changes in receptor integrity occur in individual patients, these changes may be obscured because of the large variations among individuals.

3. MK-801 Binding

In addition to [^{3}H]glutamate and [^{3}H]CPP binding, a number of investigators have examined the NMDA receptor in AD brains using MK-801 binding (Table 20.10). This dibenzocycloheptene imine is a noncompetitive antagonist of the NMDA receptor complex and binds with a high affinity to the NMDA receptor-associated ionophore. In brief, Mouradian *et al*. (1988) found no differences in [^{3}H]MK-801 binding between control and AD patients in homogenates of frontal, parietal, temporal, or occipital cortices or in the hippocampus. The authors note, however, that the use of homogenized tissue may obviate their ability to discern changes within discrete brain regions (i.e., CA subfields of the hippocampus). Procter *et al*. (1989a) also investigated the frontal cortex and observed no change in [^{3}H]MK-801 binding when comparing control to AD brain. However, despite the lack of change, Procter *et al*. (1989b) demonstrated that MK-801 binding was increased in the presence of glycine within both control and AD brains, although the magnitude of change was significantly greater in control brain. Because glycine has a modulatory effect on the NMDA receptor, Procter *et al*. (1989b) suggested that these data support the hypothesis that in AD there is an abnormality in glutamatergic transmission rather than a marked decline in receptor density.

Ulas *et al*. (1992) also investigated MK-801 binding (in the presence of glycine and glutamate). However, unlike the previous studies, these authors observed a 34% decrease in [^{3}H]MK-801 binding in the CA1 stratum pyramidale and significant, yet smaller, decreases in the dentate gyrus, parahippocampal gyrus, and subiculum. All total, decreased binding was observed in 5 out of 16 regions of the brain. In contrast, [^{3}H]glutamate binding was reduced only in the CA1 region (see previous discussion). The authors speculated that the relatively selective vulnerability of the channel-associated recognition site may be related to changes in the NMDA receptor complex or alterations in membrane lipids that are known to occur in AD.

Palmer and Burns (1994) examined the redox, polyamine, and glycine modulatory sites using [^{3}H]MK-801 to bind to the NMDA receptor in the superior frontal and superior tem-

TABLE 20.10 MK-801 Binding in Aged Humans

Age	Subject	Postmortem (hr)	Ligand	Region	Change in AD	Reference
73 ± 2	Control	16 ± 1.9	[^{3}H]MK801	Frontal cortex	No change	Mouradian *et al.* (1988)
75 ± 2	AD	13 ± 1.5		Parietal cortex	No change	
				Temporal cortex	No change	
				Hippocampus	No change	
79	Control	4–48	[^{3}H]MK801	Frontal cortex	Decrease	Procter *et al.* (1989a)
81	AD	5–48		Temporal cortex	Not Significant (ns)	
				Parietal cortex	ns	
				Cerebellar cortex	ns	
79 ± 3	Control AH[b]	11 ± 2	[^{3}H]MK801	Hippocampus	Decrease anterior[a]	Ulas *et al.* (1992)
81 ± 3	AD AH[b]	8 ± 2		Parahippocampal region	Decrease	
78 ± 4	Control PH[b]	11 ± 2		Subiculum	Decrease[a]	
80 ± 3	AD PH[b]	9 ± 3				
73 ± 8	Control	11 ± 7	[^{3}H]Dizocilpine	Superior frontal cortex	ns	Palmer and Burns (1994)
74 ± 7	AD	10 ± 6	(MK801) plus arcaine-polyamine site antagonist	Superior temporal cortex		
75.6 ± 9.8	Control	38.1 ± 11.1	[^{3}H] MK801	Frontal cortex	Decrease	Scheuer *et al.* (1996)
81.2 ± 7.5	AD	30.8 ± 15.2		Parietal cortex	ns	

[a] Significant change only in certain layers or subfields.
[b] AH: anterior hippocampus; PH: posterior hippocampus.

poral cortices of AD brains and controls. The polyamine site antagonist, arcaine, inhibited [^{3}H]MK-801 binding in a dose-dependent fashion in control brains. This binding was unchanged in AD. The inhibition of the redox site was also similar in control and AD brains. Moreover, glycine-stimulated [^{3}H]MK-801 binding was unaffected in the AD brain. Collectively, these data indicate that the NMDA receptor and its modulatory redox, polyamine, and glycine subsites are preserved in AD patients. This preservation may in part reflect a combination of compensatory and regenerative changes that occur in the AD brain, particularly during the earlier phases of the disease.

Scheuer *et al*. (1996) examined [^{3}H]MK-801 binding and observed significant reductions in binding in the frontal cortex (20%), but no changes in the parietal cortex when comparing control and AD brain. The reduction in the frontal cortex occurs independent of AD-specific neuropathologic changes and/or alterations in the composition or structure of the neuronal membrane.

4. TCP Binding

As stated previously, TCP, like MK-801, binds to the cation channel in the NMDA receptor complex. In addition, glutamate has been shown to increase the rate of binding of TCP, thus suggesting that it binds to the activated channel (Table 20.11). With this ligand, Maragos *et al*. (1987) examined the distribution of NMDA-binding sites in subjects with AD and observed significant reductions in [^{3}H]TCP binding in the strata pyramidale of CA1/CA2, CA3, and subiculum. Of note, the reductions were greatest in regions of the hippocampus containing significant AD pathology (i.e., CA1 and subiculum) and least in areas displaying less pathology (i.e., CA3). Consistent with these latter observations, no reduction was observed in the molecular layer of the DG. Because this latter region is known to contain high densities of NMDA receptors but is relatively spared of plaques and tangles (at least until the end stage of the disease), Maragos and colleagues (1987) hypothesized that plaque and/or tangle formation in this region may antedate reductions in the glutamate/TCP receptor complex.

Monaghan *et al*. (1987) also studied the density and distribution of [^{3}H]TCP-binding sites in the human hippocampus of control and AD brains. Consistent with work of Maragos *et al*. (1987), AD patients showed significant loss of binding in the CA1 strata pyramidale/radiatum (40%). No change was seen in the molecular layer of the dentate gyrus or strata pyramidale/lucidum of CA3. Collectively, these data support the theory that the reduction of TCP binding in the CA1 region of the hippocampus in AD corresponds to the selective loss of neurons in this region.

Simpson *et al*. (1988) also utilized the binding of [^{3}H]TCP in AD brains to study the NMDA receptor. Binding of [^{3}H]TCP showed a reduction only in the frontal cortex of AD subjects. Binding in the temporal cortex, head of the caudate, and hippocampus were unchanged in AD. Of interest, the temporal cortex and caudate nucleus were also characterized by a loss of glutamate terminals (i.e., [^{3}H]aspartate binding). In explaining these data, Simpson *et al*. (1988) suggested that on the one hand, the loss of [^{3}H]TCP-binding sites in the frontal cortex is indicative of the degeneration of glutamate receptor-bearing cells receiving a glutamatergic innervation. On the other hand, the integrity of these binding sites in the temporal cortex (i.e., a region of frank pathology and cell loss) suggests that if glutamate causes neuronal degeneration, it must do so in a way that spares cells bearing NMDA receptors. Moreover, the demonstration that some regions of the brain are characterized by reductions in [^{3}H]aspartate whereas [^{3}H]TCP is unchanged suggests that a loss of glutamate terminals is not necessarily linked to changes in postsynaptic NMDA receptors.

Ninomiya *et al*. (1990) also studied the binding of [^{3}H]TCP in the frontal cortex of AD and control patients. In AD brains, the total concentration (B_{max}) of binding sites was reduced significantly by 40–50%. [^{3}H]TCP binding exhibited a linear

TABLE 20.11 TCP Binding in Aged Humans

Age	Subject	Postmortem (hr)	Ligand	Region	Change in AD	Reference
60 ± 6	Control	22 ± 6	[^{3}H]TCP	Hippocampus	Decrease[a]	Maragos *et al.* (1987)
62 ± 5	Non-AD dementia	14 ± 2		Subiculum	Decrease	
74 ± 3	AD	14 ± 3		Parahippocampal region	Decrease	
	Control		[^{3}H]TCP	Hippocampus	Decrease in CA1	Monaghan *et al.* (1987)
73 ± 5	Control	37 ± 5	[^{3}H]TCP	Temporal cortex	Not significant (ns)	Simpson *et al.* (1988)
77 ± 3	AD	35 ± 9		Frontal cortex	Decrease	
				Caudate nucleus	ns	
				Hippocampus	ns	
79 ± 2	Control	9 ± 1.6	[^{3}H]TCP	Frontal cortex	Decrease	Ninomiya *et al.* (1990)
81 ± 3	AD	9 ± 1				
70 ± 5	Control	13 ± 5	[^{3}H]TCP	Hippocampus	Decrease in CA1	Jansen *et al.* (1990)
77 ± 8	AD	17 ± 7		Entorhinal cortex	Decrease	

[a]Significant change only in certain layers or subfields.

correlation with NMDA-sensitive [^{3}H]glutamate-binding sites. These data suggest that the primary change in the NMDA receptor–ion channel complex in AD brain is the reduction of its number, possibly reflecting the loss of neurons bearing these receptor complexes. In regions where [^{3}H]TCP-binding sites are spared, they also retained their affinity for glutamate and reactivity to NMDA, L-glutamate, and glycine. These latter data suggest that those receptor complexes spared in AD are functionally normal.

Jansen *et al*. (1990) also examined [^{3}H]TCP binding in the hippocampus of AD and control brains. Reductions were observed in the CA1 field (42%), CA3 field (34%), and entorhinal neocortex (36%). Because the three NMDA-linked sites (i.e., glycine, L-glutamine, and phencyclidine) show congruent changes in AD, the authors suggested that these sites are allosterically linked in this region of the brain.

5. Glycine Binding

The demonstration that glycine enhanced the *in vitro* responses of cortical neurons to NMDA led some to speculate that the NMDA type of glutamate receptor has an agonist (NMDA) site and a strychnine-insensitive glycine-binding site, which show allosteric interactions and together regulate the binding of phencyclidine and related compounds (i.e., TCP, MK-801) to a site in the open ion channel. Because of its putative important role in glutamate receptor functioning, a number of investigators have sought to determine whether binding to the glycine site is disrupted in AD (Table 20.12). For example, Jansen *et al*. (1990) examined [^{3}H]glycine binding in the hippocampus of AD and control brains and observed reductions in the CA1 (44%) and CA3 subfields (34%), as well as the entorhinal neocortex (40%). Del Bel and Slater (1991) also observed significant reductions in [^{3}H]glycine binding in membranes prepared from the frontal cortex of AD brains compared to controls. In contrast, binding in the temporal cortex, hippocampus, and caudate nucleus remained unchanged. Moreover, autoradiograms showed no significant differences between controls and AD in any of the areas examined. These latter data are consistent with the autoradiographic studies of Ulas *et al*. (1992), who, following examination of the AD and control hippocampi, failed to observe significant differences in levels of binding to the glycine modulatory site within any of seven hippocampal subfields. Similar to their results obtained for NMDA-sensitive binding sites, Ulas *et al*. (1992) noted that some AD patients exhibited [^{3}H]glycine binding below the level of binding for any control whereas levels in others were maintained or even increased. This intersubject variability underscores the marked diversity (i.e., stages) of AD subjects and may well help us understand the many conflicting reports on NMDA responses in AD.

6. Immunocytochemistry of the NMDA Receptor

Utilizing immunocytochemical methods, Aronica *et al*. (1998) investigated the localization of glutamate receptor subunits in the hippocampus of AD and control brains. In vulnerable regions of the hippocampus (i.e., CA1), NMDAR1 immunolabeling was reduced, presumably due to cell loss. Notably, of those surviving cells the intensity of staining was comparable to and, in many instances, greater than controls. An increase in NMDAR1 immunolabeling in the hippocampus was also observed by Ikonomovic *et al*. (1999). In the latter study, Ikonomovic *et al*. (1999) examined subjects presenting with a wide range of pathologic severity (i.e., Braak stages I–VI). Whereas small variations in the pattern of immunolabeling were observed in control cases, AD brains were characterized by marked intersubject variability. For example, AD cases with mild to modest AD pathology (i.e., Braak stages I–III) were largely indistinguishable from controls in the overall pattern of immunolabeling. In contrast, in those cases with more severe AD pathology (i.e., Braak stages IV–VI), the intensity of immunolabeling was often more intense than in controls or cases with mild AD pathology. Moreover, in pathologically severe cases, numerous NMDAR1-positive pyramidal cells, particularly in the CA1 and subiculum, were characterized by darkly stained, long, and often tortuous apical dendrites. In contrast to the aforementioned pattern of immunolabeling, a reduction in NMDAR1 immunolabeling was observed within the outer molecular layer (i.e., the termination zone of the perforant pathway). This latter region was also the site of a number of NMDAR1-labeled plaques. The reduction of NMDAR1 immunolabeling within the termination zone of the perforant pathway is consistent with the AD-related loss of

TABLE 20.12 Glycine Binding in Aged Humans

Age	Subject	Postmortem (hr)	Ligand	Region	Change in AD	Reference
70 ± 5	Control	13 ± 5	[^{3}H]glycine	Hippocampus	Decrease in CA1	Jansen *et al.* (1990)
7 ± 8	AD	17 ± 7		Entorhinal cortex	Decrease	
69.8 ± 4.1	Control	30 ± 4	[^{3}H]glycine	Temporal cortex	Decrease	Del Bel and Slater (1991)
71.6 ± 6.3	AD	36 ± 3		Hippocampus	Near significant	
				Frontal cortex	Not significant	
				Caudate nucleus	Not significant	
72 ± 6	Control	16 ± 4	[^{3}H]glycine	Primary visual cortex	Decrease	Carlson *et al.* (1993)
73 ± 9	AD	14 ± 6		Visual association cortex	Decrease[a]	
				Higher-order visual cortex	Decrease	

[a]Significant change only in certain layers or subfields.

perforant pathway fibers and concomitant reduction in postsynaptic glutamate receptors. Moreover, the loss of NMDA receptors within the outer molecular layer provides a strong cellular/molecular correlate underlying AD-associated memory impairment.

7. *In Situ* Hybridization of NMDA Receptor

To date, relatively few investigators have studied the expression of selected NMDA receptor subunit mRNAs in AD. For example, Ulas and Cotman (1997) examined the expression of NMDA receptor mRNAs in the hippocampus and entorhinal and perirhinal cortices in AD and control brains and observed significantly reduced levels of NMDAR1 mRNA in layer III of the entorhinal cortex as well as in layers II, III, and IV–VI of the perirhinal cortex. Notably, layer II/III of the entorhinal cortex was affected severely in AD and therefore the reduction in NMDAR1 levels was a predictable corollary to the loss of neurons in this region. However, not all vulnerable sectors of the hippocampus displayed reductions in mRNA levels. For example, the CA1 region was characterized by severe neuronal damage in AD, yet NMDAR1 mRNA levels were not reduced significantly in the AD brain. In contrast, in the granule cell layer of the dentate gyrus (i.e., a region far less affected with AD pathology), substantial, yet nonsignificant, reductions in NMDAR1 mRNA levels were observed. The lack of significance within this latter region can reasonably be attributed to a relatively small sample size as well as considerable intersubject variation in NMDAR1 mRNA levels. Comparable intersubject variations were reported by Mishizen *et al*. (1999) in studies of NMDAR1, NR2A, and NR2B mRNA levels within the hippocampus of control and AD brains. Notably, Ulas and Cotman (1997) found no significant relationship between mRNA levels and postmortem delay, tissue storage, age of the subject, or minimental status. These findings suggested that mRNA levels may be affected by additional factors such as the agonal state. To date, relatively few studies screen individual subjects in order to evaluate the integrity of specific mRNAs. Such prospective analyses may be essential in order to determine whether changes in mRNA levels are indeed disease specific or the result of nonspecified factors possibly related to death but unrelated to the disease process.

B. AMPA Receptors

1. AMPA Binding

To date, a number of investigators have examined the integrity of AMPA receptors in AD (Table 20.13). Similar to studies of the NMDA receptor, the results of these investigations are inconsistent. For example, Chalmers *et al*. (1990) examined levels of [^{3}H]AMPA within the frontal cortex of AD and control brains and observed no significant changes in either group. Moreover, [^{3}H]AMPA binding in the AD frontal cortex was unrelated to senile plaque number. Likewise, Ulas *et al*. (1994) investigated [^{3}H]AMPA binding in the striatum and nucleus accumbens of patients with AD, Parkinson's disease or both and aged-matched controls and observed no changes in binding among the various groups. As discussed in a previous section, binding to the NMDA receptor was increased substantially within the three patient groups. These latter data support a unique role for the NMDA receptor within striatothalamocortical circuits and may in fact represent a compensatory reaction following damage to this circuit.

In contrast, reductions in [H^3]AMPA binding were observed within several brain regions by Dewar *et al*. (1991). Specifically, autoradigraphic studies demonstrated reductions in [^{3}H]AMPA binding in superficial and deep layers of the parahippocampal gyrus, subiculum, and CA1 region. Furthermore, the reduction in binding was correlated with the degree of local neuronal degeneration. Additional confirmation for a reduction in [^{3}H]AMPA binding in AD brains comes from the work of Carlson *et al*. (1993). In their studies of the visual cortex, Carlson *et al*. (1993) observed reductions in [^{3}H]AMPA binding in layer II of area 17, as well as layers III–VI of area 18. No changes were observed within any layer of the higher order visual association cortex area 21. As discussed in a previous

TABLE 20.13 AMPA Binding in Aged Humans

Age	Subject	Postmortem (hr)	Ligand	Region	Change in AD	Reference
75 ± 3	Control	10 ± 2	[^{3}H]AMPA	Hippocampus	Decrease[a]	Dewar *et al.* (1991)
82 ± 3	AD	11 ± 2		Parahippocampus	Decrease	
71–91	Control	4–21	[^{3}H]AMPA	Hippocampus	Decrease[a]	Geddes *et al.* (1992)
73–91	AD	2–23				
72 ± 6	Control	16 ± 4	[^{3}H]AMPA	Visual cortex	Decrease[a]	Carlson *et al.* (1993)
73 ± 9	AD	14 ± 6				
67 ± 3	Control	10 ± 2	[^{3}H]AMPA	Caudate, putamen	Not significant	Ulas *et al.* (1994)
72 ± 3	Parkinson's disease	7 ± 3		Nucleus accumbens		
72 ± 4	AD	14 ± 3				
74 ± 4	Parkinson's disease/AD	12 ± 3				

[a]Significant change only in certain layers or subfields.

section, the visual cortex is characterized by hierarchical levels of neuropathologic severity with higher order visual cortex > association cortex > primary visual cortex. Whereas levels of NMDA binding adhered to this hierarchical scheme (i.e., reductions were greatest in regions with most intense pathology), AMPA receptor levels did not parallel the pathology. The authors suggested that these latter data may imply that AMPA receptors do not play a direct role in the AD pathology.

Geddes *et al*. (1992) investigated the density and distribution of [^{3}H]AMPA-binding sites in the hippocampus and parahippocampal gyrus in AD and control brains. On average, binding levels were similar between control and AD brains even in regions of the hippocampus characterized by considerable cell loss (i.e., CA1). Further analyses revealed no correlation between levels of [^{3}H]AMPA binding and neuronal density. Although Geddes *et al*. (1992) observed [^{3}H]AMPA binding to be preserved within the vast majority of hippocampal subfields, a couple exceptions were reported in AD. These included a 20% decrease in the outer molecular layer of the dentate gyrus and a 26% increase in the polymorphic region of the dentate gyrus. Although the mechanism underlying the increase in binding is unclear, Geddes and colleagues (1992) noted that it may reflect a subcortical pathology or seizures as both have been reported to result in the compensatory upregulation of postsynaptic receptors.

2. Immunocytochemistry of AMPA Receptors

As a corollary to receptor autoradiographic studies of AMPA receptors, a few laboratories have sought to examine the integrity of the AMPA receptor in AD using immunocytochemical techniques. For example, Hyman *et al*. (1994) examined the hippocampus and temporal cortex of AD and control brains with antibodies to the GluR1, GluR2/3, and GluR4 subunits of the AMPA receptor. On average, the pattern of staining between control and AD hippocampus was similar except for the pattern of staining for GluR2/3 in the molecular layer of the dentate gyrus. In control brains the staining was homogeneous throughout the depth of the molecular layer. However, in 3 of the 15 AD brains, GluR2/3 immunolabeling appeared more intense in the inner portion of the molecular layer than the outer two-thirds. The authors noted that the increase in staining may represent an upregulation of receptors on deafferented neurons or receptors on the terminals of axons sprouting into this region.

Additional immunohistochemical studies of AMPA receptors come from the work of Armstrong and colleagues (Armstrong *et al*., 1994, Ikonomovic *et al.*, 1995a,b, 1997; Ikonomovic and Armstrong, 1996). Initial studies of the entorhinal cortex revealed GluR2/3-labeled cells distributed abundantly throughout layers II, III, V, and VI (Armstrong *et al*., 1994). In AD, there was a profound loss of GluR2/3 labeling and protein concentration (Armstrong *et al*., 1994; Yasuda *et al*., 1995). However, adjacent Nissl-stained tissue sections revealed substantial cell loss in the entorhinal cortex in AD, thus providing a reasonable explanation for the loss of GluR2/3 protein. In contrast, brains with relatively mild AD pathology revealed a dramatic loss of GluR2/3 immunolabeling but little if any loss of layer II neurons following examination of Nissl-stained tissue sections (Armstrong *et al*., 1994). These latter findings support the notion that a decrease in GluR2/3 immunolabeling within layer II neurons precedes the loss of these cells. In subsequent studies, double-labeling techniques were employed to examine the distribution of GluR2/3 and an anti-tau monoclonal antibody (i.e., MC1) within the entorhinal cortex of cases with varying degrees of AD pathology (Ikonomovic *et al*., 1997). In addition, near adjacent sections were immunolabeled with GluR2/3 and a marker of normal neuronal cytoskeleton (i.e., MAP2). In those cases with relatively mild AD pathology, most layer II entorhinal cortex neurons were double labeled with GluR2/3 and MAP2. Occasionally, MC1-labeled cells were observed, although in no instance were these neurons double labeled with GluR2/3. In cases with moderate AD pathology, layer II neurons exhibited a substantial loss of GluR2/3-labeled neurons, whereas the number of MAP2-labeled neurons remained stable. Notably, the loss of GluR2/3-labeled neurons was accompanied by an increase in the number of MC1-labeled neurons. In no instance were GluR2/3 and MC1 colocalized within the same neurons. In cases with severe AD pathology, virtually no GluR2/3-labeled neurons were observed in the entorhinal cortex. In addition, the number of MAP2-positive cells was diminished greatly. In contrast, MC1-labeled neurons were abundant. That GluR2/3 and MC1 were not observed in the same neuron, together with the observation that the number of GluR2/3-labeled neurons decreases as the number of MC1-positive cells increases, supports the notion that a loss of GluR2/3 immunolabeling precedes the appearance of MC1 immunolabeling (Ikonomovic *et al*., 1997).

As a corollary to studies of the entorhinal cortex, Armstrong and colleagues investigated the anatomical organization of GluR2/3 and GluR1 within the hippocampus of control and AD brains (Ikonomovic *et al*., 1995a,b). Within the dentate gyrus, it is well known that glutamatergic afferents arising from layer II neurons in the entorhinal cortex terminate within the outer two-thirds of the molecular layer on the apical dendrites of granule neurons (Hyman *et al*., 1994). Within this termination zone of the perforant pathway, Ikonomovic *et al*. (1995a) observed dense immunolabeling for the AMPA receptor subunits, with GluR1 displaying a greater intensity than GluR2/3. In a number of pathologically severe AD cases, the intensity of GluR1 and GluR2/3 immunolabeling within the dentate gyrus, particularly the molecular layer, was increased substantially. Because the perforant pathway is known to be disrupted in AD, it was hypothesized that the increase in GluR immunolabeling in the dentate gyrus is compensatory in nature, resulting from the loss of glutamatergic (i.e., perforant) pathway fibers (Armstrong and Ikonomovic, 1996). In support of this hypothesis are studies in rat demonstrating increases in GluR1 and, to a lesser extent, GluR2/3 within the molecular layer of the dentate gyrus following perforant pathway lesions (Mizukami *et al*., 1997a,b).

In CA fields, pyramidal neurons also displayed intense immunolabeling for GluR1 and GluR2/3. As discussed in detail (Ikonomovic *et al*., 1995a), these receptor subunits display a differential distribution on pyramidal neurons with GluR1 largely associated with processes and GluR2/3 with somas. In vulnerable sectors of the AD hippocampus (i.e., CA1, subiculum), a variable reduction in GluR1 and GluR2/

3 immunolabeling was observed that appeared to correlate with the extent of cell loss and neurofibrillary tangles (Ikonomovic *et al*., 1995b). Despite the reduction in labeled cells, the intensity of immunolabeling within the remaining cells was comparable, if not greater, than that observed in controls. Within less vulnerable regions of the hippocampus (i.e., CA2/3), the staining pattern was comparable between control and AD brains, although the intensity of imunolabeling was increased greatly in AD cases, particularly in the stratum lucidum of the CA3 regions (i.e., termination zone of mossy fibers). Collectively, these data support that hippocampal plasticity is preserved even in cases displaying severe AD pathology and suggest a critical role for AMPA receptor subunits in this plasticity and in maintaining hippocampal functioning.

The hippocampus is also the site of intense neuropathology in AD, and therefore Ikonomovic *et al*. (1997) performed a series of double-labeled studies to examine the temporal and spatial relationship between GluR2/3- and MC1-labeled neurons within vulnerable sectors of the AD hippocampus. Similar to studies in the entorhinal cortex, cells double labeled for GluR2/3 and MC1 were never observed in any CA1 subregion (Ikonomovic *et al*., 1997). Collectively, these data support the hypothesis that the GluR2/3 subunit is lost prior to the development of neurofibrillary tangles and therefore suggest that the decrease and/or loss of this receptor subunit may be important in the evolution of neurofibrillary changes via a mechanism involving the increase of intracellular calcium.

In addition to the hippocampus, the basal forebrain is another region of the brain that is particularly vulnerable in AD. Although basal forebrain neurons are a rich source of acetylcholine throughout the neocortex and hippocampus, these neurons are densely innervated by glutamatergic fibers and therefore excitotoxic mechanisms may contribute to their death and degeneration in AD. Immunolabeling studies of basal forebrain cholinergic neurons of nondemented elderly revealed both somata and processes of magnocellular neurons within the posterior nucleus basalis of Meynert to be intensely immunoreactive to GluR1 but not GluR2/3 (Ikonomovic and Armstrong, 1996). In contrast, neurons within the more rostral diagonal band region expressed both GluR1 and GluR2/3. Notably, the posterior aspect of the basal forebrain is more vulnerable to pathologic insult than anterior regions. The fact that GluR2/3 is selectively absent from neurons in the posterior (i.e., vulnerable) nucleus basalis of Meynert is consistent with the notion that glutamate receptors assembled without GluR2 are apt to be more permeable to calcium. This affects the ability of the cell to maintain intracellular calcium homeostasis, a critical step in determining the susceptibility of a cell to glutamate toxicity and for its selective vulnerability. Notably, investigation of the basal forebrain from younger subjects (i.e., 5–55 years of age) revealed intense GluR1 and GluR2/3 immunolabeling within posterior and anterior aspects of the nucleus basalis of Meynert, thus suggesting that the paucity of GluR2/3 labeling observed in older subjects is age related. Functionally, a ''switch'' in subunit composition, such that the assembled receptor no longer contains GluR2/3, has serious implications with respect to the responsiveness of these neurons to glutamate receptor activation via the AMPA receptor.

In addition to the studies of Armstrong and colleagues, Aronica *et al*. (1998) examined the staining pattern of GluR2(4) and GluR1 in the hippocampus of AD and control brains. On average, all projection neurons in the hippocampus were immunolabeled for GluR1 and GluR2(4). Specifically, GluR2(4) was characterized by dense cytoplasmic staining, especially in the neuropil of CA1–4. In addition, intense labeling was observed in CA4 polymorphic neurons, CA3 pyramidal neurons, and pyramidal cell somata and apical dendrites of CA1 and subiculum. In most instances, the staining pattern between controls and AD brains was comparable with the exception of the molecular layer of the dentate gyrus. In this latter region, staining was relatively similar in the inner and outer layers of control brains, whereas in the AD brain the inner layer was stained more intensely compared to the outer layer. There was also a decrease in GluR1 and GluR2(4) immunoreactivity noted in the CA1 field of AD brains possibly due to cell loss. Of note, GluR1 and GluR2(4) immunolabeling was observed in novel juxtaneuronal clusters localized to dystrophic dendrites within the neuropil of the CA1 pyramidal layer. The precise neuropathologic characterization of these latter structures is unknown.

3. I*n Situ* Hybridization of AMPA Receptor

As a corollary to immunohistochemical studies of AMPA receptor subunits, a few investigators have employed *in situ* hybridization methods to examine messenger RNAs for each of the AMPA receptor subunits. In brief, Pellegrini-Giampietro *et al*. (1994) examined the expression of GluR1, GluR2, and GluR3 AMPA receptor subunits in AD and nondemented controls and observed no changes in GluR1 message levels in the AD hippocampus. Because GluR2 and GluR3 displayed relatively low hybridization signals, they could not be quantitated reliably. Of note, considerable intersubject variability was observed with some samples showing marked reductions in GluR1 mRNA within the dentate gyrus, CA1, and CA3 relative to controls whereas others showed no changes. When hybridization densities were correlated with a number of pre- and postmortem variables, a negative correlation was found between GluR1 mRNA levels in the dentate gyrus and storage duration of brain samples. These data led the authors to conclude that all differences in mean expression levels between AD and control samples could be ascribed to storage time. The latter findings underscore the importance of taking into account various pre- and postmortem variables prior to making any conclusions as to mRNA changes in brains of patients with AD or any other neurologic disease. In contrast, García-Ladona *et al*. (1994) reported reductions in GluR1–4 levels within the hippocampus of AD subjects relative to controls. However, the significance of these latter findings is not clear in light of the fact that only four AD subjects were examined and no quantitative data were presented.

In addition to *in situ* hybridization studies, Akbarian *et al*. (1995) employed reverse transcriptase-polymerase chain reaction, restriction endonuclease digestion, gel electrophoresis, and scintillation radiometry to determine the proportions of edited and unedited GluR2 RNA within the frontal neocortex of brains from patients with AD and age-matched controls. As stated previously, the posttranscriptional editing of the transcript of the GluR2 gene results in the substitution of an arginine for glutamine in the second transmembrane region (TMII/

pore loop segment) of the expressed protein. The presence of the positively charged arginine is associated with a reduction in Ca^{2+} permeability of the receptor channel. In brief, Akbarian and colleagues (1995) determined that in the prefrontal neocortex of control patients, <0.1% of all GluR2 RNA molecules were unedited (i.e., contains a CAG codon for glutamine) whereas >99.9% were edited (i.e., contains a CGG codon for arginine). In the prefrontal cortex of AD patients, ~1.0% of all GluR2 RNA molecules were unedited and 99% were edited. Although the near 11-fold increase in unedited GluR2 was highly significant, it remains true that the overwhelming majority of GluR2 molecules exist in the edited form. Nevertheless, Akbarian *et al*. (1995) suggested that disturbances in GluR2 RNA editing leading to excessive Ca^{2+} permeability may contribute to neuronal dysfunction in AD.

C. Kainate Receptors

1. Kainate Binding

To date, a number of investigators have examined kainate (KA)-binding densities within AD brains (Table 20.14). For example, Geddes and Cotman (1986) studied the density and distribution of kainate receptors in the hippocampus of AD and control brains and on average found binding density very similar between the two groups, except in the stratum lucidum where a 30% decrease was observed in the AD brain. In addition, in the inner part of the dentate molecular layer (i.e., supragranular layer), Geddes and Cotman (1986) observed a near 75% expansion of the distribution of KA-binding sites. These latter data are consistent with the notion that the AD brain maintains a considerable degree of plasticity and that the progression of the disease is not simply associated with the ongoing loss of neuronal elements. A significant AD-related loss of KA binding in the stratum lucidum of CA3 (i.e., terminal fields of mossy fibers) was likewise observed by Represa *et al*. (1988). However, in contrast to the findings of Geddes and Cotman (1986), these latter investigators failed to observe any expansion of the distribution of KA-binding sites in the supragranular layer of the dentate gyrus. Rather, within this region a significant loss of KA binding was found (Represa *et al*., 1988). To reconcile these discrepant findings, Represa *et al*. (1988) suggested that it is the severity of the neuropathology rather than any methodological issues that explains these differences. Moreover, Represa and colleagues (1988) contended that their inability to support a significant sprouting response of mossy fibers is consistent with a loss of plasticity in AD [a view contrary to the one held by Geddes and Cotman (1986)]. The authors of this chapter hold the position that neuronal plasticity is an innate property of the brain and plays a significant role in the early and moderate stages of AD. However, these compensatory reserves can be overwhelmed with the progression of the disease and, in most circumstances, are largely lost throughout the terminal (i.e., end stages) of the disease. Thus, while characterizing specific aspects of neuronal plasticity, it is critical that it is done so within the context of neuropathological staging.

TABLE 20.14 Kainate Binding in Humans

Age	Subject	Postmortem (hr)	Ligand	Region	Change in AD	Reference
78±5	Control	8±4	[^{3}H]KA	Parietal cortex	Not significant(ns)	Cowburn *et al.* (1989)
78±10	AD	10±7		Temporal cortex	ns	
				Frontal cortex	ns	
				Hippocampus	ns	
				Caudate	ns	
64.4	Control	15.3	[^{3}H]KA	Hippocampus	Decrease in CA1[a]	Penney *et al.* (1990)
70.9	AD	23.4				
68.3	Non AD-demented	12				
84±2	Control	11–23	[^{3}H]KA	Frontal cortex	Decrease[a]	Chalmers *et al.* (1990)
89±2	AD	3–15				
75±3	Control	10±2	[^{3}H]KA	Hippocampus	Decrease[a]	Dewar *et al.* (1991)
82±3	AD	11±2		Parahippocampus	Decrease	
71–91	Control	4–21	[^{3}H]KA	Hippocampus	ns increase	Geddes *et al.* (1992)
73–91	AD	2–23			in outer 2/3 DGML[b,c]	
67±3	Control	10±2	[^{3}H]KA	Caudate, putamen	No change	Ulas *et al.* (1994)
72±3	Parkinson's disease	7±3		Nucleus accumbens		
72±4	AD	14±3				
74±4	Parkinson's disease/AD	12±3				

[a] Significant change only in certain layers or subfields.
[b] DGML, dentate gyrus molecular layer.
[c] In some but not all patients.

Additional studies of KA binding were conducted by Cowburn *et al*. (1989) using membrane preparations of human neocortex, hippocampus, and caudate. No changes in either the affinity or the number of KA receptors were observed in any of the regions examined, despite the loss of neocortical and hippocampal glutamatergic terminals. These data led Cowburn *et al*. (1989) to conclude that glutamatergic dysfunction in AD is primarily a result of pyramidal cell deafferentation with a relative sparing of postsynaptic glutamate receptors. That KA receptors are preserved in AD is additionally supported by subsequent studies by Geddes and colleagues (1992) and Ulas *et al*. (1994). In fact, Geddes *et al*. (1992) noted that not only were KA receptors unchanged in the AD hippocampus, but in some individuals (i.e., 3 out of 10), increases in KA binding were observed in the outer half of the dentate gyrus. Further evidence for an increase in KA binding comes from the work of Chalmers *et al*. (1990), who observed a 70% increase in KA receptor binding density in the deep layers of the frontal neocortex compared to controls. No changes were observed in the superficial layers of the neocortex. The increases in KA binding were interpreted as representative of a regenerative change in the AD brain in response to the loss of glutamatergic afferents.

In contrast to the findings described previously, Penney *et al*. (1990) observed significant decreases in [^{3}H]KA binding in the stratum pyramidale of CA1 and the prosubiculum in AD brains (i.e., areas of severe neuropathology). However, the magnitude of this reduction was less than that observed for NMDA and AMPA receptor, possibly reflecting a differential distribution of these receptor subtypes on pyramidal cell dendrites. Dewar *et al*. (1991) also compared kainate binding in normal and AD subjects and observed in the AD brain reductions in [^{3}H]KA binding in the superficial and deep layers of the parahippocampal gyrus as well as the subiculum. Notably, NMDA and AMPA binding were variably affected in these latter regions, again possibly reflecting the localization of these three binding sites on neuronal elements, which are differentially susceptible to AD-mediated neuronal degeneration. Specifically, it is hypothesized that those binding sites localized to more distal dendritic locations will undergo a greater degree of loss as distal dendrites and spines of pyramidal neurons are thought to be affected early in AD. In addition, Aronica *et al*. (1998) confirmed that KA receptors are localized to the somatodendritic components of pyramidal neurons utilizing immunohistochemical techniques.

V. Current Topics of Glutamate Toxicity in Alzheimer's Disease

A. Balanced Actions of Excitatory and Inhibitory Systems

The role of Ca^{2+} and perturbed intracellular Ca^{2+} homeostasis ($[Ca^{2+}]_i$) has been implicated in the pathogenesis of a number of acute and chronic neurodegenerative disorders, including AD (Khachaturian, 1989; Mattson, 1992). Glutamate-related excitotoxicity is believed to account, at least in part, for the selective neuronal death within brain regions vulnerable to AD pathology (e.g., hippocampus, entorhinal neocortex) (Greenamyre and Young, 1989). The function and activity of large pyramidal neurons within these latter regions are regulated through a delicate balance between their presynaptic excitatory (i.e., glutamatergic, cholinergic) and inhibitory (i.e., GABAergic) neurotransmitter systems. This regulation largely depends on the distribution, density, and molecular composition of synaptic sites involving glutamatergic (i.e., NMDA, AMPA/kainate), cholinergic (i.e., muscarinic), and GABAergic (i.e., GABA) receptors. Notably, the balanced interaction between excitatory and inhibitory systems ensures normal physiological function of pyramidal neurons. However, the disturbed balance between these two opposing neurotransmitter systems may initiate a process of neuronal degeneration and lead to excitotoxic cell death (Mattson and Kater, 1989). Specifically, whereas individual excitatory effects of glutamatergic and cholinergic inputs may not be damaging to a cell, the additive effects of these two systems may be sufficient to cross a threshold level for the induction of excitotoxic neurodegeneration. Similarly, a disease-related hypofunction of the GABAergic inhibitory system, resulting in the dysinhibition of neurons, may significantly disturb the normal neurotransmitter balance, resulting in cells that are exposed to the damaging effects of glutamate overstimulation. Each one of these two scenarios may result in an increased flow of Ca^{2+} into a cell, which, if sustained for a prolonged period of time, disturbs $[Ca^{2+}]_i$ homeostasis and endangers cell viability. These Ca^{2+}-related events form the basis of excitotoxic cell damage (Siesjö *et al*., 1989; Mattson, 1992).

B. Glutamate Activation of Calcium-Permeable Ion Channels

Glutamate-dependent disturbances of $[Ca^{2+}]_i$ have been implicated in excitotoxic neuronal death (Choi, 1985, 1987). Figures 20.1–20.4 (see color insert) illustrate the interactions of numerous intra- and extracellular mechanisms that may participate in the regulation of $[Ca^{2+}]_i$ homeostasis. Whereas undisturbed functioning of these mechanisms is crucial for proper development, synaptogenesis, and the participation of a cell in neuronal networks, their malfunctioning may lead to a number of pathologic changes and ultimately cell death. As shown in Fig. 20.1 (see color insert), glutamate-gated Ca^{2+}-permeable receptor channels in the plasma membrane represent the main entry routes for Ca^{2+} into the cell. Whereas NMDA receptors, a class of highly Ca^{2+}-permeable ionotropic glutamate receptors, have been traditionally regarded as mediators of excitotoxic neurodegeneration, increasing attention has been given to the role that Ca^{2+}-permeable non-NMDA (i.e., AMPA/kainate) receptors may play in excitotoxic cell damage (Lu *et al*., 1996). As stated in Section I, Ca^{2+} permeability of AMPA receptor channels is largely regulated by a single gene encoding an individual receptor subunit (i.e., GluR2) (Burnashev *et al*., 1992; Jonas and Burnashev, 1995; Swanson *et al*., 1997). Specifically, the presence of an edited form of the GluR2 subunit in the AMPA receptor complex diminishes Ca^{2+} flow through the receptor-associated ion channel. The disease-related, downregulation of the GluR2 subunit has been demonstrated to participate in a number of neurodegenerative conditions, including global ischemia and limbic seizures (see Pellegrini-Giampietro *et al*., 1997). In addition, loss of the GluR2 subunit may account for the selec-

tive neuronal degeneration in vulnerable regions of AD brains (Armstrong *et al*., 1994; Ikonomovic and Armstrong, 1996; Ikonomovic *et al*., 1997). These observations have led to formulation of the ''GluR2 hypothesis'' of excitotoxic neurodegeneration (Pellegrini-Giampietro *et al*., 1997).

In addition to ionotropic glutamate receptor channels, Ca^{2+} may enter a cell through voltage-dependent Ca^{2+} channels (VDCC) and a class of metabotropic glutamate receptors (mGluR) linked to IP3 production, which induces the release of Ca^{2+} from its intracellular stores (Fig. 20.1). Because glutamate can activate multiple receptor classes, it is important to note that it is the additive effect of receptor activation and the net $[Ca^{2+}]_i$ accumulation, rather than the type of channels through which Ca^{2+} enters a cell, that is a critical determinant of excitotoxic cell injury (Lu *et al*., 1996).

C. Neuroprotective Actions of Neurotrophins

Damaging effects of sustained $[Ca^{2+}]_i$ elevations are normally countered by protective mechanisms, including those regulated by neurotrophic factors. Neurotrophic factors (nerve growth factor, basic fibroblast growth factor, and brain-derived neurotrophic factor) can protect neurons from glutamate-mediated excitotoxicity manifested through disturbed $[Ca^{2+}]_i$ and oxidative stress (Mattson *et al*., 1989, 1993a,b). By stabilizing $[Ca^{2+}]_i$, nerve growth factor and brain-derived neurotrophic factor can protect cultured neocortical neurons from damage caused, for example, by hypoglycemia (Cheng and Mattson, 1991). Protective effects of neurotrophic factors can be achieved through several mechanisms involving regulation of gene expression (Fig. 20.2, see color insert). These mechanisms include (1) increasing the expression of antioxidant enzymes (Nistico *et al*., 1992; Mattson *et al*., 1995), (2) suppressing the expression of highly Ca^{2+}-permeable glutamate receptors (Mattson *et al*., 1993c), and (3) increasing the expression of Ca^{2+}-binding proteins (Collazo *et al*., 1992). The ability of a cell to counter potentially damaging $[Ca^{2+}]_i$ increases depends partially on their normal expression of Ca^{2+}-binding proteins. Cells containing certain Ca^{2+}-binding proteins are expected to have greater Ca^{2+}-buffering capacity and to be more resistant to degenerative changes. Wernyj and colleagues (1999) demonstrated that the stable expression of the Ca^{2+}-binding protein calbindin in glial cells attenuated rises in $[Ca^{2+}]_i$ caused by either calcium ionophore or amyloid-β peptide (Aβ), resulting in increased cell survival and suppression of apoptotic cell death. Similarly, neurons overexpressing calbindin have been shown to significantly suppress Aβ-induced elevations in $[Ca^{2+}]_i$ and reactive oxygen species (Guo *et al*., 1998). These effects were potentiated by the mutant presenilin-1gene (PS-1). It should be noted that in addition to APP gene mutations, mutations in presenilin genes can also lead to early onset AD (for review, see Hardy, 1997). Notably, hippocampal neurons of mice expressing mutant PS-1 were more susceptible to Aβ toxicity (Guo *et al*., 1999).

D. Role of Apolipoprotein E

In addition to PS-1 mutations, additional factors affect Aβ toxicity. The apolipoprotein E (ApoE) genotype is one of them (for review, see Strittmatter and Roses, 1995). Inheritance of multiple allele 4 (ApoE4) copies is considered a risk for the familial form of AD (Corder *et al*., 1993). In contrast, ApoE3 (and ApoE2) may have a protective role. By binding to normal tau, they may prevent its hyperphosphorylation and aggregation into neurofibrillary tangles (Strittmatter *et al*., 1994). A positive correlation exists between the presence of ApoE4 allele and increased amounts of both amyloid deposits and neurofibrillary pathology in AD patients (Berr *et al*., 1994; Ohm *et al*., 1995; Nagy *et al*., 1995). It was proposed that binding of ApoE to Aβ is isoform specific (Strittmatter *et al*., 1994). The ApoE4 isoform was reported to enhance Aβ fibril formation more efficiently than ApoE3 (Sanan *et al*., 1994; Wisniewski *et al*., 1994). An *in vitro* study of cultured smooth muscle cells (Mazur-Kolecka *et al*., 1995) showed that both ApoE4 and ApoE3 can induce intracellular accumulation of Aβ, although ApoE3 had a less stable effect. In contrast to these reports, a study using conditioned media from ApoE3- or ApoE4-transfected HEK-293 cells showed substantially higher levels of ApoE3 binding to Aβ (LaDu *et al*., 1994). Richey *et al.* (1995), however, found no significant differences between binding characteristics of ApoE4 and ApoE3 to either senile plaques (Aβ-mediated) or neurofibrillary tangles (tau-mediated). Although the relationship of ApoE4 to glutamate toxicity remains unclear, it is important to consider that any factor that enhances Aβ formation may, directly or indirectly, contribute to the pathologic consequences of glutamate receptor-mediated neurodegeneration (see next section).

E. Role of Aβ and APP in Excitotoxic Cell Injury

In addition to the overstimulation of Ca^{2+}-permeable glutamate receptors, the effects of Aβ are also known to produce $[Ca^{2+}]_i$-destabilizing effects (Mattson *et al*., 1992, 1993d; Mattson, 1994). In AD brains, excessive accumulation of Aβ, a metabolic product of the amyloid precursor protein APP (Neve *et al*., 1990), is one of the neuropathological hallmarks of the disease (Selkoe, 1991, 1994). APP can be metabolized through the α-secretase pathway (α-secretase), resulting in the production of large amino-terminal fragments of secreted APP (APPs, or sAPPα), or through the β-secretase pathway (β-secretase), releasing both a carboxy-terminal-truncated form of APP (sAPPβ) and intact Aβ fragments (Golde *et al*., 1992) (Fig. 20.3, see color insert). Each of these metabolic products of APP has specific cellular effects (for review, see Mattson, 1997). Physiologically, the α-secretase represents a major secretory pathway of APP metabolism (Esch *et al*., 1990). Furthermore, neuronal APP mainly undergoes intracellular metabolic processing, resulting in secretion of only a small amount of Aβ (Shoji *et al*., 1992). In contrast, the aberrant β-secretase is considered a major APP-processing pathway in AD, resulting in abundant deposits of Aβ within the brain parenchyma. APPs have been shown to protect cells from excitotoxic damage through mechanisms of both reducing $[Ca^{2+}]_i$ and preventing its rise (Mattson *et al*., 1993e; Schubert and Behl, 1993). As demonstrated in cultured hippocampal neurons, one of the mechanisms by which APPs may reduce $[Ca^{2+}]_i$ is by their ability to activate high-conductance potassium channels (Furukawa *et al*., 1996a). In addition to its ability to regulate $[Ca^{2+}]_i$, APPs can attenuate glutamate-induced excitotoxicity through regulation (i.e., increased acti-

vation) of a glutamate transporter system that is responsible for clearing glutamate from the extracellular space (Masliah, 1997). This is important because decreased levels of glutamate transporter activity have been found to correlate with the extent of neuropathology in AD brains (Scott *et al*., 1995; Masliah *et al*., 1996). In brains of transgenic mice, human APP increased the uptake of glutamate/aspartate (Masliah *et al*., 1998). These results were also corroborated in an *in vitro* system of cultured astrocytes (Masliah *et al*., 1998). In contrast to the glutamate transporter-related neuroprotective effects of APPs, *in vitro* studies demonstrated that Aβ is responsible for oxidative damage of the astroglial glutamate-uptake system (Harris *et al*., 1996; Butterfield, 1997). Whereas Aβ-induced impairment of glutamate transporter can be attenuated by sAPPα (Mattson *et al*., 1999), it remains unclear whether this impairment in AD results from (1) increased Aβ production or (2) a consequent formation of functionally impaired sAPPβ . It is also of importance to note that sAPPβ is less potent than sAPPα in attenuating glutamate-evoked $[Ca^{2+}]_i$ disturbances (Furukawa *et al*., 1996b). In addition to its ability to both suppress glutamate-induced elevation of $[Ca^{2+}]_i$ and protect neurons against glutamate overstimulation, sAPPα has been shown by whole-cell patch-clamp techniques to selectively suppress NMDA currents in hippocampal neurons (Furukawa and Mattson, 1998), providing yet another mechanism of neuronal protection against excitotoxicity. Following initial reports of neurotoxic effects of Aβ (Yankner *et al*., 1989, 1990), a substantial body of research supported the relationship between Aβ and excitotoxic neuronal injury. While APPs have been mainly viewed as $[Ca^{2+}]_i$ stabilizing and neuroprotective, Aβ can destabilize $[Ca^{2+}]_i$ homeostasis and increase neuronal vulnerability to excitotoxic (Koh *et al*., 1990; Mattson *et al*., 1992, 1993f) or hypoglycemic damage (Copani *et al*., 1991). Of importance, Mattson and colleagues (1993g) demonstrated that $[Ca^{2+}]_i$ increases in cultured neurons directly correlated with increased time of exposure to Aβ as well as Aβ aggregation. Aβ may carry out this effect in several ways. As illustrated in Fig. 20.4, $[Ca^{2+}]_i$-destabilizing mechanisms of Aβ may involve either activation of existing Ca^{2+}-permeable receptors and channels or formation of *de novo* ion channels in the membrane. For example, Mattson and colleagues have proposed that Aβ, when accumulated at the cell surface, is capable of modifying the response of membrane-associated mechanisms responsible for either influx (glutamate receptors, voltage dependent calcium channels) or removal (Ca^{2+} pump, Na^+/Ca^{2+} exchanger) of Ca^{2+} (see Mattson, 1994). In support of this notion, Furukawa and colleagues (1994; Furukawa and Mattson, 1995) suggested that $[Ca^{2+}]_i$ increases can be related to binding of Aβ to the cell membrane and opening of nonselective ion channels. Furthermore, Weiss *et al.* (1994) provided additional evidence that Ca^{2+} influx through Ca^{2+}-permeable channels may be one of the mechanisms Aβ induces neuronal degeneration. This notion, however, was not corroborated by experiments applying Aβ and Ca^{2+} channel blockers in the primary culture of hippocampal neurons (Whitson and Appel, 1995). For a detailed review of studies examining ionic effects associated with APP and its metabolites (e.g., Aβ), see Fraser *et al.* (1997). Finally, an alternative mechanism of Aβ-mediated disturbance of $[Ca^{2+}]_i$ homeostasis was described by Arispe *et al.* (1993, 1994). These authors reported that Aβ incorporates into the cell membrane where it forms *de novo* Ca^{2+} channels (for review, see Pollard *et al*., 1995).

F. Free Radical Formation and Oxidative Stress

Providing additional support of the protective effect neurotrophic factors have against excitotoxic damage, an *in vitro* study by Mattson and colleagues (1993g; Mark *et al*., 1997a) demonstrated that Aβ-induced $[Ca^{2+}]_i$ disturbances and oxidative stress can be significantly attenuated by brain-derived neurotrophic factor. As stated previously, neurotrophic factors can also protect neurons from the toxic effects of free radicals (Mattson *et al*., 1993a). A substantial amount of evidence shows that free radical formation and oxidative stress are some of the mechanisms responsible for Aβ-mediated cell damage (Goodman and Mattson, 1994; Hensley *et al*., 1994; Behl *et al*., 1994; Klegeris and McGeer, 1997; Butterfield, 1997; Mark *et al*., 1997b). Specifically, Aβ can induce oxidative as well as excitotoxic cell injury, whereas these effects are also attenuated by APPs (Goodman and Mattson, 1994; Mattson and Pedersen, 1998). In a study involving rat neocortical synaptosomes, Keller *et al*. (1997) demonstrated that Aβ can induce impairment of glucose and glutamate transport, as well as mitochondrial dysfunction, all of which are oxidative stress mediated. Furthermore, the effect Aβ has on nonneuronal cells (*e.g*., microglia) may also contribute to excitotoxic neuronal damage. For example, Klegeris and McGeer (1997) showed that Aβ stimulates macrophages to produce glutamate and free oxygen radicals. These studies demonstrate that the neurotoxic effects of Aβ, either directly or indirectly, involve free radical damage and may be responsible for selective neuronal death in AD (for review, see Mark *et al*., 1996).

G. Cytoskeletal Alterations and Formation of AD-like Neurofibrillary Changes

Glutamate-related excitotoxicity is also known to induce AD-like cytoskeletal alterations. For example, the *in vitro* exposure of human neocortical neurons to excitotoxins (i.e., glutamate, aspartate) resulted in the induction of paired helical filament (PHF) formation (De Boni and Crapper-McLachlan, 1985). Furthermore, a number of more recent *in vitro* studies supported the notion that glutamate-mediated Ca^{2+} influx into cells can cause antigenic changes (i.e., increased immunoreactivity to tau and ubiquitin) similar to those observed in neurofibrillary tangles of AD brains (Mattson, 1990; Mattson *et al*., 1991; Sindou *et al*., 1992; Sautière *et al*., 1992; Hugon *et al*., 1995; Couratier *et al*., 1995, 1996). Neurofibrillary tangles consist of PHFs in which the microtubule-associated protein, tau, is aberrantly hyperphosphorylated (Grundke-Iqbal *et al*., 1986). Because activation of glutamate receptors can result in protein phosphorylation (Sholz and Palfrey, 1991), it is hypothesized that glutamate-induced Ca^{2+} influx may activate specific protein kinases, or deactivate phosphatases, to result in tau hyperphosphorylation. Specifically, it has been proposed that the mechanism responsible for conversion of normal tau into hyperphosphorylated tau (PHF-tau) may involve dysfunction of protein kinases and/or phosphatases

that are able to phosphorylate and dephosphorylate, respectively, tau protein (for review, see Iqbal *et al*., 1994; Trojanowski and Lee, 1995). A double-labeling study of AD brains (Ikonomovic *et al*., 1997), using a marker of early cytoskeletal changes (i.e., monoclonal antibody MC1), supports the concept that glutamate-induced loss of $[Ca^{2+}]_i$ homeostasis may be responsible for neurofibrillary tangle formation in vulnerable neuronal populations of AD brains (i.e., hippocampus, entorhinal neocortex).

VI. Summary

As summarized in this chapter, a number of investigators have examined the structural and functional integrity of the glutamate receptor in the aged brain and in subjects with AD. While the results of these many studies are far from uniform, a number of trends do emerge. With respect to investigations of the aged rodent brain, the majority of works support the view that NMDA receptor binding is reduced with increasing age. Notably, these reductions are observed regardless of the ligand employed to visualize the NMDA receptor (i.e., [^{3}H]glutamate, -CPP, -MK801, -TCP, -glycine). Similarly, several investigations supported the notion that kainate receptor binding is reduced in the aged rodent brain. In contrast, far less convincing evidence is provided in support of an age-related reduction in AMPA binding. Collectively, these data support the view that glutamate receptors are affected differentially by the aging process. However, not all investigations are in agreement with the majority opinion, thus raising a number of controversies concerning the participation of glutamate in the senescent brain. Contributing to the various inconsistencies in data are a host of technical issues, including strain or species, age, sex, brain region examined, and method employed to visualize the glutamate receptor subtype. For example, binding studies utilizing brain homogenates often run the risk of obscuring discrete (i.e., laminar) differences in binding due to the large size of the brain sample. The interpretation of a number of studies is likewise influenced by age of the animal. For example, while some investigations observed differences in the binding properties of receptors when comparing rodents of young and intermediate ages, no such differences were observed when comparing rodents of intermediate and old ages. Such findings raise the distinct possibility that alterations in binding properties may be related more to a maturational process than an aging one.

A number of investigators also examined the structural and functional integrity of the glutamate receptor in behaviorally characterized rodents. If, in fact, the dysfunction of glutamate neurotransmission participates in age-related declines in cognition, one would predict a positive correlation between deficits in, for example, glutamate receptor binding and poor performance in a given behavioral paradigm. Despite this prediction, deficits in glutamate receptor binding failed, in most instances, to correlate with the extent of cognitive impairment. These findings are somewhat surprising in light of considerable evidence linking the glutamate neurotransmitter system to various aspects of learning and memory, including LTP. To explain these findings, one must consider whether the brain region examined is intimately involved in the execution of the behavioral task under investigation. Moreover, it is equally important to keep in mind that glutamate receptors are expressed by cells utilizing a number of other neurotransmitters. For example, work in our laboratory demonstrates that cholinergic neurons in the basal forebrain express high levels of specific AMPA receptor subunits. The dysfunction of basal forebrain cholinergic neurons is known to result in poor performance in the Morris water maze. Given the fact that, for example, glutamatergic/cholinergic systems are so intimately linked, it may be difficult to attribute behavioral performance and/or the lack thereof to deficits in one neurotransmitter system. Rather, a specific behavior is more likely to result from the net contribution of multiple neurotransmitter systems. This latter concept is supported by a number of electrophysiological studies demonstrating, for example, age-related reductions in NMDA-stimulated dopamine and/or noradrenaline release.

The brain is also known to maintain a number of neuroplastic reserves capable of compensating for deficits in one or another neurotransmitter systems following injury, disease, and/or normal aging. Work from our laboratory provides extensive documentation in support of the plasticity of the glutamate receptor following surgical deafferentation in the rodent brain as well as in the AD brain. That other neurotransmitter systems are undergoing parallel compensatory alterations is highly tenable and may also serve to obviate the behavioral deficits resulting from the dysfunction of a single (e.g. glutamate) neurotransmitter system.

In the AD brain, an argument could also be made for deficits in glutamate receptor binding/function, although the data are somewhat less compelling than for the rodent brain. For example, in the AD brain the outcome appears to depend to a large extent on the subtype of the glutamate receptor, the ligand-binding site, and the brain region examined. Although similar to the rodent brain, the NMDA receptor in the AD brain appears to be more vulnerable than AMPA or kainate receptors, there are numerous exceptions to this rule. Contributing to various discrepant findings are a number of technical issues common to the rodent studies, as well as a number of additional factors unique to studies of postmortem human brain. Notably, a common theme throughout many of the studies is the concept of intersubject variability. Obvious contributors to this variability include postmortem interval, storage time of the tissue sample, and pathologic severity. Studies (Wolfe *et al*., personal communication) demonstrate that specific AMPA and NMDA receptor subunits are highly vulnerable to postmortem degradation whereas others are relatively resistant. For example, GluR1, GluR2/3, GluR4, and NMDAR1 exhibit marked preservation following postmortem times in excess of 18 hr, whereas NR2A and NR2B show evidence of proteolytic breakdown within the first couple of hours postmortem. In addition, Pellegrini-Giampietro *et al.* (1994) found that all differences in mean expression levels of specific mRNA transcripts between AD and control samples could be ascribed to storage time. Finally, relatively few studies of the AD brain took into account pathologic severity, yet for those who did, inconsistencies abound with respect to correlations between glutamate receptor binding and numbers of plaques and neurofibrillary tangles. Notably, the vast majority of studies examined patients with end-stage AD, thus negating any possibility of observing alterations in glutamate receptor

binding and/or function during the early (i.e., incipient) stages of the disease. As stated earlier, it is the belief of the authors that glutamate receptors undergo marked plasticity during the early phases of the disease likely in an attempt to compensate for the loss of glutamatergic innervation. In contrast, during the terminal stages of the disease these receptors are more prone to undergo neurodegenerative changes as reflected in the loss of receptor density. Whereas this loss in receptor density most likely parallels the death of neurons, no study has yet employed sterological methods to assay cell number and glutamate receptor density. The latter is critical in determining whether the loss of glutamate receptors precedes neuronal cell death or occurs concomitant with the loss of neurons. Ikonomovic *et al.* (1997) employed double-immunolabeling techniques and demonstrated that there is a loss of GluR2/3 prior to the appearance of a marker highly specific for neurofibrillary tangles (i.e., MC1) in layer II of the entorhinal neocortex and CA1 region of the hippocampus.

In considering other factors contributing to intersubject variability, one should also take into account the agonal state of the patient. The agonal state is known to contribute significantly to the stability of a number of protein and mRNA species, yet is largely discounted as a potentially confounding variable in the vast majority of studies.

Finally, in interpreting results presented in this chapter it is important to recognize that an overwhelming number of these studies rely on traditional binding methods to assay glutamate receptor levels and/or function. While these studies provide much needed information with respect to the glutamate receptor in the aged and AD brain, it is clear from our knowledge of the molecular biology of the glutamate receptor that additional techniques are needed that allow for the visualization of subunit specific mRNAs and proteins. The functional properties of the glutamate receptor, including its ability to gate calcium, are highly dependent on the subunit composition of the assembled receptor. It is our hypothesis that glutamate receptors in the aging brain and in AD undergo changes in their subunit composition, which in turn either protects the neuron or makes them more vulnerable to the pathologic consequences of glutamate receptor activation. Moreover, the vulnerability of a neuron to glutamate is enhanced significantly if a cell is in a state of disequilibrium with respect to intracellular calcium levels. Thus, factors that may contribute to the increased influx of calcium into a cell (e.g., the presence of Aβ) and/or impede the flow of calcium from the cell (i.e., impaired Na^+/Ca^{2+} exchanger) will enhance glutamate receptor-mediated toxicity. It is important to note that many of the mechanisms involved in the removal of calcium from the cell are highly energy dependent, thereby supporting the notion that any process that compromises glucose/energy metabolism may contribute to the pathologic consequences of glutamate receptor activation.

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References

Akbarian, S., Smith, M. A., and Jones, E. G. (1995). Editing for an AMPA receptor subunit RNA in prefrontal cortex and striatum in Alzheimer's disease, Huntington's disease and schizophrenia. *Brain Res.* **699**, 297–304.

Anegawa, N. J., Lynch, D. R., Verdoorn, T. A., and Prichett, D. B. (1995). Transfection of *N*-methyl-D-asparate receptors in a nonneuronal cell line leads to cell death. *J. Neurochem.* **64**, 2004–2012.

Aprikyan, G. V., and Gekchyan, K. G. (1988). Release of neurotransmitter amino acids from rat brain synaptosomes and its regulation in aging. *Gerontology* **34**, 35–40.

Arispe, N., Rojas, E., and Pollard, H. B. (1993). Alzheimer's disease amyloid β protein forms calcium channels in bilayer membranes: Blockade by tromethamine and aluminum. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 567–571.

Arispe, N., Pollard H. B., and Rojas, E. (1994). The ability of amyloid β-protein [AβP(1–40)] to form Ca^{2+} channels provides a mechanism for neuronal death in Alzheimer's disease. *Ann. N.Y. Acad. Sci.* **747**, 256–266.

Armstrong, D. M., and Ikonomovic, M. D. (1996). AMPA-selective glutamate receptor subtype immunoreactivity in the hippocampal dentate gyrus of patients with Alzheimer's disease: Evidence for hippocampal plasticity. *Mol. Chem. Neuropathol.* **28**, 59–64.

Armstrong, D. M., Ikonomovic, M. D., Sheffield, R., and Wenthold, R. J. (1994). AMPA- selective glutamate receptor subtype immunoreactivity in the entorhinal cortex of non-demented elderly and patients with Alzheimer's disease. *Brain Res.* **639**, 207–216.

Aronica, E., Dickson, D. W., Kress, Y., Morrison, J. H., and Zukin, R. S. (1998). Non-plaque dystrophic dendrites in Alzheimer hippocampus: A new pathological structure revealed by glutamate receptor immunocytochemistry. *Neuroscience* **82**, 979–991.

Bahr, B. A., Godshall, A. C., Hall, R. A., and Lynch, G. (1992). Mouse telecephalon exhibits an age-related decrease in glutamate (AMPA) receptors but no change in nerve terminal markers. *Brain Res.* **589**, 320–326.

Barnes, C. A., Rao, G., and Shen, J. (1997). Age-related decrease in the *N*-methyl-D-aspartate-mediated excitatory postsynaptic potential in hippocampal region CA1. *Neurobiol. Aging*. **18**, 445–452.

Baskys, A., Reynolds, J. N., and Carlen, P. L. (1990). NMDA depolarizations and long-term potentiation are reduced in the aged rat neocortex. *Brain Res.* **530**, 142–146.

Baudry, M., Arst, D. S., and Lynch, G. (1981). Increased [^{3}H]glutamate receptor binding in aged rats. *Brain Res.* **223**, 195–198.

Behl, C., Davis, J. B., Lesley, R., and Schubert, D. (1994). Hydrogen peroxide mediates amyloid beta protein toxicity. *Cell (Cambridge, Mass.)* **77**, 817–827.

Berr, C., Hauw, J. J., Delaère, P., Duyckaerts, C., and Amouyel, P. (1994). Apolipoprotein E allele epsilon 4 is linked to increased deposition of the amyloid beta-peptide (A-beta) in cases with or without Alzheimer's disease. *Neurosci. Lett.* **178**, 221–224.

Billard, J. M., Jouvenceau, A., Lamour, Y., and Dutar, P. (1997). NMDA receptor activation in the aged rat: Electrophysiological investigations in the CA1 area of the hippocampal slices ex vivo. *Neurobiol. Aging* **18**, 535–542.

Bonhaus, D. W., Perry, W. B., and McNamara, J. O. (1990). Decreased density, but not number, of *N*-methyl-D-aspartate, glycine and phencyclidine binding sites in hippocampus of senescent rats. *Brain Res.* **532**, 82–86.

Burnashev, N., Monyer, H., Seeburg, P. H., and Sakmann, B. (1992). Divalent ion permeability of AMPA receptor channel is dominated by the edited form of a single subunit. *Neuron* **8**, 189–198.

Burnashev, N., Villarroel A., and Sakmann, B. (1996). Dimensions and ion selectivity of recombinant AMPA and kainate receptor channels and their dependence on Q/R site residues. *J. Physiol. (London)* **496**, 165–173.

Butterfield, D. A. (1997). Beta-amyloid-associated free radical oxidative stress and neurotoxicity: Implications for Alzheimer's disease. *Chem. Res. Toxicol.* **10**, 495–506.

Carlson, M. D., Penney, J. B., Jr., and Young, A. B. (1993). NMDA, AMPA, and benzodiazepine binding site changes in Alzheimer's disease visual neocortex. *Neurobiol. Aging* **14**, 343–352.

Castorina, M., Ambrosini, A. M., Ramacci, M. T., and Angelucci, L. (1994). Age-dependent loss of NMDA receptors in hippocampus, striatum, and frontal cortex of the rat: Prevention by acetyl-L-carnitine. *Neurochem. Res.* **19**, 795–798.

Cepeda, C., Li, Z., and Levine, M. S. (1996). Aging reduces neostriatal responsiveness to *N*-methyl-D-aspartate and dopamine: An *in vitro* electrophysiological study. *Neuroscience* **73**, 733–750.

Chalmers, D. T., Dewar, D., Graham, D. I., Brooks, D. N., and McCulloch, J. (1990). Differential alterations of cortical glutamatergic binding sites in senile dementia of the Alzheimer type. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 1352–1356.

Cheng, B., and Mattson, M. P. (1991). NGF and bFGF protect rat hippocampal and human cortical neurons against hypoglycemic damage by stabilizing calcium homeostasis. *Neuron* **7**, 1031–1041.

Choi, D. W. (1985). Glutamate neurotoxicity in neocortical cell culture is calcium dependent. *Neurosci. Lett.* **58**, 293–297.

Choi, D. W. (1987). Ionic dependence of glutamate neurotoxicity. *J. Neurosci.* **7**, 369–379.

Choi, D. W. (1988). Glutamate neurotoxicity and disease of the nervous system. *Neuron* **1**, 623–634.

Cimino, M., Marini, P., Colombo, S., Cattabeni, F., and Meldolesi, J. (1992). [^{3}H]-CGP 39653 mapping of glutamatergic *N*-methyl-D-aspartate receptors in the brain of aged rats. *Neurosci. Res. Commun.* **12**, 31–39.

Clark, A. S., Magnusson, K. R., and Cotman, C. W. (1992). In vitro autoradiography of hippocampal excitatory amino acid binding in aged Fischer 344 rats: Relationship to performance on the Morris water maze. *Behav. Neurosci.* **106**, 324–335.

Cohen, S. A., and Müller, W. E. (1992). Age-related alterations of NMDA-receptor properties in the mouse forebrain: Partial restoration by chronic phosphatidylserine treatment. *Brain Res.* **584**, 174–180.

Collazo, D., Takahashi, H., and McKay, R. D. G. (1992). Cellular targets and trophic functions of neurotrophin-3 in the developing rat hippocampus. *Neuron* **9**, 643–656.

Copani, A., Koh, J.-Y., and Cotman, C. W. (1991). β amyloid increases neuronal susceptibility to injury by glucose deprivation. *NeuroReport* **2**, 763–765.

Corder, E. H., Saunders, A. M., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G. W., Roses, A. D., Haines, J. L., and Pericak-Vance, M. A. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261**, 921–923.

Couratier, P., Sindou, P., Tabaraud, F., Diop, A. G., Spencer, P. S., and Hugon, J. (1995). Modulation of tau neuronal expression induced by NMDA, non-NMDA and metabotropic glutamate receptor agonists. *Neurodegeneration* **4**, 33–41.

Couratier, P., Lesort, M., Sindou, P., Esclaire, F., Yardin, C., and Hugon, J. (1996). Modifications of neuronal phosphorylated tau immunoreactivity induced by NMDA toxicity. *Mol. Chem. Neuropathol.* **27**, 259–273.

Cowburn, R., Hardy, J., Roberts, P., and Briggs, R. (1988). Regional distribution of pre-and postsynaptic glutamatergic function in Alzheimer's disease. *Brain Res.* **452**, 403–407.

Cowburn, R. F., Hardy, J. A., Briggs, R. S., and Roberts, P. J. (1989). Characterization, density, and distribution of kainate receptors in normal and Alzheimer's diseased human brain. *J. Neurochem.* **52**, 140–147.

D'Aniello, A., Lee, J. M., Petrucelli, L., and Di Fiore, M. M. (1998). Regional decreases of free D-aspartate levels in Alzheimer's disease. *Neurosci. Lett.* **250**, 131–134.

Davis, S., Markowska, A. L., Wenk, G. L., and Barnes, C. A. (1993). Acetyl-L-carnitine: Behavioral, electrophysiological, and neurochemical effects. *Neurobiol. Aging* **14**, 107–115.

Dawson, R., Jr., Wallace, D. R., and Meldrum, M. J. (1989). Endogenous glutamate release from frontal cortex of adult and aged rats. *Neurobiol. Aging* **10**, 665–668.

De Boni, U., and Crapper-McLachlan, D. R. (1985). Controlled induction of paired helical filaments of the Alzheimer type in cultured human neurons by glutamate and aspartate. *J. Neurol. Sci.* **68**, 105–118.

Del Bel, E. A., and Slater, P. (1991). Binding to the glycine site of the NMDA receptor complex in brain of patients with Alzheimer's disease. *Neurosci. Lett.* **131**, 75–78.

Dewar, D., Chalmers, D. T., Graham, D. I., and McCulloch, J. (1991). Glutamate metabotropic and AMPA binding sites are reduced in Alzheimer's disease: An autoradiographic study of the hippocampus. *Brain Res.* **553**, 58–64.

Durand, G. M., Bennett, M. V. L., and Zukin, R. S. (1993). Splice variants of the *N*-methyl-D-asparate receptor NR1 identify domains involved in regulation by polyamines and protein kinase C. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 6731–6735.

Ehlers, M. D., Tingley, W. G., and Huganir, R. L. (1995). Regulated subcellular distribution of NR1 subunit of the NMDA receptor. *Science* **269**, 1734–1737.

Esch, F. S., Keim, P. S., Beattie, E. C., Blacher, R. W., Culwell, A. R., Oltersdorf, T., McClure, D., and Ward, P. J. (1990). Cleavage of amyloid beta peptide during constitutive processing of its precursor. *Science* **248**, 1122–1124.

Fonnum, F. (1991). Neurochemical studies on glutamate-mediated neurotransmission. In, "Excitatory Amino Acids". (B. S. Meldrum, F. Moroni, R. P. Simon, and J. H. Woods, eds.), FIDIA Res. Found. Symp. Ser., No 5, pp. 15–25, Raven Press, New York.

Fraser, S. P., Suh, Y. H., and Djamgoz, M. B. A. (1997). Ionic effects of the Alzheimer's disease β-amyloid precursor protein and its metabolic fragments. *Trends Neurosci.* **20**, 67–72.

Freeman, G. B., and Gibson, G. E. (1987). Selective alteration of mouse brain neurotransmitter release with age. *Neurobiol. Aging* **8**, 147–152.

Furukawa, K., and Mattson, M. P (1995). Cytochalasins protect hippocampal neurons against amyloid beta-peptide toxicity: Evidence that actin depolymerization suppresses Ca^{2+} influx. *J. Neurochem.* **65**, 1061–1068.

Furukawa, K., and Mattson, M. P. (1998). Secreted amyloid precursor protein alpha selectively suppress *N*-methyl-D-aspartate currents in hippocampal neurons: Involvement of cyclic GMP. *Neuroscience* **83**, 429–438.

Furukawa, K., Abe, Y., and Akaike, N. (1994). Amyloid beta protein-induced irreversible current in rat neocortical neurones. *NeuroReport* **5**, 2016–2018.

Furukawa, K., Barger, S. W., Blalock, E. M., and Mattson, M. P. (1996a). Activation of K^+ channels and suppression of neuronal activity by secreted beta-amyloid-precursor protein. *Nature (London)* **379**, 74–78.

Furukawa, K., Sopher, B. L., Rydel, R. E., Begley, J. G., Pham, D. G., Martin, G. M., Fox, M., and Mattson, M. P. (1996b). Increased activity-regulating and neuroprotective efficacy of alpha-secretase-derived secreted amyloid precursor protein conferred by a carboxy-terminal heparin-binding domain. *J. Neurochem.* **67**, 1882–1896.

García-Ladona, F. J., Palacios, J. M., Probst, A., Wieser, H. G., and Mengod, G. (1994). Excitatory amino acid AMPA receptor mRNA localization in several regions of normal and neurological disease affected human brain. An *in situ* hybridization study. *Mol. Brain Res.* **21**, 75–84.

Geddes, J. W., and Cotman, C. W. (1986). Plasticity in hippocampal excitatory amino acid receptors in Alzheimer's disease. *Neurosci. Res.* **3**, 672–678.

Geddes, J. W., Chang-Chui, H., Cooper, S. M., Lott, I. T., and Cotman, C. W. (1986). Density and distribution of NMDA receptors in the

human hippocampus in Alzheimer's disease. *Brain Res.* **399**, 156–161.

Geddes, J. W., Ulas, J., Brunner, L. C., Choe, W., and Cotman, C. W. (1992). Hippocampal excitatory amino acid receptors in elderly, normal individuals and those with Alzheimer's disease: Non-*N*-methyl-D-aspartate receptors. *Neuroscience* **50**, 23–34.

Golde, T. E., Estus, S., Younkin, L. H., Selkoe, D. J., and Younkin, S. G. (1992). Processing of the amyloid protein precursor to potentially amyloidogenic derivatives. *Science* **255**, 728–730.

Gonzales, R. A., Brown, L. M., Jones, T. W., Trent, R. D., Westbrook, S. L., and Leslie, S. W. (1991). *N*-methyl-D-aspartate mediated responses decrease with age in Fischer 344 rat brain. *Neurobiol. Aging* **12**, 219–225.

Goodman, Y., and Mattson, M. P. (1994). Secreted forms of β-amyloid precursor protein protect hippocampal neurons against amyloid β-peptide induced oxidative injury. *Exp. Neurol.* **128**, 1–12.

Greenamyre, J. T., and Young, A. B. (1989). Excitatory amino acids and Alzheimer's disease. *Neurobiol. Aging* **10**, 593–602.

Greenamyre, J. T., Penney, J. B.,Young, A. B., D'Amato, C. J., Hicks, S. P., and Shoulson, I. (1985). Alterations in L-glutamate binding in Alzheimer's and Huntington's disease. *Science* **227**, 1496–1499.

Greenamyre, J. T., Penney J. B., D'Amato, C. J., and Young, A. B. (1987). Dementia of the Alzheimer's type: Changes in hippocampal L-[^{3}H]Glutamate binding. *J. Neurochem.* **48**, 543–551.

Grundke-Iqbal, I., Iqbal, K., Tung, Y.-C., Quinlan, M., Wisniewski, H. M., and Binder, L. I. (1986). Abnormal phosphorylation of the microtubule associated protein tau in Alzheimer cytoskeletal pathology. *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4913–4917.

Guo, Q., Christakos, S., Robinson, N., and Mattson, M. P. (1998). Calbindin D28k blocks the proapoptotic actions of presenilin 1: Reduced oxidative stress and preserved mitochondrial function. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 3227–3232.

Guo, Q., Sebastian, L., Sopher, B. L., Miller, M. W., Ware, C. B., Martin, G. M., and Mattson, M. P. (1999). Increased vulnerability of hippocampal neurons from presenilin-1 mutant knock-in mice to amyloid [β]-peptide toxicity: Central roles of superoxide production and caspase activation. *J. Neurochem.* **72**, 1019–1029.

Hardy, J. (1997). Amyloid, the presenilins and Alzheimer's disease. *Trends Neurosci.* **20**, 154–159.

Harris, M. E., Wang, Y., Pedigo, N. W., Jr., Hensley, K., Butterfield, D. A., and Carney, J. M. (1996). Amyloid [β]-peptide (25–35) inhibits Na^+-dependent glutamate uptake in rat hippocampal astrocyte cultures. *J. Neurochem.* **67**, 277–286.

Hensley, K., Carney, J., Mattson, M. P., Aksenova, M., Harris, M., Wu, J. F., Floyd, R., and Butterfield, D. A. (1994). A new model for β-amyloid aggregation and neurotoxicity based on the free radical generating capacity of the peptide. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 3270–3274.

Hollmann, M., Hartley, M., and Heinemann, S. (1991). Ca^{2+} permeability of KA-AMPA-gated glutamate channels depends on subunit composition. *Science* **252**, 851–853.

Hugon J., Sindou, P., Lesort, M., Couratier, P., Esclaire, F., and Yardin, C. (1995). Modifications of phosphorylated tau immunoreactivity linked to excitotoxicity in neuronal cultures. *In* "Alzheimer's Disease: Lessons from Cell Biology" (K. S. Kosik, Y. Christen, and D. J. Selkoe, eds.), pp. 172–179, Springer-Verlag, Berlin.

Hyman, B. T., Penney, L. B., Jr., Blackstone, C. D., and Young, A. B. (1994). Localization of Non-*N*-Methyl-D-Aspartate glutamate receptors in normal and Alzheimer hippocampal formation. *Ann. Neurol.* **35**, 31–37.

Ikonomovic, M. D., and Armstrong, D. M. (1996). Distribution of AMPA receptor subunits in the nucleus basalis of Meynert in aged humans: Implications for selective neuronal degeneration. *Brain Res.* **716**, 229–232.

Ikonomovic, M. D., Sheffield, R., and Armstrong, D. M. (1995a). AMPA-selective glutamate receptor subtype immunoreactivity in the aged human hippocampal formation. *J. Comp. Neurol.* **359**, 239–252.

Ikonomovic, M. D., Sheffield, R., and Armstrong, D. M. (1995b). AMPA-selective glutamate receptor subtype immunoreactivity in the hippocampal formation of patients with Alzheimer's disease. *Hippocampus* **5**, 469–486.

Ikonomovic, M. D., Mizukami, K., Davies, P., Hamilton, R., Sheffield, R., and Armstrong, D. M. (1997). The loss of GluR2(3) immunoreactivity precedes neurofibrillary tangle formation in the entorhinal cortex and hippocampus of Alzheimer brains. *J. Neuropathol. Exp. Neurol.* **56**, 1018–1027.

Ikonomovic, M. D., Mizukami, K., Warde, D., Hamilton, R., Wenthold, R. J., Sheffield, R., and Armstrong, D. M. (1999). Distribution of glutamate receptor subunit NMDAR1 in the hippocampus of normal elderly and patients with Alzheimer's disease. *Exp. Neurol.* **160**, 194–204.

Ingram, D. K., Garofalo, P., Spangler, E. L., Mantione, C. R., Odano, I., and London, E. D. (1992). Reduced density of NMDA receptors and increased sensitivity to dizocilpine-induced learning impairment in aged rats. *Brain Res.* **580**, 273–280.

Iqbal, K., Alonso Adel, C., Gong, C.-X., Khatoon, S., Singh, T. J., and Grundke-Iqbal, I. (1994). Mechanism of neurofibrillary degeneration in Alzheimer's disease. *Mol. Neurobiol.* **9**, 119–123.

Jansen, K. L. R., Faull, R. L., Dragunow, M., and Synek, B. L. (1990). Alzheimer's disease: Changes in hippocampal *N*-Methyl-D-asparate, quisqualate, neurotensin, adenosine, benzodiazepine, serotonin and opioid receptors: An autoradiographic study. *Neuroscience* **39**, 613–627.

Jasek, M. C., and Griffith, W. H. (1998). Pharmacological characterization of ionotropic excitatory amino acid receptors in young and aged rat basal forebrain. *Neuroscience* **82**, 1179–1194.

Jonas, P., and Burnashev, N. (1995). Molecular mechanisms controlling calcium entry through AMPA-type glutamate receptor channels. *Neuron* **15**, 987–990.

Jouvenceau, A., Dutar, P., and Billard, J. M. (1998). Alteration of NMDA receptor-mediated synaptic responses in CA1 area of the aged rat hippocampus: Contribution of GABAergic and cholinergic deficits. *Hippocampus* **8**, 627–637.

Keller, E. A., Borghese, C. M., Carrer, H. F., and Ramirez, O. A. (1992). The learning capacity of high or low performance rats is related to the hippocampus NMDA receptors. *Brain Res.* **576**, 162–164.

Keller, J. N., Pang, Z., Geddes, J. W., Begley, J. G., Germeyer, A., Waeg, G., and Mattson, M. P. (1997). Impairment of glucose and glutamate transport and induction of mitochondrial oxidative stress and dysfunction in synaptosomes by amyloid β-peptide: Role of the lipid peroxidation product 4-hydroxynonenal. *J. Neurochem.* **69**, 273–284.

Khachaturian, Z. S. (1989). The role of calcium regulation in brain aging: Reexamination of a hypothesis. *Aging* **1**, 17–34.

Kitamura, Y., Zhao, X.-H., Ohnuki, T., Takie, M., and Nomura, Y. (1992). Age-related changes in transmitter glutamate and NMDA receptor/channels in the brain of senescence-accelerated mouse. *Neurosci. Lett.* **137**, 169–172.

Kito, S., Miyoshi, R., and Nomoto, T. (1990). Influence of age on NMDA receptor complex in rat brain studied by in vitro autoradiography. *J. Histochem. Cytochem.* **38**, 1725–1731.

Klegeris, A., and McGeer, P. L. (1997). β-amyloid protein enhances macrophage production of oxygen free radicals and glutamate. *J. Neurosci. Res.* **49**, 229–235.

Koh, J. Y., Yang, L. L., and Cotman, C. W. (1990). β-amyloid protein increases the vulnerability of cultured neocortical neurons to excitotoxic damage. *Brain Res.* **533**, 315–320.

LaDu, M. J., Falduto, M. T., Manelli, A. M., Reardon, C. A., Getz, G. S., and Frail, D. E. (1994). Isoform-specific binding of apolipoprotein E to beta-amyloid. *J. Biol. Chem.* **269**, 23403–23406.

Le Jeune, H., Cécyre, D., Rowe, W., Meaney, M. J., and Quirion, R. (1996). Ionotropic glutamate receptor subtypes in the age memory-impaired and unimpaired Long-Evans rat. *Neuroscience* **74**, 349–363.

Lipartiti, M., Fadda, E., Savoini, G., Siliprandi, R., Sautter, J., Arban, R., and Manev, H. (1993). In rats, the metabotropic glutamate receptor-triggered hippocampal neuronal damage is strain-dependent. *Life Sci.* **52**, 85–90.

Lu, Y. M., Yin, H. Z., Chiang, J., and Weiss, J. H. (1996). Ca^{2+}-permeable AMPA/Kainate and NMDA channels: High rate of Ca^{2+} influx underlies potent induction of injury. *J. Neurosci.* **16**, 5457–5465.

Magnusson, K. R. (1995). Differential effects of aging on binding sites of the activated NMDA receptor complex in mice. *Mech. Ageing Dev.* **84**, 227–243.

Magnusson, K. R. (1997). Influence of dietary restriction on iontropic glutamate receptors during aging in C57B1 mice. *Mech. Ageing Dev.* **95**, 187–202.

Magnusson, K. R., and Cotman, C. W. (1993a). Age-related changes in excitatory amino acid receptors in two mouse strains. *Neurobiol. Aging* **14**, 197–206.

Magnusson, K. R., and Cotman, C. W. (1993b). Effects of aging on NMDA and MK801 binding sites in mice. *Brain Res.* **604**, 334–337.

Mano, I., and Teichberg, V. I. (1998). A tetrameric subunit stoichiometry for a glutamate receptor-channel complex. *NeuroReport* **9**, 327–331.

Maragos, W. F., Chu, D. C. M., Young, A. B., D'Amato, C. J., and Penney, J. B., Jr., (1987). Loss of hippocampal [^{3}H]TCP binding in Alzheimer's disease. *Neurosci. Lett.* **74**, 371–376.

Mark, R. J., Blanc, E. M., and Mattson, M. P. (1996). Amyloid beta-peptide and oxidative cellular injury in Alzheimer's disease. *Mol. Neurobiol.* **12**, 211–224.

Mark, R. J., Keller, J. N., Kruman, I., and Mattson, M. P. (1997a). Basic FGF attenuates amyloid beta-peptide-induced oxidative stress, mitochondrial dysfunction, and impairment of Na^+/K^+-ATPase activity in hippocampal neurons. *Brain Res.* **756**, 205–214.

Mark, R. J., Lovell, M. A., Markesbery, W. R., Uchida, K., and Mattson, M. P. (1997b). A role for 4-hydroxynonenal, an aldehydic product of lipid peroxidation, in disruption of ion homeostasis and neuronal death induced by amyloid β-peptide. *J. Neurochem.* **68**, 255–264.

Masliah, E. (1997). Role of amyloid precursor protein in the mechanisms of neurodegeneration in Alzheimer's disease. *Lab. Invest.* **77**, 197–209.

Masliah, E., Alford, M., DeTeresa, R., Mallory, M., and Hansen, L. (1996). Deficient glutamate transport is associated with neurodegeneration in Alzheimer's disease. *Ann. Neurol.* **40**, 759–766.

Masliah, E., Raber, J., Alford, M., Mallory, M., Mattson, M. P., Yang, D., Wong, D., and Mucke, L. (1998). Amyloid protein precursor stimulates excitatory amino acid transport. Implications for roles in neuroprotection and pathogenesis. *J. Biol. Chem.* **273**, 12548–12554.

Matsumoto, H., Kikuchi, S., and Ito, M. (1982). Age-related changes in the glutamate metabolism of cerebral neocortical slices from rats. *Neurochem. Res.* **7**, 679–685.

Mattson, M. P. (1990). Antigenic changes similar to those seen in neurofibrillary tangles are elicited by glutamate and calcium influx in cultured hippocampal neurons. *Neuron* **4**, 105–117.

Mattson, M. P. (1992). Calcium as sculptor and destroyer of neuronal circuitry. *Exp. Gerontol.* **27**, 29–49.

Mattson, M. P. (1994). Calcium and neuronal injury in Alzheimer's disease. Contribution of β-amyloid precursor protein mismetabolism, free radicals, and metabolic compromise. *Ann. N.Y. Acad. Sci.* **747**, 50–76.

Mattson, M. P. (1997). Cellular actions of beta-amyloid precursor protein and its soluble and fibrillogenic derivatives. *Physiol. Rev.* **77**, 1081–1132.

Mattson, M. P., and Kater, S. B. (1989). Excitatory and inhibitory neurotransmitters in the generation and degeneration of hippocampal neuroarchitecture. *Brain Res.* **478**, 337–348.

Mattson, M. P., and Pedersen, W. A. (1998). Effects of amyloid precursor protein derivatives and oxidative stress on basal forebrain cholinergic systems in Alzheimer's disease. *Int. J. Dev. Neurosci.* **16**, 737–753.

Mattson, M. P., Murrain, M., Guthrie, P. B., and Kater, S. B. (1989). Fibroblast growth factor and glutamate: Opposing roles in the generation and degeneration of hippocampal neuroarchitecture. *J. Neurosci.* **9**, 3728–3740.

Mattson, M. P., Engle, M. G., and Rychlik, B. (1991). Effects of elevated intracellular calcium levels on the cytoskeleton and tau in cultured human neocortical neurons. *Mol. Chem. Neuropathol.* **15**, 117–142.

Mattson, M. P., Cheng, B., Davis, D., Bryant, K., Lieberburg, I., and Rydel, R. E. (1992). β-amyloid peptides destabilize calcium homeostasis and render human neocortical neurons vulnerable to excitotoxicity. *J. Neurosci.* **12**, 379–389.

Mattson, M. P., Cheng, B., and Smith-Swintosky, V. L. (1993a). Neurotrophic factor mediated protection from excitotoxicity and disturbances in calcium and free radical metabolism. *Semin. Neurosci.* **5**, 295–307.

Mattson, M. P., Zhang, Y., and Bose, S. (1993b). Growth factors prevent mitochondrial dysfunction, loss of calcium homeostasis and cell injury, but not ATP depletion in hippocampal neurons deprived of glucose. *Exp. Neurol.* **121**, 1–13.

Mattson, M. P., Kumar, K., Wang, H., Cheng, B., and Michaelis, E. K. (1993c). Basic FGF regulates the expression of a functional 71 kDa NMDA receptor protein that mediates calcium influx and neurotoxicity in hippocampal neurons. *J. Neurosci.* **13**, 4575–4588.

Mattson, M. P., Barger, S. W., Cheng, B., Lieberburg, I., Smith-Swintosky, V. L., and Rydel, R. E. (1993d). β-amyloid precursor protein metabolites and loss of neuronal calcium homeostasis in Alzheimer's disease. *Trends Neurosci.* **16**, 409–414.

Mattson, M. P., Cheng, B., Culwell, A. R., Esch, F. S., Lieberburg, I., and Rydel, R. E. (1993e). Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of β-amyloid precursor protein. *Neuron* **10**, 243–254.

Mattson, M. P., Cheng, B., Davis, D., Bryant, K., Lieberberg I., and Rydel, R. E. (1993f). β-amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity. *J. Neurosci.* **12**, 379–389.

Mattson, M. P., Tomaselli, K. J., and Rydel, R. E. (1993g). Calcium-destabilizing and neurodegenerative effects of aggregated β-amyloid peptide are attenuated by basic FGF. *Brain Res.* **621**, 35–49.

Mattson, M. P., Lovell, M. A., Furukawa, K., and Markesbery, W. R. (1995). Neurotrophic factors attenuate glutamate-induced accumulation of peroxides, elevation of intracellular Ca^{2+} concentration, and neurotoxicity and increase antioxidant enzyme activities in hippocampal neurons. *J. Neurochem.* **65**, 1740–1751.

Mattson, M. P., Guo, Z. H., and Geiger, J. D. (1999). Secreted form of amyloid precursor protein enhances basal glucose and glutamate transport and protects against oxidative impairment of glucose and glutamate transport in synaptosomes by a cyclic GMP-mediated mechanism. *J. Neurochem.* **73**, 532–537.

Mazur-Kolecka, B., Frackowiak, J., and Wisniewski, H. M. (1995). Apolipoproteins E3 and E4 induce, and transthyretin prevents accumulation of the Alzheimer's beta-amyloid peptide in cultured vascular smooth muscle cells. *Brain Res.* **698**, 217–222.

Mishizen, A., Sheffield, R., Nissanov, J., and Armstrong, D. M. (1999). *N*-methyl-D-aspartate mRNA expression in the hippocampus of normal elderly and patients with Alzheimer's disease pathology. *Soc. Neurosci. Abstr.* **29**, 840.

Mitchell, J. J., and Anderson, K. J. (1998). Age-related changes in [^{3}H]MK-801 binding in the Fischer 344 rat brain. *Neurobiol. Aging* **19**, 259–265.

Miyoshi, R., Kito, S., Doudou, N., and Nomoto, T. (1991a). Effect of age on α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) binding sites in the rat brain studied by in vitro autoradiography. *Neurochem Res.* **16**, 849–854.

Miyoshi, R., Kito, S., Doudou, N., and Nomoto, T. (1991b). Influence of age on *N*-methyl-D-aspartate antagonist binding sites in the rat brain studied by in vitro autoradiography. *Synapse* **8**, 212–217.

Mizukami, K., Mishizen, A., Ikonomovic, M. D., Sheffield, R., and Armstrong, D. M. (1997a). Alterations of AMPA-selected glutamate subtype immunoreactivity in the dentate gyrus after perforant pathway lesion. *Brain Res.* **768**, 354–360.

Mizukami, K., Ikonomovic, M. D., Mishizen, A., Sheffield, R., and Armstrong, D. M. (1997b). Alterations of $GABA_A$ $\beta 2/3$ immunoreactivity in the dentate gyrus after perforant pathway lesion. *NeuroReport* **8**, 3379–3383.

Monaghan, D. T., Geddes, J. W., Yao, D., Chung, C., and Cotman, C. W. (1987). [^{3}H]TCP binding sites in Alzheimer's disease. *Neurosci. Lett.* **73**, 197–200.

Monyer, H., Burnashev, N., Laurie, D. J., Sakmann, B., and Seeburg, P. H. (1994). Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron* **12**, 529–540.

Mouradian, M. M., Contreras, P. C., Monahan, J. B., and Chase, T. N. (1988). [^{3}H]MK-801 binding in Alzheimer's disease. *Neurosci. Lett.* **93**, 225–230.

Müller, W. E., Scheuer, K., and Stoll, S. (1994a). Glutamatergic treatment strategies for age-related memory disorders. *Life Sci.* **55**, 2147–2153.

Müller, W. E., Stoll, S., Scheuer, K., and Meichelbock, A. (1994b). The function of the NMDA – receptor during normal brain aging. *J. Neural Transm.* **44**(Suppl.), 145–158.

Nagahara, A. H., Nicolle, M. M., and Gallagher, M. (1993). Alterations in [^{3}H]-kainate receptor binding in the hippocampal formation of aged Long-Evans rats. *Hippocampus* **3**, 269–277.

Nagy, Z., Esiri, M. M., Jobst, K. A, Johnston, C., Litchfield, S., Sim, E., and Smith, A. D. (1995). Influence of the apolipoprotein E genotype on amyloid deposition and neurofibrillary tangle formation in Alzheimer's disease. *Neuroscience* **69**, 757–761.

Nakanishi, S. (1992). Molecular characterization of the family of metabotropic glutamate receptors. *In* "Molecular Characterization of the Family of Metabotropic Glutamate Receptors" (R. P. Simon ed.), pp. 15–16. Thieme, New York.

Neve, R. L., Dawes, L. R., Yankner, B. A., Benowitz, L. I., Rodriguez, W., and Higgins, G. A. (1990). Genetics and biology of the Alzheimer amyloid precursor. *Prog. Brain Res.* **86**, 257–267.

Nicoletti, V. G., Dell'Albani, C. P., Ragusa, N., and Stella, A. M. G. (1995). AMPA-Selective glutamate receptor subunits in the rat hippocampus during aging. *J. Neurosci. Res.* **40**, 220–224.

Nicolle, M. M., Bizon, J. L., and Gallagher, M. (1996). *In vitro* autoradiography of ionotropic glutamate receptors in hippocampus and striatum of aged-Long-Evans rats: Relationship to spatial learning. *Neuroscience* **74**, 741–756.

Ninomiya, H., Fukunaga, R., Taniguchi, T., Fujiwara, M., Shimohana, S., and Kameyama, M. (1990). [^{3}H]N-[1-(2-Thienyl)cyclohexyl]-3,4-piperidine ([^{3}H]TCP). binding in human frontal cortex: Decreases in Alzheimer-type dementia. *J. Neurochem.* **54**, 526–532.

Nistico, G., Ciriolo, M. R., Riskinm, D., Iannone, M., DeMartino, A., and Rotillio, G. (1992). NGF restores decrease in catalase activity and increases superoxide dismutase and glutathione peroxidase activity in the brain of aged rats. *Free Radical Biol. Med.* **12**, 177–181.

Nixon, R. A., Saito, K. I., Grynspan, F., Griffin, W. R., Katayama, S., Honda, T., Mohan, P. S., Shea, T. B., and Beermann, M. (1994). Calcium-activated neutral proteinase (calpain) system in aging and Alzheimer's disease. *Ann. N. Y. Acad. Sci.* **747**, 77–91.

Nomura, Y., Kitamura, Y., Ohnuki, T., Arima, T., Yamanaka, Y., Sasaki, K., and Oomura, Y. (1997). Alterations in acetylcholine, NMDA, benzodiazepine receptors and protein kinase C in the brain of the senescence-accelerated mouse: An animal model useful for studies on cognitive enhancers. *Behav. Brain Res.* **83**, 51–55.

Ohm, T. G., Kirca, M., Bohl, J., Scharnagl, H., Gross, W., and Marz, W. (1995). Apolipoprotein E polymorphism influences not only cerebral senile plaque load but also Alzheimer-type neurofibrillary tangle formation. *Neuroscience* **66**, 583–587.

Olney, J. W. (1990). Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. *Annu. Rev. Pharmacol. Toxicol.* **30**, 47–71.

Pagliusi, S. R., Gerrard, P., Abdallah, M., Talabot, D., and Catsicas, S. (1994). Age-related changes in expression of AMPA-selective glutamate receptor subunits: Is calcium-permeability altered in hippocampal neurons? *Neuroscience* **61**, 429–433.

Palmer, A. M., and Burns, M. A. (1994). Preservation of redox, polyamine, and glycine modulatory domains of the *N*-methyl-D-asparate receptor in Alzheimer's disease. *J. Neurochem.* **62**, 187–196.

Pellegrini-Giampietro, D. E., Bennett, M. V. L., and Zukin, S. (1994). AMPA/Kainate receptor Gene expression in normal and Alzheimer's disease hippocampus. *Neuroscience* **61**, 41–49.

Pellegrini-Giampietro, D. E, Gorter, J. A., Bennett, M. V. L., and Zukin, R. S. (1997). The GluR2 (GluR-B) hypothesis: Ca^{2+}-permeable AMPA receptors in neurological disorders. *Trends Neurosci.* **20**, 464–470.

Pelleymounter, M. A., Beatty, G., and Gallagher, M. (1990). Hippocampal ^{3}H-CPP binding and spatial learning deficits in aged rats. *Psychobiology* **18**, 298–304.

Penney, J. B., Maragos, W. F., Greenamyre, J. T., Debowey, D. L., Hollingsworth, Z., and Young, A. B. (1990). Excitatory amino acid binding sites in the hippocampal region of Alzheimer's disease and other dementias. *J. Neurol. Neurosurg. Psychiatry* **53**, 314–320.

Peterson C., and Cotman, C. W. (1989). Strain-dependent decrease in glutamate binding to the *N*-methyl-D-Aspartic acid receptor during aging. *Neurosci. Lett.* **104**, 309–313.

Pittaluga, A., Fedele, E., Risiglione, C., and Raiteri, M. (1993). Age-related decrease of the NMDA receptor-mediated noradrenaline release in rat hippocampus and partial restoration by D-cycloserine. *Eur. J. Pharmacol.* **231**, 129–134.

Pollard, H. B., Arispe, N., and Rojas, E. (1995). Ion channel hypothesis for Alzheimer amyloid peptide neurotoxicity. *Cell. Mol. Neurobiol.* **15**, 513–526.

Porter, R. H. P., Cowburn, R. F., Alasuzoff, I., Briggs, R. S. J., and Roberts, P. J. (1992). Heterogeneity of NMDA receptors labelled with [^{3}H]3-(($\pm$)-2-carboxypiperazin-4-yl). propyl-1-phosphonic acid ([^{3}H]CPP): Receptor status in Alzheimer's disease brains. *Eur. J. Pharmacol.* **225**, 195–201.

Porter, R. H. P., Roberts, P. J., and Briggs, R. S. J. (1993). NMDA receptor status in elderly normal individuals and those with Alzheimer's disease. *Ann. N. Y. Acad. Sci.* **695**, 50–53.

Price, M. T., Olne, J. W., and Haft, R. (1981). Age-related changes in glutamate concentration and synaptosomal glutamate uptake in adult rat striatum. *Life Sci.* **28**, 1365–1370.

Procter, A. W., Wong, E. H. F., Stratmann, G. C., Lowe, S. L., and Bowen, D. M. (1989a). Reduced glycine stimulation of [^{3}H]MK-801 binding in Alzheimer's disease. *J. Neurochem.* **53**, 698–704.

Procter, A. W. Stirling, J. M., Stratmann, G. C., Cross, A. J., and Bowen, D. M. (1989b). Loss of glycine-dependent radioligand binding to the *N*-methyl-D-aspartate-phencyclidine receptor complex in patients with Alzheimer's disease. *Neurosci. Lett*. **101**, 62–66.

Represa, A., Duyckaerts, C., Tremblay, E., Hauw, J. J., and Ben-Ari, Y. (1988). Is senile dementia of the Alzheimer type associated with hippocampal plasticity? *Brain Res.* **457**, 355–359.

Richey, P. L, Siedlak, S. L., Smith, M. A., and Perry, G. (1995). Apolipoprotein E interaction with the neurofibrillary tangles and senile plaques in Alzheimer disease: Implications for disease pathogenesis. *Biochem. Biophys. Res. Commun.* **208**, 657–663.

Sanan, D. A., Weisgraber, K., Huang, D. Y., Saunders, A., Schmechel, D., Wisniewski, T., Frangione, B., Roses, A. D., and Strittmatter, W. J. (1994). Apolipoprotein E associates with Aβ amyloid peptide to form novel monofibrils: Isoform apoE4 associates more efficiently than apoE3. *J. Clin. Invest.* **94**, 880–889.

Sautière, P. E., Sindou, P., Couratier, P., Hugon, J., Wattez, A., and Delacourte, A. (1992). Tau antigenic changes induced by glutamate in

rat primary culture model: A biochemical approach. *Neurosci. Lett.* **140**, 206–210.

Scheuer, K., Maras, A., Gattaz, W. F., Cairns, N., Förstl, H., and Müller, W. E. (1996). Cortical NMDA receptor properties and membrane fluidity are altered in Alzheimer's disease. *Dementia* **7**, 210–214.

Schiffer, H. H., Swanson, G. T., and Heineman, S. F. (1997). Rat GluR7 and a carboxy-terminal splice variant, GluR7b, are functional kainate receptor subunits with a low sensitivity to glutamate. *Neuron* **19**, 1141–1146.

Schubert, D., and Behl, C. (1993). The expression of amyloid beta precursor protects nerve cells from β-amyloid and glutamate toxicity and alters their interaction with the extracellular matrix. *Brain Res.* **629**, 275–282.

Scott, H. L., Tannenberg, A. E., and Dodd, P. R. (1995). Variant forms of neuronal glutamate transporter sites in Alzheimer's disease cerebral cortex. *J. Neurochem.* **64**, 2193–2202.

Seeburg, P. H. (1993). The molecular biology of mammalian glutamate receptor channels. *Trends Neurosci.* **16**, 359–365.

Selkoe, D. J. (1991). The molecular pathology of Alzheimer's disease. *Neuron* **6**, 487–498.

Selkoe, D. J. (1994). Alzheimer's disease: A central role for amyloid. *J. Neuropathol. Exp. Neurol.* **53**, 438–447.

Serra, M., Ghiani, C. A., Foddi, M. C., Motzo, C., and Biggio, G. (1994). NMDA receptor function is enhanced in the hippocampus of aged rats. *Neurochem. Res.* **19**, 483–487.

Shoji, M., Golde, T. E., Ghiso, J., Cheung, T. T., Estus, S., Shaffer, L. M., Cai, X. D., McKay, D. M., Tintner, R., Frangione, B., and Younkin, S. G. (1992). Production of the Alzheimer amyloid beta protein by normal proteolytic processing. *Science* **258**, 126–129.

Sholz, W. K., and Palfrey, H. C. (1991). Glutamate-stimulated protein phosphorylation in cultured hippocampal pyramidal neurons. *J. Neurosci.* **11**, 2422–2432.

Siesjo, B. K., Bengtsson, F., Grampp, W., and Theander, S. (1989). Calcium, excitotoxins, and neuronal death in the brain. *Ann. N. Y. Acad. Sci.* **568**, 234–251.

Simpson, M. D. C., Royston, M. C., Deakin, J. F. W., Cross, A. J., Mann, D. M. A., and Slater, P. (1988). Regional changes in [^{3}H]D-aspartate and [^{3}H]TCP binding sites in Alzheimer's disease brains. *Brain Res.* **462**, 76–82.

Sindou, P., Couratier, P., Barthe, D., and Hugon, J. (1992). A dose-dependent increase of Tau immunostaining is produced by glutamate toxicity in primary neuronal cultures. *Brain Res.* **572**, 242–246.

Sommer, B., Kohler, M., Sprengel, R., and Seeburg, P. H. (1991). RNA editing in brain controls a determinant of ion flow in glutamate-gated channels. *Cell (Cambridge, Mass.)* **67**, 11–29.

Strittmatter, W. J., and Roses, A. D. (1995). Apolipoprotein E and Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 4725–4727.

Strittmatter, W. J., Saunders, A. M., Goedert, M., Weisgraber, K. H., Dong, L-M., Jakes, R., Huang, D. Y., Pericak-Vance, M., Schmechel, D., and Roses A. D. (1994). Isoform-specific interactions of apolipoprotein E with microtubule-associated protein tau: Implications for Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 11183–11186.

Swanson, G. T., Kamboj, S. K., and Cull-Candy, S. G. (1997). Single-channel properties of recombinant AMPA receptors depend on RNA editing, splice variation, and subunit composition. *J. Neurosci.* **17**, 58–59.

Tamaru, M., Yoneda, Y., Ogita, K., Shimizu, J., and Nagata, Y. (1991). Age-related decreases of the *N*-methyl-D-aspartate receptor complex in the rat cerebral cortex and hippocampus. *Brain Res.* **542**, 83–90.

Trojanowski, J. Q., and Lee V. M.-Y. (1995). Phosphorylation of paired helical filament tau in Alzheimer's disease neurofibrillary lesions: Focusing on phosphatases. *FASEB J.* **9**, 1570–1576.

Ulas, J., and Cotman, C. W. (1997). Decreased expression of *N*-methyl-D-Aspartate receptor 1 message RNA in select regions of Alzheimer brain. *Neuroscience* **79**, 973–982.

Ulas, J., Brunner, L. C., Geddes, J. W., Choe, W., and Cotman C. W. (1992). *N*-Methyl-D-Aspartate receptor complex in the hippocampus of elderly, normal individuals and those with Alzheimers's disease. *Neuroscience* **49**, 45–61.

Ulas, J., Weihmulle, F. B., Brunner, L. C., Joyce, J. N., Marshall, J. F., and Cotman, C. W. (1994). Selective increase of NMDA-sensitive glutamate binding in the striatum of Parkinson's disease, and mixed Parkinson's disease/Alzheimer's disease patients: An autoradiographic study. *J. Neurosci.* **14**, 6317–6324.

Wardas, J., Pietraszek, M., Schulze, G., Ossowska, K., and Wolfarth, S. (1997). Age-related changes in glutamate receptors: An autoradiographic analysis. *Pol. J. Pharmacol.* **49**, 401–410.

Weiss, J. H., Pike, C. J., and Cotman, C. W. (1994). Ca^{2+} channel blockers attenuate beta-amyloid peptide toxicity to cortical neurons in culture. *J. Neurochem.* **62**, 372–375.

Wenk, G. L. (1993). Age-related changes in monkey and rodent neurochemistry. *Neurobiol. Aging* **14**, 689–690.

Wenk, G. L., Walker, L. C., Price, D. L., and Cork, L. C. (1991). Loss of NMDA, but not Gaba-A, binding in the brains of aged rats and monkeys. *Neurobiol. Aging* **12**, 93–98.

Wernyj, R. P., Mattson, M. P., and Christakos, S. (1999). Expression of calbindin-D28k in C6 glial cells stabilizes intracellular calcium levels and protects against apoptosis induced by calcium ionophore and amyloid beta-peptide. *Mol. Brain Res.* **64**, 69–79.

Whitson, J. S., and Appel, S. H. (1995). Neurotoxicity of A beta amyloid protein in vitro is not altered by calcium channel blockade. *Neurobiol. Aging* **16**, 5–10.

Wisniewski, T., Castano, E. M., Golabek, A., Vogel, T., and Frangione, B. (1994). Acceleration of Alzheimer's fibril formation by apolipoprotein E in vitro. *Am. J. Pathol.* **145**, 1030–1035.

Yankner, B. A., Dawes, L. R., Fisher, S., Villa-Komaroff, L., Oster-Granite, M. L., and Neve, R. L. (1989). Neurotoxicity of a fragment of the amyloid precursor associated with Alzheimer's disease. *Science* **245**, 417–420.

Yankner, B. A., Duffy, L. K., and Kirschner, D. A. (1990). Neurotrophic and neurotoxic effects of amyloid beta protein: Reversal by tachykinin neuropeptides. *Science* **250**, 279–282.

Yasuda, R. P., Ikonomovic, M., Sheffield, R., Rubin, R. T., Wolfe, B. B., and Armstrong, D. M. (1995). Reduction of AMPA-selective glutamate receptor subunits in the entorhinal cortex of patients with Alzheimer's disease pathology: A biochemical study. *Brain Res.* **678**, 161–167.

Luc Buée and André Delacourte

21

Tau Phosphorylation

I. Introduction

The emergence of molecular biology and genetics in neurobiology allows for new classifications of neurodegenerative disorders. Among them, one is based on the nature of abnormal protein aggregates. For instance, it is possible to distinguish α-synucleopathies (Lewy body disease, multiple system atrophy, Parkinson's disease), tauopathies [e.g., Alzheimer's disease (AD) and related disorders with neurofibrillary tangles (NFT), Pick's disease], and poly-Glu inclusions (e.g., ataxia, Huntington's disease) (Goedert *et al*., 1998). This new classification emphasizes the role of these proteins or their modifications in the physiopathological process. This chapter focuses on tauopathies that include many dementing neurodegenerative disorders exhibiting intracellular inclusions made of phosphorylated microtubule-associated tau proteins (Delacourte and Buée, 1997; Goedert, 1998).

Neurodegenerative disorders are characterized by neuronal loss and intraneuronal accumulations of fibrillary materials. Neuropathologists distinguish several intracellular inclusions such as Hirano bodies, Lewy bodies, Pick bodies, and NFT. Hyperphosphorylated microtubule-associated tau proteins are the main components of the aggregated filaments found in NFT and Pick bodies. These tau aggregates are consistently found in AD (Brion *et al.*, 1985), amyotrophic lateral sclerosis/parkinsonism–dementia complex of Guam (ALS/PDC) (Joachim *et al*., 1987; Buée-Scherrer *et al*., 1995), progressive supranuclear palsy (PSP) (Pollock *et al*., 1986; Hauw *et al*., 1990), and Pick's disease (Pollock *et al*., 1986; Hof *et al*., 1994a). They have been described in patients with myotonic dystrophy (Kiuchi *et al*., 1991; Vermersch *et al*., 1996) and in other rare conditions. They are also seen in normal aging (Delaère *et al*., 1989).

We will first describe what is known about tau structure at the gene and protein levels. We will then discuss tau phosphorylation among neurodegenerative disorders focusing on AD, as most of our knowledge on tau proteins derives from data obtained in AD. Finally, we will discuss how the study of modified tau proteins in these disorders allows for a better understanding of the disease process and how tau phosphorylation may be possibly considered as a biological marker of tauopathies.

II. Tau Proteins

A. Structure and Roles

Tau proteins belong to the microtubule-associated family (Weingarten *et al*., 1975) and are involved in microtubule assembly and stabilization. In human, they are found in neurons, although nonneuronal cells also have trace amounts (Gu *et al*., 1996).

In the adult brain, six tau isoforms are produced from a single gene, located on chromosome 17q21, by alternative mRNA splicing (Fig. 21.1). Exons 2, 3, and 10 are alternatively spliced and allow for six combinations ($2-3-10-$; $2+3-10-$; $2+3+10-$; $2-3-10+$; $2+3-10+$; $2+3+\ 10+$) (Goedert *et al*., 1989a,b; Kosik *et al*., 1989).

At the protein level, tau proteins constitute a family of six isoforms ranging from 352 to 441 amino acids with molecular masses ranging from 45 to 65 kDa when run on SDS–PAGE (Fig. 21.1A). The tau variants differ from each other by the presence or absence of 29 or 58 amino acid inserts located in the amino-terminal part and a 31 amino acid repeat located in the carboxy-terminal part. In absence of the latter, which is encoded by exon 10, the spliced products give rise to three tau isoforms with three repeats (3R). The three other tau isoforms contain this 31 amino acid repeat and thus have four repeats (4R). These repeats and their adjacent domains constitute the microtubule-binding domains of tau (Lee *et al*., 1988, 1989; Goedert *et al*., 1989a). In normal cerebral cortex, 3R-tau isoforms are slightly more predominant than 4R-tau isoforms (Goedert *et al*., 1989a). Furthermore, the two tau isoforms with the 58 amino acid insert are weakly expressed (Hong *et al*., 1998; Mailliot *et al*., 1998a). Finally, tau isoforms may be distributed differentially in neuronal subpopulations. For instance, 4R-tau isoforms are not detected by *in situ* hybridization in granular cells of the dentate gyrus (Goedert *et al*., 1989a). These variations indicate that the different domains of tau are likely to be involved in various physiological functions.

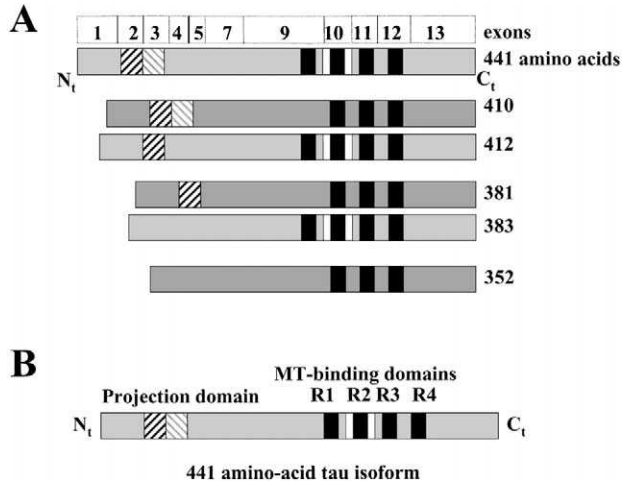

FIG. 21.1. (A) Schematic representation of the six brain tau isoforms (ranging from 352 to 441 amino acids). Constitutive and cassette exons code for different tau regions. Alternative splicing of the exons 2, 3, and 10 allows the six combinations (2−3−10−; 2+3−10−; 2+3+10−; 2−3−10+; 2+3−10+; 2+3+10+). They differ from each other by the addition of one or two 29 amino acid inserts [encoded by exons 2 (dark hatched box) and 3 (light hatched box)] in the amino-terminal domain in combination with either three (R1, R3, and R4) or four (R1–R4) microtubule-binding domains [referred to as 3R (light gray) and 4R (dark gray), respectively]. The fourth microtubule-binding domain (R2) is encoded by exon 10 (white box). (B) Protein domains of the longest brain tau isoform. The amino-terminal region is highly acidic and is referred to as the projection domain. The four repeat domains (R1–R4) constitute the microtubule-binding domains in the carboxy midpart of tau protein.

The two 29 amino acid sequences encoded by exons 2 and 3 are highly acidic and give different lengths to the amino-terminal part of tau proteins. They are referred to as the projection domain as this amino-terminal part projects from the microtubule surface and interacts with other cytoskeletal elements, cytoplasmic organelles, and plasma membrane (Fig. 21.1) (Delacourte and Buée, 1997; Mandelkow and Mandelkow, 1998).

As indicated earlier, the carboxy-terminal part of tau proteins is characterized by the presence of three or four microtubule-binding domains (Fig. 21.1). These repetitive domains are the repeats encoded by exons 9–12. The 3R or 4R is made of a highly conserved 18 amino acid repeat separated from each other by less conserved 13 or 14 amino acid interrepeat domains. It has been demonstrated that adult 4R tau isoforms are more efficient at promoting microtubule assembly than 3R tau isoforms. Data indicate that a heptapeptide (K_{224}KVAVVR$_{230}$) located in the proline-rich region has a high microtubule-binding activity in combination with the repeats regions (Fig. 21.2) (Goode *et al*., 1997). However, microtubule assembly also depends partially on the phosphorylation state of tau proteins: phosphorylated tau proteins are less effective than nonphosphorylated tau on microtubule polymerization.

B. Tau Phosphorylation and Physiology

1. Phosphorylation Sites

There are 80 putative Ser or Thr phosphorylation sites on the longest human brain tau isoform (441 amino acids), and tau proteins can be phosphorylated at multiple sites, some of which regulate their microtubule-binding properties. The phosphorylation sites can be divided in two classes: Ser/Thr-Pro and non-Ser/Thr-Pro sites. The first class includes sites mostly located in both regions flanking the microtubule-binding domains, whereas the second one includes sites all along the tau molecule.

Using phosphorylation-dependent monoclonal antibodies against tau, mass spectrometry, and sequencing, at least 30 Ser/Thr sites have been found to be phosphorylated (Table 21.1; Fig. 21.3) (Hasegawa *et al*., 1992; Morishima-Kawashima *et al*., 1995; Lovestone and Reynolds, 1997; Roder *et al*., 1997; Hanger *et al*., 1998; Johnson and Hartigan, 1998). All of these sites are localized outside the microtubule-binding domains with the exception of Ser262 (R1), Ser285 (R1-R2 IR), Ser305 (R2-R3 IR), Ser324 (R3), Ser352 (R4), and Ser356 (R4) (Goedert *et al*., 1989a,b; Seubert *et al*., 1995; Roder *et al*., 1997). Most of these phosphorylation sites are on Ser-Pro and Thr-Pro motives. A number of sites on non-Ser/Thr-Pro sites have also been identified. The different states of tau phosphorylation result from the activity of specific kinases and phosphatases.

2. Kinases

Most of the kinases involved in phosphorylation of the first class of tau sites are part of the proline-directed protein kinases

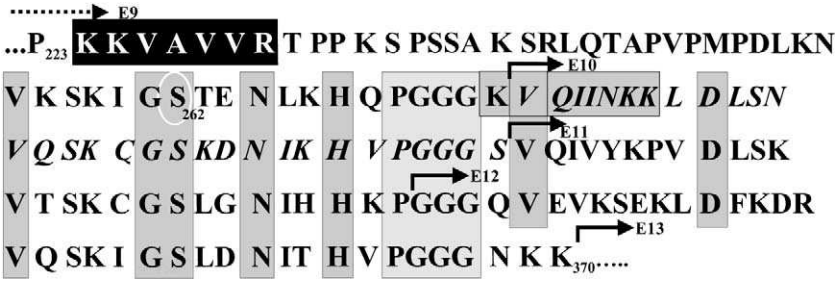

FIG. 21.2. Partial sequence of the 441 amino acid tau isoform (Pro223-Lys370), including microtubule-binding domains. Consensus sequences among the four microtubule-binding domains are in gray. Other major sequences with microtubule-binding properties are the heptapeptide in exon 9 (white characters/black box), R1–R2 inter-repeat (gray characters/gray box), and the phosphorylation site Ser262 (circle). The sequence encoded by exon 10 is in italic. The beginning of sequences encoded by exons 10, 11, 12, and 13 is indicated by an arrow.

TABLE 21.1 Tau Phosphorylation, Anti-tau antibodies, and Kinases[a]

Ser/Thr sites	AD-type Ab	Antibodies	*CaMKII*	*Cdc2*	*cdk5*	*CKI*	*CKII*	*GSK3*	*MAPK*	*MARK*	*PhK*	*pKA*	*pKC*	*SAPK*
T39						+								
S46P							+	+						
T50P							+	+						
T153P				+					+					
T181P		AT270			+				+					+
S184														
S188				+										
S199P								+	+					
S202P		*AT8*		+	+			+	+					+
T205P				+	+			+	+					+
T212P	***AT100***			+	+				+					
S214				+							+			
T217P									+					
T231P	***PHF-27*** ***TG-3***	*AT180* M4 PHF-6 PHF-41		+	+			+						+
S235P		*AT180* MC-6		+	+			+	+					
S237										+				
S262		12E8	+							+	+	+		
S285										+				
S293									+					
S305										+	+			
S324									+			+		
S352										+	+			
S356		12E8	+							+				
S396P		AD2 PHF-1 PHF-13 PHF-47 T3P			+	+		+	+					+
S404P		AD2 PHF-1		+	+	+		+						+
S409	***PG-5***										+			
S413							+							
S416		+									+			
S422P	**988/AP422**								+					+

[a]Ser/Thr sites are numbered according to the longest human tau isoform (441 amino acids). Ser/Thr sites followed by a proline are indicated by a P after the numbering. Antibodies that label phosphorylated sites are in columns 2 (abnormal phosphorylation, in bold face) and 3. The (+) signs indicate that this kinase phosphorylates tau on this particular site. CaMKII, calcium/calmodulin protein kinase II; CKI/CKII, casein kinases I and II; GSK3, glycogen synthase kinase 3; MAPK, mitogen-activated protein kinases; MARK, microtubule affinity regulating kinases; PhK, phosphorylase kinase; SAPK, stress-activated protein kinases.

(PDPK), which include mitogen-activated protein (MAP) kinases [erk1/2 and stress-activated protein kinases (SAPK)] (Drewes *et al*., 1992; Roder *et al*., 1997; Goedert *et al*., 1997), glycogen synthase kinase 3 (GSK3) (Hanger *et al*., 1992), and cyclin-dependent kinases, including cdc2 and cdk5 (Baumann *et al*., 1993). The second class includes non-Ser/Thr-Pro sites and can be phosphorylated by many other protein kinases, including microtubule affinity-regulating kinase (MARK) (Drewes *et al*., 1997), Ca^{2+}/calmodulin-dependent protein kinase II (CaMPK II) (Baudier and Cole, 1987), cyclic AMP-

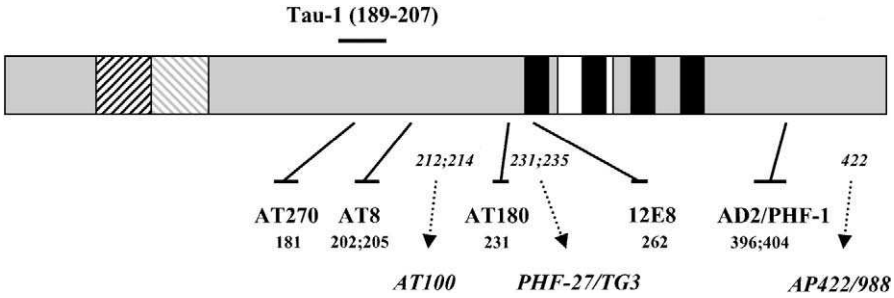

FIG. 21.3. Binding sites of anti-tau antibodies. The different well-known antibodies and their binding sites are represented on the schematic map of the 441 amino acid tau isoform (color codes are similar to those described in Fig. 21.1). With the exception of Tau-1, which recognizes the dephosphorylated 189–207 amino acid sequence, all antibodies bind to phosphorylated epitopes. Antibodies that recognize abnormal tau phosphorylation are in italic.

dependent kinase (PKA) (Litersky and Johnson, 1992), casein kinase II (Greenwood *et al*., 1994), and phosphorylase K (Paudel, 1997) (Table 21.1).

Numerous kinases, proline directed and non-proline directed, have to be used in tandem in order to obtain a complete phosphorylation of recombinant tau, which may be positively modulated at the substrate level by non-PDPK-catalyzed phosphorylations (Singh *et al*., 1996; Mandelkow and Mandelkow, 1998).

3. Phosphatases

Tau proteins from brain tissue or neuroblastoma cells are dephosphorylated rapidly by endogenous phosphatases (Matsuo *et al*., 1994; Buée-Scherrer *et al*., 1996a; Sontag *et al*., 1996; Soulié *et al*., 1996), such as Ser/Thr phosphatase proteins 1, 2A, 2B (calcineurin), and 2C, which are present in the brain (Cohen, 1997). Purified phosphatase proteins 1, 2A, and 2B can dephosphorylate tau proteins (Goedert *et al*., 1992a; Fleming and Johnson, 1995; Goedert *et al*., 1995). Like kinases, phosphatases have many direct or indirect physiological effects and counterbalance the action of kinases. Phosphatases acting on tau are regulated developmentally and may be associated with microtubules (Dudek and Johnson, 1995). For instance, protein phosphatase 1 is targeted to microtubules by tau proteins (Liao *et al*., 1998).

4. Phosphorylation Regulates Different Roles of Tau Proteins

a. Tau Phosphorylation and Microtubule Assembly. Tau proteins bind microtubules through their microtubule-binding domains. However, microtubule assembly depends partially on the phosphorylation state of tau proteins: phosphorylated tau proteins are less effective than nonphosphorylated tau on microtubule polymerization (Lindwall and Cole, 1984; Drubin and Kirschner, 1986). Phosphorylation of Ser262 alone reduces the affinity of tau for microtubules *in vitro* dramatically (Biernat *et al*., 1993). Nevertheless, this site alone, which is present in fetal tau and adult tau, as well as in hyperphosphorylated tau proteins found in NFT, is insufficient to eliminate tau binding to microtubules (Seubert *et al*., 1995). The proline-rich, amino-terminal region of tau proteins is highly phosphorylated, and hyperphosphorylation may also compete for ionic interactions that are important for structural–functional interactions between proline-rich and repeat regions (Goode *et al*., 1997). Thus, phosphorylation outside the microtubule-binding domains can strongly influence tubulin assembly by modifying tau-microtubule affinity.

b. Tau Phosphorylation in Development and Cell Sorting. Phosphorylation of tau proteins is regulated developmentally (Pope *et al*., 1993; Mawal-Dewan *et al*., 1994; Dudek and Johnson, 1995; Rösner *et al*., 1995; Saito *et al*., 1995). It is high in fetal and decreases with age due to phosphatase activation (Mawal-Dewan *et al*., 1994; Rösner *et al*., 1995). Also, there is likely an independent regulation of multiple phosphorylation sites within subcellular domains of developing neurons (Szendrei *et al*., 1993; Rebhan *et al*., 1995; Black *et al*., 1996; Kempf *et al*., 1996). However, compared to other MAPs, tau proteins are preferentially axonal (Litman *et al*., 1993, 1994). It was also shown that microtubules play a crucial role in axonal sorting of tau mRNA (Litman *et al*., 1994; Behar *et al*., 1995). Thus, both tau phosphorylation and microtubules may be involved in tau trafficking and cell sorting (nuclear, axonal, or somatodendritic) (Litman *et al*., 1993, 1994; Hirokawa *et al*., 1996).

In summary, these observations show that tau proteins are found in all cell compartments, but in different phosphorylation states. Phosphorylation seems to affect several sites simultaneously. Furthermore, within the same compartment, variability in the degree of phosphorylation is observed during development, due to the expression of several adult isoforms, and because the ratio between kinases and phosphatases is modified during development (Pope *et al*., 1993; Mawal-Dewan *et al*., 1994; Dudek and Johnson, 1995; Buée-Scherrer *et al*., 1996a). Phosphorylation, in combination with the type of isoform, can modulate the properties of tau proteins. In turn, tau proteins provide the microtubule with its own identity and physical characters (rigidity, length, stability, interactive capacity with other organelles). Therefore, by regulating microtubule assembly, tau proteins play a role in modulating the functional organization of the neuron, particularly in axonal morphology, growth, and cell polarity.

III. Tau Phosphorylation and Pathology

Phosphorylation is a key posttranslational modification in tau metabolism. However, in pathological conditions, hyper-

phosphorylation occurs and modifies tau biochemical properties, in that tau proteins become longer and stiffer (Hagestedt *et al*., 1989). In neurodegenerative disorders, hyperphosphorylated tau proteins are found aggregated into filamentous neuronal inclusions.

A. Nonhereditary Disorders

1. Sporadic Alzheimer's Disease

In AD, these filaments are named paired helical filaments (PHF). The major antigenic components of PHF are tau proteins (Brion *et al*., 1985), and several groups have reported phosphorylation as the major modification in these proteins (Grundke-Iqbal *et al*., 1986; Ihara *et al*., 1986, Flament and Delacourte, 1989; Greenberg *et al*., 1992). Their biochemical characterization by immunoblotting reveals the presence of a triplet of proteins (tau 55, 64, and 69) also referred to as A68, or PHF-tau (Delacourte *et al*., 1990; Lee *et al*., 1991; Goedert *et al*., 1992b; Greenberg *et al*., 1992). However, a 72 to 74 kDa component is also present in only very low amounts (Fig. 21.4A) (Mulot *et al*., 1994; Sergeant *et al*., 1997a). Using PHF-tau preparations, Goedert and colleagues (1992b) showed that dephosphorylated PHF-tau proteins have a more similar electrophoretic mobility than the six recombinant tau isoforms. The following scheme is now well established (Fig. 21.5): tau 55 results from the phosphorylation of the fetal isoform (2−, 3−, 10−), tau 64 from the phosphorylation of tau variants with one cassette exon (2+, 3−, 10− and/or 2−, 3−, 10+), and tau 69 from the phosphorylation of tau variants with two cassette exons (2+, 3+, 10− and/or 2+, 3−, 10+). Phosphorylation of the longest tau isoform (2+, 3+, 10+) induces the formation of the additional hyperphosphorylated tau 74 variant (Sergeant *et al*., 1995, 1997a; Mailliot *et al*., 1998a).

After death, native tau proteins from brain tissue are dephosphorylated rapidly by endogenous phosphatases, whereas PHF-tau are not (Matsuo *et al*., 1994; Mawal-Dewan *et al*., 1994; Buée-Scherrer *et al*., 1996a). Thus, in autopsy-derived materials, tau phosphorylation is a good marker of a pathological process. In this regard, tau proteins may be divided into four states: dephosphorylated, phosphorylated, hyperphosphorylated, and abnormally phosphorylated, which reflect their differences in both autopsy- and biopsy-derived materials (Table 21.2). Indeed, despite the fact that many phosphorylation sites are common to aggregated tau proteins, referred to as PHF-tau in AD, and native tau obtained from biopsy-derived materials, there are biochemical differences that distinguish them and support the concept of ''pathological tau.'' First, two-dimensional immunoblot analysis reveals that PHF-tau are more acidic than native tau (Sergeant *et al*., 1995). Second, the main difference between biopsy and postmortem tissues is that PHF-tau are aggregated, whereas tau from biopsies are not. Therefore, insoluble polymers of tau are present exclusively in AD brain extracts, which are visualized as ''smears'' on Western blots. Third, hyperphosphorylation generates differences that can be visualized by a few phosphorylation-dependent antibodies such as AT100 (Matsuo *et al*., 1994), AP422 (Hasegawa *et al*., 1996), 988 (Bussière *et al*., 1999), PHF-27 (Hoffmann *et al*., 1997), or the TG/MC antibodies (i.e., TG3) (Vincent *et al*., 1996) (Table 21.2; Fig. 21.3).

Altogether, these results show that the main feature of pathological tau is their aggregation into polymers that constitute neurofibrillary lesions. In addition, and possibly in association with the aggregation process, specific abnormal phosphorylation sites are also present on PHF-tau. Both this abnormal tau phosphorylation and the tau hyperphosphorylation (Table 21.2) are the ones that are analyzed in the next paragraphs. We do not consider normal tau phosphorylation, which is found only in biopsy-derived materials, as all neuropathological analyses are performed on autopsy-derived materials with more than 3 hr postmortem delays.

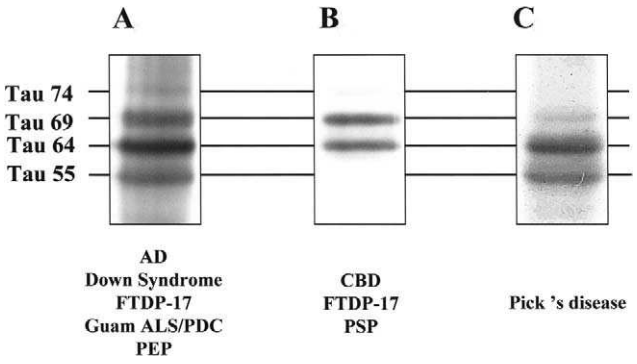

FIG. 21.4. Typical Western blots using the phosphorylation-dependent monoclonal antibody AD2 exhibiting the electrophoretic tau profiles encountered in tauopathies: the triplet tau 55, 64, and 69 and the minor 74 kDa tau variant (A), the tau doublet 64 and 69 and the minor 74 kDa variant (B), and the other doublet tau 55 and 64 and the minor 69 kDa tau variant (C). PEP, postencaphalitic parkinsonism.

2. Corticobasal Degeneration and Progressive Supranuclear Palsy

Tau aggregation is also observed in NFT in corticobasal degeneration (CBD) and progressive supranuclear palsy (see Chapter 13). However, in PSP and CBD, tau aggregates are characterized biochemically by a doublet (tau 64 and 69) and a minor variant at 74 kDa (Fig. 21.4B) (Flament *et al*., 1991; Ksiezak-Reding *et al*., 1994; Vermersch *et al*., 1994; Buée-Scherrer *et al*., 1996b; Feany and Dickson, 1996). In recent studies, tau isoforms with a sequence encoded by exon 10 were found in CBD and PSP, whereas tau isoforms without exon 10 were not detected. These data suggested that only phosphorylated tau isoforms with four microtubule-binding domains aggregate into filaments in CBD and PSP, (Fig. 21.6) (Mailliot *et al*., 1998a; Sergeant *et al*., 1999). The specific pathological tau epitopes found in AD are also observed on aggregated tau proteins in PSP and CBD, as visualized by antibodies AT100 (Schmidt *et al*., 1996; Mailliot *et al*., 1998a; Sergeant *et al*., 1999) and 988 (Bussière *et al*., 1999).

3. Pick's Disease

In Pick's disease, intraneuronal tau aggregates assemble into characteristic spherical Pick bodies (see Chapter 12). Tau from Pick bodies correspond to another doublet (tau 55 and 64) with a minor variant at 69 kDa (Fig. 21.4C) (Delacourte *et al*., 1996). The 55 and 64- kDa doublet is characteristic of Pick's disease because it is different from the AD profile or the CBD/

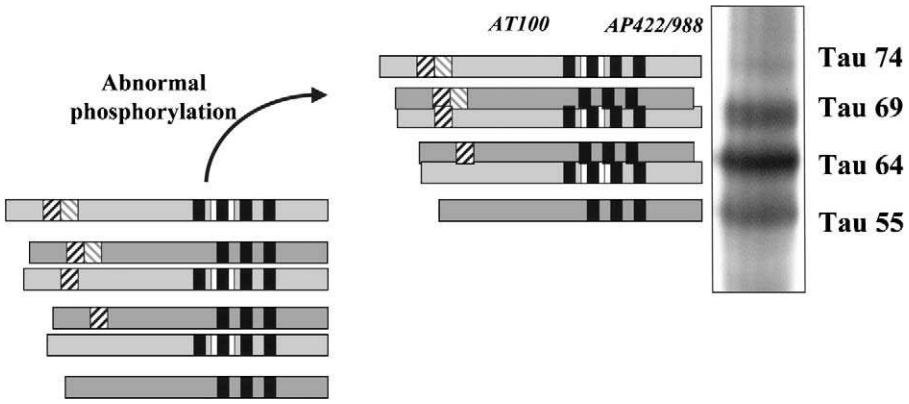

FIG. 21.5. Schematic representation of abnormal phosphorylation of the six brain tau isoforms in AD leading to higher molecular weight tau variants (tau 55, 64, and 69 and the minor tau 74 variant). It should be noted that the two tau isoforms with the 58 amino acid insert are expressed more weakly than the others. Tau 55 results from the phosphorylation of the shortest isoform (2−, 3−, 10−), tau 64 from the phosphorylation of tau variants with one cassette exon (2+, 3−, 10− and 2−, 3−, 10+), and tau 69 from the phosphorylation of tau variants with two cassette exons (2+, 3+, 10− and 2+, 3−, 10+). Phosphorylation of the longest tau isoform (2+, 3+, 10+) induces the formation of the additional hyperphosphorylated tau 74 variant. The color codes are similar to those used in Fig. 21.1. (Right) A typical immunoblot using the phosphorylation-dependent monoclonal antibody AD2, which recognizes phosphorylated Ser396 and 404, allowing the visualization of the AD-type electrophoretic profile (tau 55, 64, and 69 and the minor tau 74 variant). Antibodies AT100 and AP422/988 also labeled the tau triplet.

TABLE 21.2 Relative Tau Immunoreactivity among Neurodegenerative Disorders Using Phosphorylaltion-Dependent Antibodies

	AD2/PHF-1	12E8	AT100	988/AP422
AD	+++	+++	+ ++	+++
CBD/PSP	++	++	++	++
Pick	++	−	++	++
Biopsy-C	+	+	−	−
Autopsy-C	−	−	−	−

[a] +++, strong immunoreactivity; ++, medium immunoreactivity; +, immunoreactivity; −, lack of staining. Biopsy-C; biopsy-derived control materials; autopsy-C, autopsy-derived control materials.

PSP profile (Fig. 21.4) (Buée-Scherrer *et al*., 1996b; Mailliot *et al*., 1998a). The characteristic electrophoretic pattern of pathological tau in Pick's disease is well correlated with the presence of Pick bodies (Delacourte *et al*., 1996). As indicated previously, these neuronal cells do not contain tau isoforms with exon 10 (Goedert *et al*., 1989a). Interestingly, Pick bodies and the tau doublet tau 55 and 64 are not labeled with immunological probes directed against the sequence encoded by exon 10 (Sergeant *et al*., 1997b; Delacourte *et al*., 1998a; Mailliot *et al*., 1998a), suggesting that only 3R-tau isoforms aggregate into Pick bodies (Fig. 21.7). Moreover, Pick bodies and the Pick's disease tau doublet could not be detected by the monoclonal antibody 12E8 raised against the phosphorylated residue Ser 262 (Probst *et al*., 1996; Mailliot *et al*., 1998a),

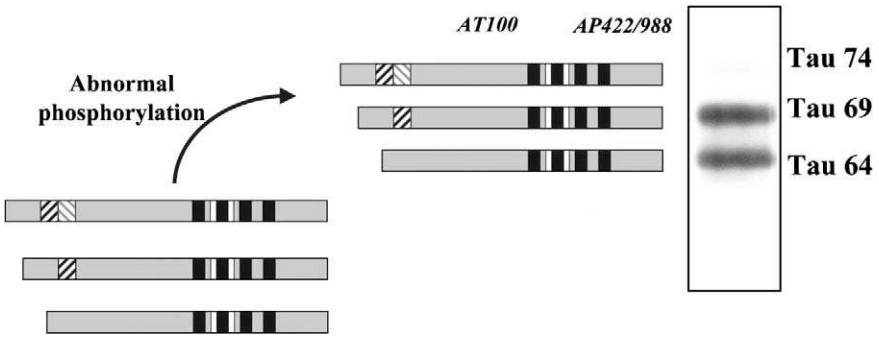

FIG. 21.6. Schematic representation of abnormal phosphorylation of the three brain 4R-tau isoforms in CBD and PSP leading to higher molecular weight tau variants (tau 64 and 69 and the minor tau 74 variant). Tau 64 results from the phosphorylation of the tau isoform with one cassette exon (2−, 3−, 10+) and tau 69 from the phosphorylation of the tau isoform with two cassette exons (2+, 3−, 10+). Phosphorylation of the longest tau isoform (2+, 3+, 10+), which is expressed weakly, induces the formation of the additional hyperphosphorylated tau 74 variant. The color codes are similar to those used in Fig. 21.1. (Right) A typical immunoblot using the phosphorylation-dependent monoclonal antibody AD2, which recognizes phosphorylated Ser396 and 404, allowing the visualization of the CBD/PSP-type electrophoretic profile (tau55, 64, and 69 and the minor tau 74 variant). Antibodies AT100 and AP422/988 also labeled the tau doublet.

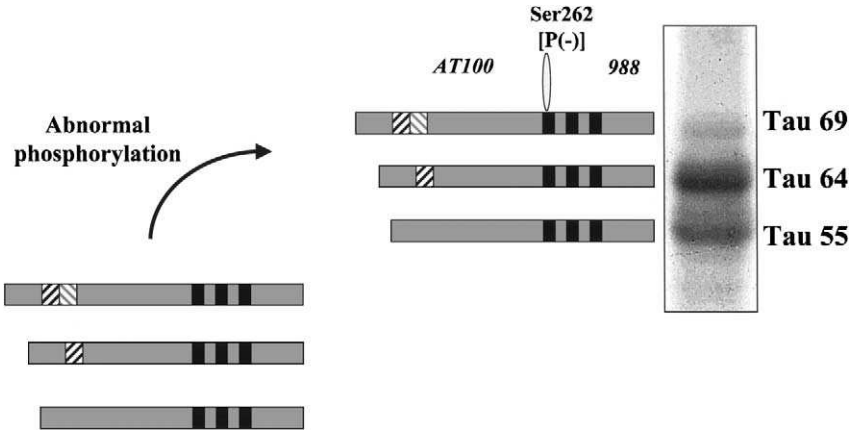

FIG. 21.7. Schematic representation of abnormal phosphorylation of the three brain 3R-tau isoforms in Pick's disease leading to higher molecular weight tau variants (tau 55 and 64 and the minor tau 69 variant). Tau 55 results from the phosphorylation of the shortest isoform (2−, 3−, 10−) and tau 64 from the phosphorylation of the tau isoform (2+, 3−, 10−). Phosphorylation of the weakly expressed tau isoform with two cassette exons (2+, 3+, 10+) induces the formation of the additional hyperphosphorylated tau 69 variant. The color codes are similar to those used in Fig. 21.1. (Right) A typical immunoblot using the phosphorylation-dependent monoclonal antibody AD2, which recognizes phosphorylated Ser396 and 404, allowing the visualization of the Pick-type electrophoretic profile (tau 55 and 64, and the minor tau 69 variant). Antibodies AT100 and 988 also labeled the tau doublet, whereas the 12E8 antibody, which recognizes phosphorylated Ser262, does not label it.

whereas in cells transfected with 3R tau isoforms, this site was found phosphorylated (Mailliot *et al*., 1998a). These data suggested that either Pick bodies bearing cells do not express kinases phosphorylating at Ser 262 or these kinases and tau proteins are not expressed in the same cell compartments. However, specific antibodies to pathological tau, including AT100 and 988, labeled the Pick's disease tau doublet (Sergeant *et al*., 1997b; Bussière *et al*., 1999).

Thus, particular sets of tau isoforms that aggregate in one given neurodegenerative disorder may lead to a specific electrophoretic tau profile (Delacourte *et al*., 1998a; Mailliot *et al*., 1998a). The abnormal phosphorylation visualized in AD using specific immunological tools, including AT100 and 988, is also observed on aggregated tau isoforms found in other neurodegenerative disorders. These data indicate that abnormal tau phosphorylation is a good biochemical marker of the neurofibrillary degeneration processes. This observation is still valuable in hereditary disorders as demonstrated in the following paragraphs.

B. Examples of Hereditary Disorders

1. Myotonic Dystrophy

Myotonic dystrophy (MD) is a slowly progressive multisystemic disorder characterized principally by myotonia and muscular atrophy. Impairment of intellectual and cognitive function in MD has also been reported (Jaspert *et al*., 1995). Myotonic dystrophy is an autosomal dominant defect in which the molecular basis is an unstable CTG trinucleotide repeat in the 3′-untranslated region of a gene encoding a putative serine/threonine protein kinase (myotonic dystrophy protein kinase: DMPK) located on chromosome 19 (Groenen and Wieringa, 1998).

Typically, neuropathological observations of the brain in MD show reduced brain weight, minor abnormalities in gyral architecture, and, microscopically, a disordered cortical cellular arrangement with neurons also present in subcortical white matter and intracytoplasmic inclusion bodies in cortical and subcortical structures (Ono *et al*., 1987). The presence of abnormally frequent NFT has also been reported in the temporal lobe, especially in the hippocampal cortex from patients with MD (Kiuchi *et al*., 1991). By immunoblotting, phosphorylated tau proteins are also detected in the hippocampus, the entorhinal cortex, and in most of the temporal areas. The amounts of pathological tau proteins are higher in the most severely affected MD cases but are always lower than in AD brain homogenates. Their profile differs from the characteristic triplet of AD, with low amounts of the tau 64 and 69 variants but high amounts of the tau 55 variant (Vermersch *et al*., 1996). The interesting observation about this pathology is the possible link between the genetic dysfunction of the Ser/Thr protein kinase and the presence of pathological tau protein. These observations demonstrate that changes in kinase expression can provoke a cascade of pathological events, including the aggregation of one tau isoform in specific brain areas (Vermersch *et al*., 1996).

Different tau electrophoretic profiles are found among neurodegenerative disorders. They are related to a combination of specific tau isoforms and particular phosphorylation sites. Because the myotonin-protein kinase is expressed in subsets of neurons (Balasubramanyam *et al*., 1998), the tau profile in MD may result from the phosphorylation of the shortest tau isoform by the myotonin-protein kinase in these subpopulations of neurons.

2. Frontotemporal Dementia and Parkinsonism Linked to Chromosome 17 (FTDP-17)

Some familial cases with frontotemporal dementia or related disorders, including disinhibition, atypical dementia, parkin-

sonism, and amyotrophy complex, familial progressive subcortical gliosis, and progressive parkinsonism dementia with pallidopontonigral degeneration exhibit a linkage with chromosome 17q21-22 and have been included in a group of pathologies referred to as frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) (Foster *et al*., 1997; Spillantini *et al*., 1998a).

Several mutations have been described on the tau gene in FTDP-17 (Clark *et al*., 1998; Dumanchin *et al*., 1998; Hutton *et al*., 1998; Poorkaj *et al*., 1998; Spillantini *et al*., 1998b; D'Souza *et al*., 1999; Murrell *et al*., 1999; Rizzu *et al*., 1999). Among the families studied, about 10 mismutations are found in coding regions and other mutations affect the splicing region after exon 10 (Table 21.3; Fig. 21.8). The presence of tau-immunoreactive intraneuronal and glial inclusions is a very common neuropathological feature.

The most prominent effect of mutations is a reduced ability of the mutated tau proteins to bind to microtubules. This reduced ability may be related to either a mutation in the microtubule-binding regions or close to a phosphorylation site involved in the regulation of tau binding to microtubules. Finally, other mutations (intronic and exonic close to a splicing site) enhance the formation of tau isoforms with 4R.

Tau isoforms (mutated, overexpressed, etc.) aggregate into filaments. Analysis of these filaments (straight, twisted ribbons, PHF) always reveals that aggregated tau isoforms leads to particular electrophoretic profiles (the doublet tau 64, and 69 and the triplet tau 55, 64 and 69) and are phosphorylated (Goedert *et al*., 1998; Spillantini *et al*., 1998a,b). These data indicate that in hereditary disorders where a tau mutation is involved, hyperphosphorylation of tau proteins still occurs and thus is a good biochemical marker of the fibrillary degeneration process.

TABLE 21.3 Tau Mutations in FTDP-17

Tau mutations	Location	Tau pathology	Reference
K257T	Exon 9	Pick doublet	Unpublished data
1260V	Exon 9	Pick doublet	Unpublished data
G272V	Exon 9	AD triplet	Hutton *et al*. (1998)
N279K	Exon 10	PSP doublet	Clark *et al*. (1998)
ΔK280	Exon 10		Rizzu *et al*. (1999)
L284L	Exon 10	PSP doublet	D'Souza *et al*. (1999)
P301L	Exon 10	PSP doublet	Hutton *et al*. (1998)
P301S	Exon 10	PSP doublet	Bugiani *et al*. (1999)
S305N	Exon 10	PSP doublet	D'Souza *et al*. (1999)
+3	Intronic	PSP doublet	Spillantini *et al*. (1998a,b)
+13	Intronic	PSP doublet	Hutton *et al*. (1998)
+14	Intronic	PSP doublet	Hutton *et al*. (1998)
+16	Intronic	PSP doublet	Hutton *et al*. (1998)
V337M	Exon 12	AD triplet	Poorkaj *et al*. (1998)
G389R	Exon 13	AD triplet	Murrell *et al*. (1999)
R406W	Exon 13	AD triplet	Hutton *et al*. (1998)

C. Combination of Tau Isoforms and Phosphorylation: A Better Understanding of the Degenerating Process

Tau isoforms with 3R and 4R may be expressed differentially and their aggregation may lead to different biochemical signatures characterized by tau doublets and the tau triplet. First, in hereditary disorders such as FTDP-17, the role of some tau mutations clearly emphasizes these differences of expression. Second, in the absence of mutations, Goedert *et al*. (1989a) showed that neurons do not express 3R and 4R tau isoforms equally (e.g., granule cells of the dentate gyrus), and Delacourte and colleagues (1998a) clearly demonstrated that only 3R tau isoforms are found in Pick bodies bearing cells such as granule cells. Third, tau proteins are found principally in axons. However, in some neurodegenerative disorders, hyperphosphorylated tau proteins accumulate in somatodendritic compartments. As indicated previously, tau trafficking is also phosphorylation dependent. Altogether, these observations indicate that in many tauopathies, different processes, including tau mutations/polymorphisms, aberrant cell trafficking, and cell vulnerability, act to affect tau metabolism leading to degeneration (Fig. 21.9). Whatever these processes might be, they lead to abnormal tau phosphorylation. Thus, it may be interesting to use abnormal tau phosphorylation as a biochemical marker of neurofibrillary degeneration.

IV. Abnormal Tau Phosphorylation as a Biochemical Marker

A. Kinases/Phosphatases Involved in Tau Abnormal Phosphorylation

Because phosphorylation of tau proteins is a common feature of neurodegenerative disorders referred to as tauopathies, a quantitative analysis of this phosphorylation may be useful in defining the degree of neurodegeneration in these disorders. Nevertheless, as described earlier, most of the phosphorylation sites found on pathological tau proteins are also detected in tau proteins obtained from biopsy-derived materials. However, tau proteins are hyperphosphorylated in pathological conditions and exhibit abnormally phosphorylated epitopes as shown by using particular antibodies such as 988, AP422, AT100, PHF-27, and TG3 (Matsuo *et al*., 1994; Hasegawa *et al*., 1996; Hoffmann *et al*., 1997; Jicha *et al*., 1999; Bussière *et al*., 1999). This hyperphosphorylation may lead to tau aggregation and may be related to either an increase in kinase activity or a decrease in phosphatase activity (Trojanowski and Lee, 1995). Many kinases have been involved (Lovestone and Reynolds, 1997). For instance, glycogen synthase kinase 3 (GSK3) is a kinase candidate (Hanger *et al*., 1992; Baum *et al*., 1996). However, these data are controversial as other studies do not support an involvement of GSK3 in tau hyperphosphorylation (Harr *et al*., 1996; Hasegawa *et al*., 1996). Mitotic protein kinases may also play a major role in tau phosphorylation, as many mitosis-specific epitopes are found in NFT (Kondratick and Vandre, 1996; Vincent *et al*., 1996). Stress-activated protein kinases are also of interest (Kyriakis *et al*., 1994; Cuenda *et al*., 1997). All SAP kinases (JNK/SAPK1, p38/SAPK2, SAPK3, SAPK4) have been shown to phosphorylate tau pro-

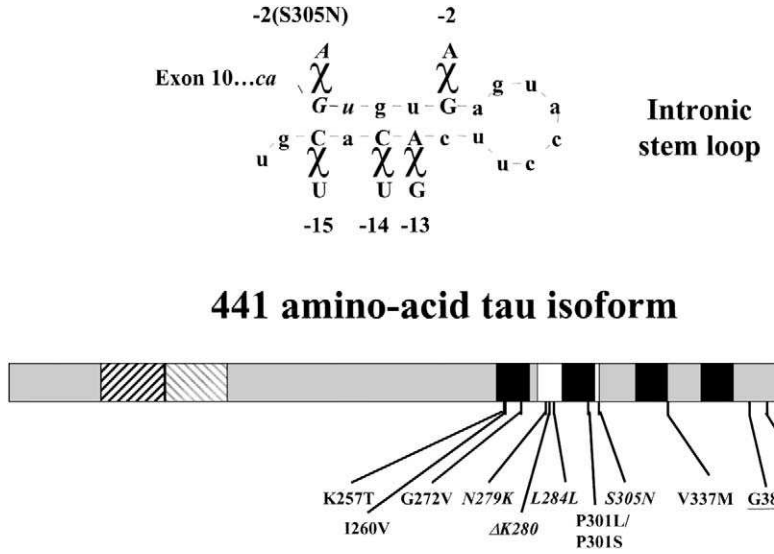

FIG. 21.8. (Top) Nucleotidic sequence of the end of exon 10 (italic black letters) and its 3′ intronic region. All FTDP-17-mutated nucleotides are in capital letters. Mutations are only shown in the stem loop structure. (Bottom) Schematic representation of the longest brain tau isoform and location of FTDP-17 mutations in the coding region. FTDP-17 mutations affecting alternative splicing are in italic, those affecting microtubule-binding domains are in bold, and those outside the microtubule-binding domains are underlined. S305N corresponds to the −2 mutation of the stem-loop structure (see top part of figure).

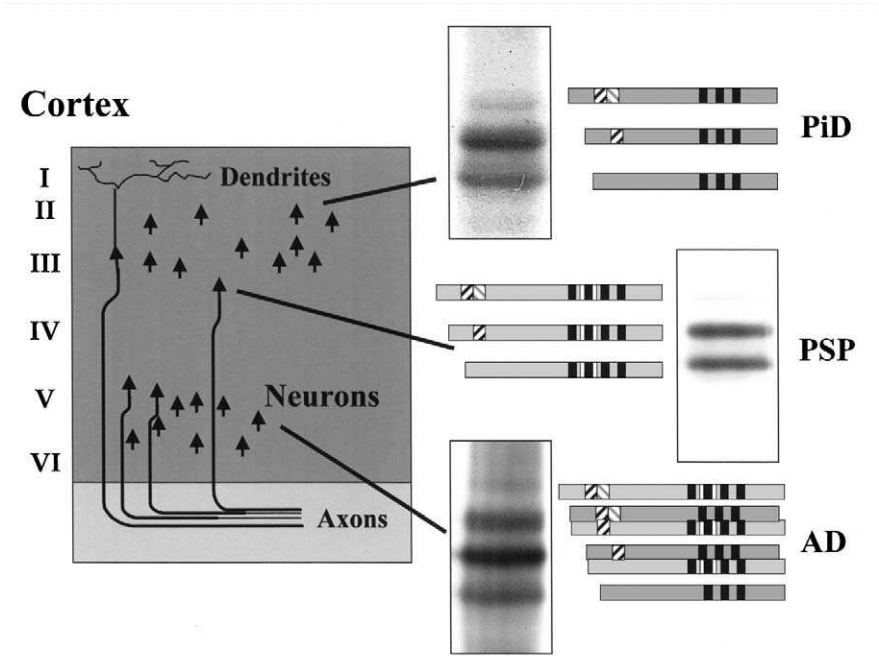

FIG. 21.9 Schematic representation of the laminar distribution of neurons in human isocortex. Because laminar and regional distributions of NFT are different among the dementing conditions, the presence of a pathologic tau triplet or doublet may be specific of a subtype of neurons. Thus, we suggest that different tau isoforms (with or without exon 10) are expressed in subsets of neurons that exhibit different vulnerability in addition to different sets of enzymes (kinases, phosphatases, etc.). Following abnormal phosphorylation, tau isoforms aggregate into filaments and display a particular electrophoretic profile when analyzed by immunoblotting. The color codes for tau isoforms are similar to those used in Fig. 21.1. (Right) A typical immunoblot using the phosphorylation-dependent monoclonal antibody AD2, which recognizes phosphorylated Ser396 and 404, allowing the visualization of the different electrophoretic tau profiles (triplet and doublets). PiD, Pick's disease.

teins *in vitro* (Goedert *et al*., 1997). Altogether, these data suggest that the SAPK family is an interesting candidate for the pathological phosphorylation of tau proteins. Conversely, tau hyperphosphorylation may be related to a decrease in phosphatase activity. Data suggest that phosphatase activities may be decreased in AD brains (Gong *et al*., 1995; Trojanowski and Lee, 1995). Furthermore, phosphatase inhibition in cell models allows the formation of specific AD-type epitopes, such as those recognized by AT100 and AP422/988 antibodies (Caillet-Boudin and Delacourte, 1996; Mailliot *et al*., 1998b).

Actually, the most interesting hypothesis is a sequential phosphorylation of tau proteins in the presence of cofactors (polyanions) leading to conformational changes (Mandelkow and Mandelkow, 1998). Such conclusions are supported by experiments about the AT100 epitope. The appearance of the AT100 epitope on tau proteins is obtained after a sequential phosphorylation of GSK3 in the presence of polyanions (glycosaminoglycans, RNA or lipids) and protein kinase A (Zheng-Fischhöfer *et al*., 1998). Protein kinase A may be a key kinase to obtain abnormal phosphorylation on tau proteins (Jicha *et al*., 1999).

B. Tau Phosphorylation Is a Reliable Marker of Neurofibrillary Degeneration in Aging and AD: Correlates with Cognitive Impairment

Using immunological probes specific of tau phosphorylation sites, it is possible to investigate biochemically NFT in postmortem brain materials from AD patients. There is a strong correlation between the immunohistochemical detection of NFT and the presence of the tau triplet, showing that tau phosphorylation is a reliable and early marker of the degenerating process. Therefore, tau phosphorylation can be used to quantify neurofibrillary degeneration in autopsy-derived brain materials (Flament *et al*., 1990a). Biochemical mappings using immunoblotting and/or ELISA have been performed in several cortical areas of the brain from patients with senile dementia of the Alzheimer type (Vermersch *et al*., 1992a; Holzer *et al*., 1994). Tau phosphorylation is detected in all areas studied, with the exception of some regions such as primary motor and visual cortices (Brodmann areas 4 and 17). The detection is particularly strong in association cortex compared to primary sensory cortex, with the highest levels in temporal neocortical and limbic areas. However, for a given brain area, tau immunoreactivity differs among cases (Vermersch *et al*., 1992a; Holzer *et al*., 1994).

A study of 130 cases ranging from normal aging to severe AD quantified hyperphosphorylated tau by immunoblotting in different cortical areas (Delacourte *et al*., 1998b, 1999). This study shows a sequential progression of neurofibrillary degeneration in cortical brain areas and allows for a classification into 10 stages (S0–S10) (Fig. 21.10). In this scheme, tau hyperphosphorylation is seen consistently in the transentorhinal and entorhinal cortex of nondemented individuals older than 75 years (S1 and S2). In addition, the hippocampus is also frequently affected (S3). This tau phosphorylation is similar to that found in AD brains, with the characteristic tau triplet or PHF-tau (Vermersch *et al*., 1992b; Delacourte *et al*., 1998b; 1999). However, lower amounts of PHF-tau are present in aged controls. In early AD, the neurofibrillary degeneration pathway is highly specific at the beginning of the disease, spreading from the hippocampal formation (S3) to the anterior (S4), inferior (S5), and midtemporal cortex (S6). Then, the disease progresses into association areas of the temporal (superior), parietal, and frontal cortices (S7). Finally, primary motor or sensory areas such as the primary motor cortex (Brodmann area 4) or the primary visual cortex (area 17) are affected (S9a-c). This study shows that neurofibrillary degeneration has to involve almost the entire temporal cortex to induce clear clinical manifestations (Fig. 21.10) (Delacourte *et al*., 1998b, 1999). Comparable data were obtained using a classical immunohistochemical approach on different populations with fewer brain regions investigated (Arnold *et al*., 1991; Dickson *et al*., 1991; Hof *et al*., 1992a; Bouras *et al*., 1993, 1994; Giannakopoulos *et al*., 1994, 1995, 1997; Bierer *et al*., 1995; Duyckaerts *et al*., 1997). Because AD is a disease of the long corticocortical connections, such a hierarchical pathway of neurofibrillary degeneration is not surprising. In fact, these are specifically affected with a well-defined pattern, involving subsets of pyramidal neurons responsible for these connections, which are found mainly in layers III and VI of the neocortex (Morrison and Hof, 1997).

C. Tau Phosphorylation Correlates with Cognitive Impairment in Various Disorders

1. Down Syndrome

Due to the trisomy of chromosome 21, Down syndrome patients have numerous somatic dysfunctions that occur during development. They also develop a variable degree of cognitive impairment, usually leading to dementia. Amyloid deposition is observed in Down syndrome after 15 years, senile plaques are present, followed by neurofibrillary degeneration after 35 years, with tau accumulation (Mann *et al*., 1989; Hof *et al*., 1995). Using immunoblotting, it has been demonstrated that a triplet of pathological tau proteins similar to that found in AD is observed in the neocortex of Down syndrome patients older than 35 years and follows the cognitive impairment (Fig. 21.4A) (Flament *et al*., 1990b). This shows that biochemical dysfunctions linked to tau phosphorylation during Down syndrome are very similar to those found in AD.

2. Parkinsonism

a. Parkinsonism with Dementia. The most characteristic clinical features of Parkinson's disease include resting tremor, expressionless face, rigidity, and slowness in initiating and performing voluntary movements. Approximatively 20% of patients with Parkinson's disease develop dementia (Marder *et al*., 1995), and the cognitive changes evoke a frontal lobe dysfunction that may be a primary event in the disease (Pillon *et al*., 1995; Taylor *et al*., 1986). Neuropathologically, Parkinson's disease is characterized by neuronal loss, especially in substantia nigra and locus coeruleus, and by the presence of intracellular inclusions called Lewy bodies and Lewy neurites (Forno, 1996). Studies suggest that α-synuclein is the major component of Lewy body filaments (Goedert *et al*., 1998). Using immunoblotting, qualitative and quantitative analyses were performed, and tau abnormal phosphorylation

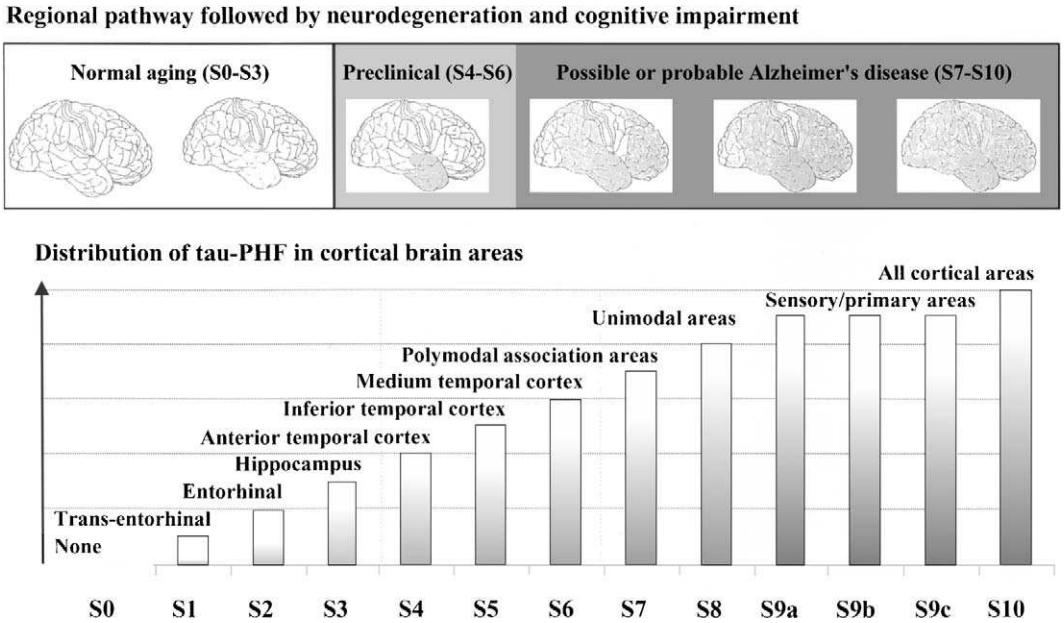

FIG. 21.10. Pathway of neurofibrillary degeneration in aging and Alzheimer's disease (see text). (Top) When PHF-tau are not detected or restricted to the hippocampal formation (S1–S3), subjects are nondemented. When PHF-tau are found in the temporal lobe, AD has began but it could be asymptomatic, as it is the preclinical stage of the disease (S4–S6). When the entire temporal cortex is affected (S7) and later, other brain regions, patients usually exhibit cognitive impairment and are diagnosed with possible or probable AD (S7–S10). (Bottom) PHF-tau distribution is never random. It is stereotyped and hierarchical. It corresponds to 10 brain regions that are successively affected (S1–S10) from the transentorhinal cortex (S1) to all cortical brain areas (S10).

corresponding to a pathological tau triplet similar to that described in AD was found in all demented cases without cortical Lewy bodies (Vermersch *et al*., 1993). The degree of abnormal tau phosphorylation is particularly high in the prefrontal, temporal and entorhinal cortices of Parkinson's disease patients with dementia. Therefore, abnormal tau phosphorylation is found in Parkinson's disease with dementia, but its cortical distribution differs from the pattern seen in AD, with a predominant involvement of prefrontal areas (Vermersch *et al*., 1993). In cases without dementia, tau abnormal phosphorylation is restricted to the hippocampal formation as described in elderly individuals (Vermersch *et al*., 1992b).

b. Postencephalitic Parkinsonism. Some patients who survived the influenza pandemic in the years 1916–1926 later developed postencephalitic parkinsonism (PEP) (Hof *et al*., 1992b; Buée-Scherrer *et al*., 1997). Extrapyramidal symptoms are the major clinical features and affected patients do not exhibit any cognitive changes and are usually neither aphasic nor apraxic. The immunohistochemical analysis of PEP cases demonstrated that NFT are found in variable densities in the hippocampus and entorhinal cortex, in neocortical areas 4, 9, and 20, and in subcortical regions. Higher NFT densities are observed in the hippocampal formation and area 20, compared to areas 4 and 9, and the putamen, indicating that some regions are affected preferentially by the degenerative process (Hof *et al*., 1992b).

Biochemical studies have shown that PEP cases display a tau pathology comparable to AD (Fig. 21.4A). However, the tau 55, 64, and 69 triplet is found in cortical and subcortical brain regions, including primary motor cortex and basal ganglia, in contrast to AD cases where this triplet is mainly restricted to hippocampal formation and association neocortex. Only patients with cognitive impairment display a tau pathology characterized by abnormal phosphorylation in association areas (Buée-Scherrer *et al*., 1997).

c. Guamanian ALS/PDC. The amyotrophic lateral sclerosis/parkinsonism–dementia complex of Guam is a chronic neurodegenerative disorder highly prevalent in the native Chamorro population of Guam island in the western Pacific (Hirano *et al*., 1961) (see Chapter 15). Neuropathologically, Guamanian ALS/PDC shows a severe cortical atrophy and neuronal loss. The neuropathological hallmark of ALS/PDC is the widespread NFT formation (Hirano *et al*., 1961, 1968). Immunohistochemical studies have also revealed that pathological tau proteins are present in NFT of ALS/PDC patients (Hof *et al*., 1994b; Buée-Scherrer *et al*., 1995; Bussière *et al*., 1999). By using immunoblotting and numerous phosphorylation-dependent antibodies, these proteins can be visualized as a triplet tau 55, 64, and 69 (Fig. 21.4A) (Buée-Scherrer *et al*., 1995; Mawal-Dewan *et al*., 1996). According to neuropathological data, and in contrast to AD patients where the tau triplet is found mostly in cortical regions, the Guamanian tau triplet is detected in both cortical and subcortical areas. Finally, Guam ALS/PDC patients are demented only when tau abnormal phosphorylation is found in asssociation areas (Buée-Scherrer *et al*., 1995).

ALS/PDC of Guam and PEP have been linked to external factors such as viruses and toxins that may induce similar neuropathologic changes characterized by the same tau electrophoretic profile. However, it is not known whether tau pathology in Guamanian ALS/PDC is also related to mutations in tau gene in the Chamorro population. Linkage analyses and

genetic studies do not support the involvement of tau as a primary cause for the disease, although it does not rule out the possibility of tau, particularly tau phosphorylation, being involved downstream in the process (Pérez-Tur *et al*., 1999).

d. Progressive Supranuclear Palsy. Progressive supranuclear palsy is a late-onset atypical parkinsonian disorder described by Steele *et al*., in 1964. Dementia is also a common feature at the end stage of the disease (Hauw *et al*., 1990).

The localization of NFT was first described in subcortical structures (Steele *et al*., 1964). Later, the degenerating process was described in cortical areas, with the same features as subcortical NFT (Hauw *et al*., 1990; Hof *et al*., 1992c). These studies demonstrated that the primary motor cortex is affected more severely than neocortical association areas compared to AD (Hauw *et al*., 1990; Hof *et al*., 1992c).

In PSP, the characteristic tau doublet (tau 64 and 69) described in Section III,A,2 was quantified (Vermersch *et al*., 1994). Biochemical mapping performed on several cortical and subcortical areas from PSP brain has revealed that this tau doublet is first detected in the subcortical regions where NFT are found, with neocortical areas being affected later (Vermersch *et al*., 1994). It is interesting to note that abnormal tau phosphorylation in cortical areas is always correlated to dementia. For instance, in a nondemented young PSP patient (33 years-old), abnormally phosphorylated tau proteins were found in both basal ganglia and thalamus, whereas they were absent in all of the other areas studied, including amygdala, hippocampus, and Brodmann's areas 4, 9, 11, 17, 18, 20, and 24 (Vermersch *et al*., 1997). Conversely, the other PSP cases with dementia studied contained large amounts of pathological tau proteins in the neocortex, especially in Brodmann areas 4 and 6 and in subcortical structures (Vermersch *et al*., 1997).

In all dementing disorders, detection of abnormal tau phosphorylation in association cortical areas appears to be always strongly correlated with cognitive impairment and dementia severity.

V. Factors That Modulate Tau Phosphorylation

Tau protein hyperphosphorylation may be modulated by a number of factors, including glucose metabolism, hypoxia, oxidation, and stress.

A. Glucose Metabolism

One of the key enzymes in tau phosphorylation is GSK3β (Hanger *et al*., 1992; Zheng-Fishhoffer *et al*., 1998). It is a protein, serine kinase implicated in the hormonal control of several regulatory proteins including glycogen synthase and the transcription factor c-jun. In AD, some brain regions are not properly supplied in glucose (see Chapters 16, 17, and 63). This hypometabolism may lead to a downregulation of insulin receptors. It is known that insulin and insulin-like growth factor 1 may activate 3-phosphoinositide-dependent protein kinase, which then activates protein kinase B, which in turn inactivates GSK3β (Hong and Lee, 1997). Thus, in case of low glucose concentrations and thus downregulation of insulin receptors, GSK3β is still active and phosphorylates tau proteins. This scheme may explain a first step in the pathology.

B. Ischemia

Ischemia disrupts the neuronal cytoskeleton both by promoting proteolysis of its components and by affecting kinase and phosphatase activities that alter its assembly (Dewar *et al*., 1994; Buée *et al*., 1996). In a reversible model of spinal cord ischemia in rabbits, tau has been found to be dephosphorylated in response to ischemia with a time course that closely matches the installation of permanent paraplegia. In a similar manner, Ca^{2+}/calmodulin-dependent kinase II activity is reduced only in the ischemic region. Thus, dephosphorylation of tau is an early marker of ischemia, as is the rapid loss of Ca^{2+}/calmodulin-dependent kinase II activity (Shackelford and Nelson, 1996). In a canine model of cardiac arrest, the effects of global brain ischemia/reperfusion (Rosenthal *et al*., 1992) on tau proteins were analyzed. Tau proteins are completely dephosphorylated on Ser/Thr-Pro sites, but after resuscitation and 2 hs of reperfusion, there is a full restoration of phosphorylation on Ser/Thr-Pro sites, whereas Ser262 phosphorylation is not restored (Buée *et al*., 1996; Mailliot *et al*., 1998c). Because phosphorylation that detaches tau protein from microtubules (Ser262, Ser214) also protects it against aggregation into PHF (Schneider *et al*., 1999), alterations in phosphorylation or degradation of tau may affect microtubule stability, possibly contributing to the disruption of axonal transport (Dewar and Dawson, 1995; Buée *et al*., 1996; Shackelford and Nelson, 1996; Mailliot *et al*., 1998c).

C. Stress

Stress-activated protein kinases have been involved in tau phosphorylation (Goedert *et al*., 1997; Reynolds *et al*., 1997a,b). They may also explain a number of observations. Cold water stress induces an immediate (30–90 min) two to threefold increase in the phosphorylation of tau proteins in rat brain, without direct involvement of the hypothalamus–pituitary–adrenal axis (Korneyev *et al*., 1995). Heat-shock stress also induces modifications of tau phosphorylation (Papasozomenos, 1996). Furthermore, JNK/SAPK1 and p38 are able to phosphorylate tau proteins (Reynolds *et al*., 1997a,b) and are linked to AD pathology (Mohit *et al*., 1995; Hensley *et al*., 1999).

D. Glycation and Oxidation

Glycation is the reaction between the NH_2 of a side chain of an amino acid and the aldehyde group (CHO) of a carbohydrate. Advanced glycation end-products generate oxygen-free radicals that could activate transcription via nuclear factor-κ B, increase βPP, and cause the release of 4-kDa amyloid peptides similar to Aβ. Therefore, glycated tau could induce oxidative stress, which may contribute to the pathogenesis of AD (Yan *et al*., 1995). In AD, antibodies against glycation products label NFT (Ledesma *et al*., 1994). The most suitable residues for glycation, lysines, which are present in the tubulin-binding motif of tau protein, seem to be preferentially modified compared to lysines in other regions. Among the modified lysines, those located in the sequence comprising residues 318–336 (in the largest human tau isoform) are found to be glycated, as determined by the reaction with an antibody that recognizes a glycated peptide containing this sequence. Because those

lysines are present in a tubulin-binding motif of tau protein, their modification could result in a decreased interaction of tau with tubulin (Ledesma *et al*., 1995). Furthermore, *in vitro* assembly of recombinant tau-derived constructs into PHF depends on intermolecular disulfide bridges formed by the single Cys322. Blocking the SH group, mutating Cys for Ala, or keeping tau in a reducing environment all inhibit assembly. These data imply that the redox potential in neurons is crucial for PHF assembly, independently or in addition to pathological phosphorylation reactions (Schweers *et al*., 1995). The tau regions affected in this oxidation process are those implicated in microtubule binding. It is interesting to note that these regions are also affected by mutations in FTDP-17 and that in all disorders, tau isoforms are found aggregated in a phosphorylated form.

VI. Tau Phosphorylation as Peripheral Marker

As described earlier, tau proteins are the major constituent of NFT, and their biochemical or immunohistochemical detection in the central nervous system is a reliable marker of the degenerative process. Therefore, their presence has been investigated in peripheral tissues and CSF in the hope it could represent a potential diagnostic tool. However, in AD, tau proteins are not found phosphorylated in peripheral tissues, including the olfactory system (Yamagishi and Ishizuka, 1994) and fibroblats (Ingelson *et al*., 1996).

The presence of tau proteins in CSF has been reported in numerous studies using a sandwich ELISA method (Vandermeeren *et al*., 1993), which permits the quantitation of both PHF-tau and total tau proteins (PHF-tau and normal tau). A significant increase in tau concentration has been shown in CSF of patients with AD and other tauopathies compared to control cases. However, this specificity is not sufficient to discriminate between CSF tau concentrations of AD patients and other disorders including vascular dementia and brain trauma (Andreasen *et al*., 1998; Zemlan *et al*., 1999). Regarding AD, an improved discrimination can be obtained by measuring out both Aβ and tau levels (Hulstaert *et al*., 1999).

Data suggest that tau-immunoreactive materials in CSF are mostly made of dephosphorylated and truncated N-terminal tau fragments (Johnson *et al*., 1997).

Altogether, these data indicate that there is an increase in tau protein concentration in CSF in tauopathies, but proteins do not bear the pathological sites of phosphorylation. However, an extensive and accurate analysis of CSF could be helpful in defining the tau protein species present at physiological state and released during the progression of a neurodegenerative disease. Thus, specific immunological probes against tau protein species could be developed and could lead to a sensitive and specific test to diagnosis AD and other neurodegenerative disorders.

VII. Concluding Remarks

In conclusion, among the different neurodegenerative disorders discussed in this chapter, differences in the regional tau distribution are observed. Because laminar and regional distributions of NFT are different among the dementing conditions, the presence of a pathologic tau triplet or doublet may be specific of a subtype of neurons. Thus, we suggest that different tau isoforms (with or without exon 10) are expressed in subsets of neurons that exhibit different vulnerability in addition to different sets of enzymes (kinases, phosphatases, etc.). However, it is also clear that tau mutations/polymorphisms may also emphasize this cell vulnerability by enhancing the expression of particular tau isoforms. Finally, it cannot be ruled out that abnormal tau phosphorylation may affect cell trafficking of tau isoforms and lead to neurofibrillary degeneration. Finally, because abnormal tau phosphorylation is a common feature to all tauopathies, a better understanding of this phosphorylation leading to neuronal degeneration in these dementing illnesses will be of crucial importance to develop strategies aimed at the therapeutic protection of the vulnerable neuronal populations.

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References

Andreasen, N., Vanmechelen, E., Van de Voorde, A., Davidsson, P., Hesse, C., Tarvonen, S., Raiha, I., Sourander, L., Winblad, B., and Blennow, K. (1998). Cerebrospinal fluid tau protein as a biochemical marker for Alzheimer's disease: A community based follow up study. *J. Neurol. Neurosurg. Psychiatry* **64**, 298–305.

Arnold, S. E., Hyman, B. T., Flory, J., Damasio, A. R., and Van Hoesen, G. W. (1991). The topographical and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer's disease. *Cereb. Cortex* **1**, 103–116.

Balasubramanyam, A., Iyer, D., Stringer, J. L., Beaulieu, C., Potvin, A., Neumeyer, A. M., Avruch, J., and Epstein, H. F. (1998). Developmental changes in expression of myotonic dystrophy protein kinase in the rat central nervous system. *J. Comp. Neurol*. **394**, 309–325.

Baudier, J., and Cole, R. D. (1987). Phosphorylation of Tau proteins to a state like that in Alzheimer's brain is catalyzed by a calcium/calmodulin-dependant kinase and modulated by phospholipids. *J. Biol. Chem.* **262**, 17577–17583.

Baum, L., Hansen, L., Masliah, E., and Saitoh, T. (1996). Glycogen synthase kinase 3 alteration in Alzheimer disease is related to neurofibrillary tangle formation. *Mol. Chem. Neuropathol*. **29**, 253–261.

Baumann, K., Mandelkow, E. M., Biernat, J., Piwnica-Worms, H., and Mandelkow, E. (1993). Abnormal Alzheimer-like phosphorylation of tau-protein by cyclin-dependent kinases cdk2 and cdk5. *FEBS Lett*. **336**, 417–424.

Behar, L., Marx, R., Sadot, E., Barg, J., and Ginzburg, I. (1995). cis-acting signals and trans-acting proteins are involved in tau mRNA targeting into neurites of differentiating neuronal cells. *Int. J. Dev. Neurosci*. **13**, 113–127.

Bierer, L. M., Hof, P. R., Purohit, D. P., Carlin, L., Schmeidler, J., Davis, K. L., and Perl, D. P. (1995). Neocortical neurofibrillary tangles correlate with dementia severity in Alzheimer's disease. *Arch. Neurol. (Chicago)* **52**, 81–88.

Biernat, J., Gustke, N., Drewes, G., Mandelkow, E. M., and Mandelkow, E. (1993). Phosphorylation of Ser(262) strongly reduces binding of tau proteins to microtubules—distinction between PHF-like immunoreactivity and microtubule binding. *Neuron* **11**, 153–163.

Black, M. M., Slaughter, T., Moshiach, S., Obrocka, M., and Fischer, I. (1996). Tau is enriched on dynamic microtubules in the distal region of growing axons. *J. Neurosci*. **16**, 3601–3619.

Bouras, C., Hof, P. R., and Morrison, J. H. (1993). Neurofibrillary tangle densities in the hippocampal formation in a non-demented population define subgroups of patients with differential early pathologic changes. *Neurosci. Lett*. **153**, 131–135.

Bouras, C., Hof, P. R., Giannakopoulos, P., Michel, J.-P., and Morrison, J. H. (1994). Regional distribution of neurofibrillary tangles and senile plaques in the cerebral cortex of elderly patients: A quantitative evaluation of a one-year autopsy population from a geriatric hospital. *Cereb. Cortex* **4**, 138–150.

Braak, H., and Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* **82**, 239–259.

Brion, J. P., Passareiro, H., Nuñez, J., and Flament-Durand, J. (1985). Immunological detection of tau protein in neurofibrillary tangles of Alzheimer's disease. *Arch. Biol*. **95**, 229–235.

Buée, L., Hof, P. R., Rosenthal, R. E., Delacourte, A., and Fiskum, G. (1996). Tau proteins phosphorylation and proteolysis in a canine model of cerebral ischemia/ reperfusion. *Soc. Neurosci. Abstr.* **22**, 1897.

Buée-Scherrer, V., Buée, L., Hof, P. R., Leveugle, B., Gilles, C., Loerzel, A. J., Perl, D. P., and Delacourte, A. (1995). Neurofibrillary degeneration in amyotrophic lateral sclerosis/ parkinsonism-dementia complex of Guam—Immunochemical characterization of Tau proteins. *Am. J. Pathol*. **146**, 924–932.

Buée-Scherrer, V., Condamines, O., Mourton-Gilles, C., Jakes, R., Goedert, M., Pau, B., and Delacourte, A. (1996a). AD2, a phosphorylation-dependent monoclonal antibody directed against Tau proteins found in Alzheimer's disease. *Mol. Brain Res*. **39**, 79–88.

Buée-Scherrer, V., Hof, P. R., Buée, L., Leveugle, B., Vermersch, P., Perl, D. P., Olanow, C. W., and Delacourte, A. (1996b). Hyperphosphorylated Tau proteins differentiate corticobasal degeneration and Pick's disease. *Acta Neuropathol*. **91**, 351–359.

Buée-Scherrer, V., Buée, L., Leveugle, B., Perl, D. P., Vermersch, P., Hof, P. R., Delacourte, A. (1997). Pathological tau proteins in postencephalitic parkinsonism: Comparison to Alzheimer's disease and other neurodegenerative disorders. *Ann. Neurol*. **42**, 356–359.

Bugiani, O., Murrell, J. R., Giaccone, G., Hasegawa, M., Ghigo, G., Tabaton, M., Morbin, M., Primavera, A., Carella, F., Solaro, C., Grisoli, M., Savoiardo, M., Spillantini, M. G., Tagliavini, F., Goedert, M., and Ghetti, B. (1999). Frontotemporal dementia and corticobasal degeneration in a family with a P301S mutation in tau. *J. Neurophathol. Exp. Neurol.* **58**, 667–677.

Bussière, T., Hof, P. R., Mailliot, C., Brown, C., Caillet-Boudin, M. L., Perl, D. P., Buée, L., and Delacourte, A. (1999). Phosphorylated serine-422 on tau proteins is a pathological epitope found in several diseases with neurofibrillary degeneration. *Acta Neuropathol*. **97**, 221–230.

Caillet-Boudin, M. L., and Delacourte, A. (1996). Induction of a specific tau Alzheimer epitope in SY5Y neuroblastoma cells. *NeuroReport* **8**, 307–310.

Clark, L. N., Poorkaj, P., Wszolek, Z., Geschwind, D. H., Nasreddine, Z. S., Miller, B., Li, D., Payami, H., Awert, F., Markopoulou, K., Andreadis, A., D'Souza, I., Lee, V. M., Reed, L., Trojanowski, J. Q., Zhukareva, V., Bird, T., Schellenberg, G., and Wilhelmsen, K. C. (1998). Pathogenic implications of mutations in the tau gene in pallido-ponto-nigral degeneration and related neurodegenerative disorders linked to chromosome 17. *Proc. Natl. Acad. Sci. U.S.A*. **95**, 13103–13107.

Cohen, P. T. (1997). Novel protein serine/threonine phosphatases: Variety is the spice of life. *Trends Biochem. Sci*. **22**, 245–251.

Cuenda, A., Cohen, P., Buée-Scherrer, V., and Goedert, M. (1997). Activation of Stress-Activated Protein kinase-3 (SAPK3) by cytokines and cellular stresses is mediated via SAPKK3 (MKK6); comparison of the specificities of SAPK3 and SAPK2 (RK/p38). *EMBO J*. **16**, 295–305.

Delacourte, A., and Buée, L. (1997). Normal and pathological tau proteins as factors for microtubule assembly. *Int. Rev. Cytol*. **171**, 167–224.

Delacourte, A., Flament, S., Dibe, E. M., Hublau, P., Sablonnière, B., Hémon, B., Scherrer, V., and Défossez, A. (1990). Pathological proteins tau 64 and 69 are specifically expressed in the somatodendritic domain of the degenerating cortical neurons during Alzheimer's disease, demonstration with a panel of antibodies against tau proteins. *Acta Neuropathol*. **80**, 111–117.

Delacourte, A., Robitaille, Y., Sergeant, N., Buée, L., Hof, P. R., Wattez, A., Laroche-Cholette, A., Mathieu, J., Chagnon, P., and Gauvreau, D. (1996). Specific pathological Tau protein variants characterize Pick's disease. *J. Neuropathol. Exp. Neurol*. **55**, 159–168.

Delacourte, A., Sergeant, N., Wattez, A., Gauvreau, D., and Robitaille, Y. (1998a). Vulnerable neuronal subsets in Alzheimer's and Pick's disease are distinguished by their tau isoform distribution and phosphorylation. *Ann. Neurol*. **43**, 193–204.

Delacourte, A., Buée, L., David, J. P., Sergeant, N., Wattez, A., Vermersch, P., Pasquier, F., Lebert, F., Ghozali, F., Fallet-Bianco, C., and DiMenza, C. (1998b). Lack of continuum between aging and Alzheimer's disease as revealed by PHF-tau and Aβ biochemistry. *Alzheimer's Reports*. **1**, 101–110.

Delacourte, A., David, J. P., Sergeant, N., Buée, L., Wattez, A., Vermersch, P., Ghozali, F., Fallet-Bianco, C., Pasquier, F., Lebert, F., Petit, H., and DiMenza, C. (1999). The biochemical pathway of neurofibrillary degeneration in aging and Alzheimer's disease. *Neurology* **52**, 1158–1165.

Delaère, P., Duyckaerts, C., Brion, J. P., Poulain, V., and Hauw, J. J. (1989). Tau, paired helical filaments and amyloid in the neocortex: A morphometric study of 15 cases with graded intellectual status in aging and senile dementia of Alzheimer type. *Acta Neuropathol*. **77**, 645–653.

Dewar, D., and Dawson, D. (1995). Tau protein is altered by focal cerebral ischaemia in the rat: An immunohistochemical and immunoblotting study. *Brain Res*. **684**, 70–78.

Dewar, D., Graham, D. I., Teasdale, G. M., and McCulloch, J. (1994). Cerebral ischemia induces alterations in Tau and ubiquitin proteins. *Dementia* **5**, 168–173.

Dickson, D. W., Crystal, H. A., Mattiace, L. A., Masur, D. M., Blau, A. D., Davies, P., Yen, S. H., and Aronson, M. K. (1991). Identification of normal and pathological aging in prospectively studied non-demented elderly humans. *Neurobiol. Aging* **13**, 179–189.

Drewes, G., Lichtenberg-Kraag, B., Döring, F., Mandelkow, E. M., Biernat, J., Dorée, M., and Mandelkow, E. (1992). Mitogen activated protein (MAP) kinase transforms tau protein into an Alzheimer-like state. *EMBO J*. **11**, 2131–2138.

Drewes, G., Ebneth, A., Preuss, U., Mandelkow, E. M., and Mandelkow, E. (1997). MARK, a novel family of protein kinases that phosphorylate microtubule-associated proteins and trigger microtubule disruption. *Cell (Cambridge, Mass.)* **89**, 297–308.

Drubin, D. G., and Kirschner, M. W. (1986). Tau protein function in living cells. *J. Cell Biol.* **103**, 2739–2746.

D'Souza, I., Poorkaj, P., Hong, M., Nochlin, D., Lee, V. M. Y., Bird, T. D., and Schellenberg, G. D. (1999). Missense and silent tau gene mutations cause frontotemporal dementia with parkinsonism-chromosome 17 type, by affecting multiple alternative RNA splicing regulatory elements. *Proc. Natl. Acad. Sci. U.S.A*. **96**, 5598–5603.

Dudek, S. M., and Johnson G. V. W. (1995). Postnatal changes in Ser/Thr protein phosphatases and their association with microtubules. *Dev. Brain Res*. **90**, 54–61.

Dumanchin, C., Camuzat, A., Campion, D., Verpillat, P., Hannequin, D., Dubois, B., Saugier-Veber, P., Martin, C., Penet, C., Charbonnier, F., Agid, Y., Frebourg, T., and Brice, A. (1998). Segregation of a missense mutation in the microtubule-associated protein tau gene with familial frontotemporal dementia and parkinsonism. *Hum. Mol. Genet*. **7**, 1825–1829.

Duyckaerts, C., Bennecib, M., Grignon, Y., Uchihara, T., He, Y., Piette, F., and Hauw, J. J. (1997). Modeling the relation between neurofibrillary tangles and intellectual status. *Neurobiol. Aging* **18**, 267–273.

Feany, M. B., and Dickson, D. W. (1996). Neurodegenerative disorders with extensive tau pathology: A comparative study and review. *Ann. Neurol*. **40**, 139–148.

Flament, S., and Delacourte, A. (1989). Abnormal tau species are produced during Alzheimer's disease neurodegenerating process. *FEBS Lett*. **247**, 213-216.

Flament, S., Delacourte, A., Delaère, P., Duyckaerts, C., and Hauw, J. J. (1990a). Correlation between microscopical changes and tau 64 and 69 biochemical detection in senile dementia of the Alzheimer-type. Tau 64 and 69 are reliable markers of the neurofibrillary degeneration. *Acta Neuropathol*. **80**, 212–215.

Flament, S., Delacourte, A., and Mann, D. M. A. (1990b). Phosphorylation of tau proteins: A major event during the process of neurofibrillary degeneration. Acomparative study between Alzheimer's disease and Down's syndrome. *Brain Res*. **516**, 15–19.

Flament, S., Delacourte, A., Verny, M., Hauw, J. J., and Javoy-Agid, F. (1991). Abnormal tau proteins in progressive supranuclear palsy. Similarities and differences with the neurofibrillary degeneration of the Alzheimer type. *Acta Neuropathol*. **81**, 591–596.

Fleming, L. M., and Johnson, G. V. W. (1995). Modulation of the phosphorylation state of Tau in situ: The roles of calcium and cyclic AMP. *Biochem. J*. **309**, 41–47.

Forno, L. S. (1996). Neuropathology of Parkinson's disease. *J. Neuropathol. Exp. Neurol*. **55**, 259–272.

Foster, N. L., Wilhelmsen, K., Sima, A. A., Jones, M. Z., D'Amato, C. J., and Gilman, S. (1997). Frontotemporal dementia and parkinsonism linked to chromosome 17: A consensus conference. *Ann. Neurol*. **41**, 706–715.

Giannakopoulos, P., Hof, P. R., Mottier, S., Michel, J. P., and Bouras, C. (1994). Neuropathological changes in the cerebral cortex of 1258 cases from a geriatric hospital: Retrospective clinicopathological evaluation of a 10-year autopsy evaluation. *Acta Neuropathol*. **87**, 456–468.

Giannakopoulos, P., Hof, P. R., Giannakopoulos, A. S., Herrmann, F. R., Michel, J. P., and Bouras, C. (1995). Regional distribution of neurofibrillary tangles and senile plaques in the cerebral cortex of very old patients. *Arch. Neurol. (Chicago)* **52**, 1150–1159.

Giannakopoulos, P., Hof, P. R., Michel, J.-P., Guimon, J., and Bouras, C. (1997). Cerebral cortex pathology in aging and Alzheimer's disease: A quantitative survey of large hospital-based geriatric and psychiatric cohorts. *Brain Res. Rev.* **25**, 217–245.

Goedert, M. (1998). Neurofibrillary pathology of Alzheimer's disease and other tauopathies. *Prog. Brain Res*. **117**, 287–306.

Goedert, M., Spillantini, M. G., Potier, M. C., Ulrich, J. and Crowther, R. A. (1989a). Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing 4 tandem repeats—Differential expression of tau protein messenger RNAs in human brain. *EMBO J*. **8**, 393–399.

Goedert, M., Spillantini, M. G., Jakes, R., Rutherford, D., and Crowther, R. A. (1989b). Multiple isoforms of human microtubule-associated protein tau: Sequences and localization in neurofibrillary tangles of Alzheimer's disease. *Neuron* **3**, 519–526.

Goedert, M., Spillantini, M. G., Cairns, N. J., and Crowther, R. A. (1992a). Tau proteins of Alzheimer paired helical filaments: Abnormal phosphorylation of all six brain isoforms. *Neuron* **8**, 159–168.

Goedert, M., Cohen, E. S., Jakes, R., and Cohen, P. (1992b). p42 MAP kinase phosphorylation sites in microtubule associated protein tau are dephosphorylated by protein phosphatase 2A1. *FEBS Lett*. **312**, 95–99; erratum: *Ibid*. **313**, 203.

Goedert, M., Jakes, R., Qi, Z., Wang, J. H., and Cohen, P. (1995). Protein phosphatase 2A is the major enzyme in brain that dephosphorylates tau protein phosphorylated by proline-directed protein kinases or cyclic AMP-dependent protein kinase. *J. Neurochem*. **65**, 2804–2807.

Goedert, M., Hasegawa, M., Jakes, R., Lawler, R., Cuenda, A., and Cohen, P. (1997). Phosphorylation of microtubule-associated protein tau by stress-activated protein kinases. *FEBS Lett*. **409**, 57–62.

Goedert, M., Spillantini, M. G., and Davies, S. W. (1998). Filamentous nerve cell inclusions in neurodegenerative diseases. *Curr. Opin. Neurobiol*. **8**, 619–632.

Gong, C. X., Shaikh, S., Wang, J. Z., Zaidi, T., Grundke-Iqbal, I., and Iqbal, K. (1995). Phosphatase activity toward abnormally phosphorylated tau: Decrease in Alzheimer disease brain. *J. Neurochem*. **65**, 732–738.

Goode, B. L., Denis, P. E., Panda, D., Radeke, M. J., Miller, H. P., Wilson, L., and Feinstein, S. C. (1997). Functional interactions between the proline-rich and repeat regions of tau enhance microtubule binding and assembly. *Mol. Biol. Cell* **8**, 353–365.

Greenberg, S. G., Davies, P., Schein, J. D., and Binder, L. I. (1992). Hydrofluoric acid-treated tau-PHF proteins display the same biochemical properties as normal tau. *J. Biol. Chem*. **267**, 564–569.

Greenwood, J. A., Scott, C. W., Spreen, R. C., Caputo, C. B., and Johnson, G. V. W. (1994). Casein kinase II preferentially phosphorylates human tau isoforms containing an amino-terminal insert—identification of threonine 39 as the primary phosphate acceptor. *J. Biol. Chem*. **269**, 4373–4380.

Groenen, P., and Wieringa, B. (1998). Expanding complexity in myotonic dystrophy. *BioEssays* **20**, 901–912.

Grundke-Iqbal, I., Iqbal, K., Tung, Y-C., Zaidi, M. S., Wisniewski, H. M., and Binder, L. I. (1986). Abnormal phosphorylation of the microtubule-associated protein tau in Alzheimer cytoskeletal pathology. *Proc. Natl. Acad. Sci. U.S.A*. **83**, 4913–4917.

Gu, Y., Oyama, F., and Ihara, Y. (1996). Tau is widely expressed in rat tissues. *J. Neurochem*. **67**, 1235–1244.

Hagestedt, G., Lichtenberg, B., Wille, H., Mandelkow, E. M., and Mandelkow, M. (1989). Tau protein becomes long and stiff upon phosphorylation: Correlation between paracrystalline structure and degree of phosphorylation. *J. Cell Biol*. **109**, 1643–1651.

Hanger, D. P., Hughes, K., Woodgett, J. R., Brion, J. P., and Anderton, B. H. (1992). Glycogen synthase kinase-3 induces Alzheimers disease-like phosphorylation of tau—generation of paired helical filament epitopes and neuronal localization of the kinase. *Neurosci Lett*. **147**, 58–62.

Hanger, D. P., Betts, J. C., Loviny, T. L., Blackstock, W. P., and Anderton, B. H. (1998). New phosphorylation sites identified in hyperphosphorylated tau (paired helical filament-tau) from Alzheimer's disease brain using nanoelectrospray mass spectrometry. *J. Neurochem*. **71**, 2465–2476.

Harr, S. D., Hollister, R. D., and Hyman, B. T. (1996). Glycogen synthase kinase 3 alpha and 3 beta do not colocalize with neurofibrillary tangles. *Neurobiol. Aging* **17**, 343–348.

Hasegawa, M., Morishima-Kawashima, M., Takio, K., Suzuki, M., Titani, K., and Ihara, Y. (1992). Protein sequence and mass spectrometric analyses of tau in the Alzheimer's disease brain. *J. Biol. Chem*. **267**, 17047–17054.

Hasegawa, M., Jakes, R., Crowther, R. A., Lee, V. M. Y., Ihara, Y., and Goedert, M. (1996). Characterization of mAb AP422, a novel phosphorylation-dependent monoclonal antibody against Tau protein. *FEBS Lett*. **384**, 25–30.

Hauw, J. J., Verny, M., Delaère, P., Cervera, P., He, Y., and Duyckaerts, C. (1990). Constant neurofibrillary changes in the neocortex in progressive supranuclear palsy — Basic differences with Alzheimer's disease and aging. *Neurosci. Lett*. **119**, 182–186.

Hensley, K., Floyd, R. A., Zheng, N. Y., Nael, R., Robinson, K. A., Nguyen, X., Pye, Q. N., Stewart, C. A., Geddes, J., Markesbery, W. R., Patel, E., Johnson, G. V., and Bing, G. (1999). p38 kinase is activated in the Alzheimer's disease brain. *J. Neurochem*. **72**, 2053–2058.

Hirano, A., Kurland, L. T., Krooth, R. S., and Lessel, S. (1961). Parkinsonism-dementia complex, and endemic disease on the island of Guam: I. Clinical features. *Brain* **84**, 642–661.

Hirano, A., Dembitzer, H. M., and Kurland, L. Y. (1968). The fine structure of some intraganglionic alterations. Neurofibrillary tangles, granulovacuolar bodies and ''rod-like'' structures as seens in Guam amyotrophic lateral sclerosis and Parkinson's dementia complex. *J. Neuropathol. Exp. Neurol*. **67**, 167–182.

Hirokawa, N., Funakoshi, T., Satoharada, R., and Kanai, Y. (1996). Selective stabilization of Tau in axons and microtubule-associated protein 2C in cell bodies and dendrites contributes to polarized localization of cytoskeletal proteins in mature neurons. *J Cell Biol*. **132**, 667–679.

Hof, P. R., Bierer, L. M., Perl, D. P., Delacourte, A., Buée, L., Bouras, C., and Morrison, J. H. (1992a). Evidence for early vulnerability of the medial and inferior temporal lobe in an 82-year-old patient with possible preclinical signs of dementia: Regional and laminar distribution of neurofibrillary tangles and senile plaques. *Arch. Neurol. (Chicago)* **49**, 946–953.

Hof, P. R., Charpiot, A., Delacourte, A., Buée, L., Purohit, D., Perl, D. P., and Bouras, C. (1992b). Distribution of neurofibrillary tangles and senile plaques in the cerebral cortex of postencephalitic parkinsonism. *Neurosci. Lett*. **139**, 10–14.

Hof, P. R., Delacourte, A., and Bouras, C. (1992c). Distribution of cortical neurofibrillary tangles in progressive supranuclear palsy. A quantitative analysis of 6 cases. *Acta Neuropathol*. **84**, 45–51.

Hof, P. R., Bouras, C., Perl, D. P., and Morrison, J. H. (1994a). Quantitative neuropathologic analysis of Picks disease cases—cortical distribution of Pick bodies and coexistence with Alzheimers disease. *Acta Neuropathol*. **87**, 115–124.

Hof, P. R., Nimchinsky, E. A., Buée-Scherrer, V., Buée, L., Nasrallah, J., Hottinger, A. F., Purohit, D. P., Loerzel, A. J., Steele, J. C., Delacourte, A., Bouras, C., Morrison, J. H., and Perl, D. P. (1994b). Amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam: Quantitative neuropathology, immunohistochemical analysis of neuronal vulnerability, and comparison with related neurodegenerative disorders. *Acta Neuropathol*. **88**, 397–404.

Hof, P. R., Bouras, C., Perl, D. P., Sparks, D. L., Mehta, N., and Morrison, J. H. (1995). Age-related distribution of neuropathologic changes in the cerebral cortex of patients with Down's syndrome: Quantitative regional analysis and comparison with Alzheimer's disease. *Arch. Neurol. (Chicago)* **52**, 379–391.

Hoffmann, R., Lee, V. M. Y, Leight, S., Varga, I., and Otvos, L. (1997). Unique Alzheimer's disease paired helical filaments specific epitopes involve double phosphorylation at specific sites. *Biochemistry* **36**, 8114–8124.

Holzer, M., Holzapfel, H. P., Zedlick, D., Bruckner, M. K., and Arendt, T. (1994). Abnormally phosphorylated tau protein in Alzheimer's disease: Heterogeneity of individual regional distribution and relationship to clinical severity. *Neuroscience* **63**, 499–516.

Hong, M., and Lee, V. M. Y. (1997). Lithium reduces tau phosphorylation by inhibition of glycogen synthase kinase-3. *J. Biol. Chem*. **272**, 19547–19553.

Hong, M., Zhukareva, V., Vogelsberg-Ragaglia, V., Wszolek, Z., Reed, L., Miller, B. I., Geschwind, D. H., Bird, T. D., McKeel, D., Goate, A., Morris, J. C., Wilhelmsen, K. C., Schellenberg, G. D., Trojanowski, J. Q., and Lee, V. M. (1998). Mutation-specific functional impairments in distinct tau isoforms of hereditary FTDP-17. *Science* **282**, 1914–1917.

Hulstaert, F., Blennow, K., Ivanoiu, A., Schoonderwaldt, H. C., Riemenschneider, M., De Deyn, P. P., Bancher, C., Cras, P., Wiltfang, J., Mehta, P. D., Iqbal, K., Pottel, H., Vanmechelen, E., and Vanderstichele, H. (1999). Improved discrimination of AD patients using beta-amyloid (1-42) and tau levels in CSF. *Neurology* **52**, 1555–1562.

Hutton, M., Lendon, C. L., Rizzu, P., Baker, M., Froelich, S., Houlden, H., Pickering-Brown, S., Chakraverty, S., Isaacs, A., Grover, A., Hackett, J., Adamson, J., Lincoln, S., Dickson, D., Davies, P., Petersen, R. C., Stevens, M., de Graaff, E., Wauters, E., van Baren, J., Hillebrand, M., Joosse, M., Kwon, J. M., Nowolny, P., Che, L. K., Norton, J., Morris, J. C., Reed, L. A., Trojanowski, J. Q., Basun, H., Lannfelt, L., Neystat, M., Fahn, S., Dark, F., Tannenberg, T., Dodd, P., Hayward, N., Kwok, J. B. J., Schofield, P. R., Andreadis, A., Snowden, J., Craufurd, D., Neary, D., Owen, F., Oostra, B. A., Hardy, J., Goate, A., van Swieten, J., Mann, D. M. A., Lynch, T., and Heutink, P. (1998). Coding and 5' splice site mutations in *TAU* associated with inherited dementia (FTDP-17). *Nature (London)* **393**, 702–705.

Ihara, Y., Nukina, N., Miura, R., and Ogawara, M. (1986). Phosphorylated tau protein is integrated into paired helical filaments in Alzheimer's disease. *J. Biochem. (Tokyo)* **99**, 1807–1810.

Ingelson, M., Vanmechelen, E., and Lannfelt, L. (1996). Microtubule-associated protein tau in human fibroblasts with the Swedish Alzheimer mutation. *Neurosci. Lett*. **220**, 9–12.

Jaspert, A., Fahsold, R., Grehl, H., and Claus, D. (1995). Myotonic dystrophy: Correlation of clinical symptoms with the size of the CTG trinucleotide repeat. *J. Neurol*. **242**, 99–104.

Jicha, G. A., O'Donnell, A., Weaver, C., Angeletti, R., and Davies, P. (1999). Hierarchical phosphorylation of recombinant tau by the paired-helical filament-associated protein kinase is dependent on cyclic AMP-dependent protein kinase. *J. Neurochem*. **72**, 214–224.

Joachim, C. L., Morris, J. H., Kosik, K. S., and Selkoe, D. J. (1987). Tau antisera recognize neurofibrillary tangles in a range of neurodegenerative disorders. *Ann. Neurol*. **22**, 514–520.

Johnson, G. V. W., and Hartigan, J. A. (1998). Tau protein in normal and Alzheimer's disease brain: An update. *Alzheimer's Dis. Rev.* **3**, 125–141.

Johnson, G. V., Seubvert, P., Cox, T. M., Motter, R., Brown, J. P., and Galasko, D. (1997). The tau protein in human cerebrospinal fluid in Alzheimer's disease consists of proteolytically derived fragments. *J. Neurochem.* **68**, 430–433.

Kempf, M., Clement, A., Faissner, A., Lee, G., and Brandt, R. (1996). Tau binds to the distal axon early in development of polarity in a microtubule- and microfilament-dependent manner. *J. Neurosci*. **16**, 5583–5592.

Kiuchi, A., Otsuka, N., Namba, Y., Nakano, I., and Tomonaga, M. (1991). Presenile appearance of abundant Alzheimer's neurofibrillary tangles without senile plaques in the brain in myotonic dystrophy. *Acta Neuropathol*. **82**, 1–5.

Kondratick, C. M., and Vandre, D. D. (1996). Alzheimer's disease neurofibrillary tangles contain mitosis-specific phosphoepitopes. *J. Neurochem.* **67**, 2405–2416.

Korneyev, A., Binder, L., and Bernardis, J. (1995). Rapid reversible phosphorylation of rat brain Tau proteins in response to cold water stress. *Neurosci. Lett*. **191**, 19–22.

Kosik, K. S., Orecchio, L. D., Bakalis, S., and Neve, R. L. (1989). Developmentally regulated expression of specific tau sequences. *Neuron* **2**, 1389–1397.

Ksiezak-Reding, H., Morgan, K., Mattiace, L. A., Davies, P., Liu, W. K., Yen, S. H., Weidenheim, K., and Dickson, D. W. (1994). Ultrastructure and biochemical composition of paired helical filaments in corticobasal degeneration. *Am J Pathol*. **145**, 1496–1508

Kyriakis J. M., Banerjee P., Nikolakaki E., Dai T., Rubie E. A., Ahmad M. F., Aavruch J., and Woodgett J. R. (1994). The stress-activated protein kinase subfamily of c-jun kinases. *Nature (London)* **369**, 156–160.

Ledesma, M. D. Bonay, P., Colaco, C., and Avila, J. (1994). Analysis of microtubule-associated protein Tau glycation in paired helical filaments. *J. Biol. Chem.* **269**, 21614–21619.

Ledesma, M. D., Bonay, P., and Avila, J. (1995). Tau protein from Alzheimer's disease patients is glycated at its tubulin-binding domain. *J. Neurochem.* **65**, 1658–1664.

Lee, G., Cowan, N., and Kirschner, M. (1988). The primary structure and heterogeneity of Tau protein from mouse brain. *Science* **239**, 285–289.

Lee, G., Neve, R. L., and Kosik, K. S. (1989). The microtubule binding domain of tau protein. *Neuron* **2**, 1615–1624.

Lee, V. M.-Y., Balin, B. J., Otvos, L., Jr, and Trojanowski, J. Q. (1991). A68: A major subunit of paired helical filaments and derivatized forms of normal tau. *Science* **251**, 675–678.

Liao, H., Li, Y., Brautigan, D. L., and Gundersen, G. G. (1998). Protein phosphatase 1 is targeted to microtubules by the microtubule-associated protein Tau. *J. Biol. Chem.* **273**, 21901–21908.

Lindwall, G., and Cole, R. D. (1984). Phosphorylation affects the ability of Tau protein to promote microtubule assembly. *J. Biol. Chem.* **255**, 5301–5305.

Litersky, J. M., and Johnson, G. V. W. (1992). Phosphorylation by cAMP-dependent protein kinase inhibits the degradation of tau by calpain. *J. Biol. Chem.* **267**, 1563–1568.

Litman, P., Barg, J., Rindzoonski, L., and Ginzburg, I. (1993). Subcellular localization of tau mRNA in differentiating neuronal cell culture: Implications for neuronal polarity. *Neuron* **10**, 627–638.

Litman, P., Barg, J., and Ginzburg, I. (1994). Microtubules are involved in the localization of tau mRNA in primary neuronal cell cultures. *Neuron* **13**, 1463–1474.

Lovestone, S., and Reynolds, C. H. (1997). The phosphorylation of tau: A critical stage in neurodevelopment and neurodegenerative processes. *Neuroscience* **78**, 309–324.

Mailliot, C., Sergeant, N., Bussière, T., Caillet-Boudin, M. L., Delacourte, A., and Buée, L. (1998a). Phosphorylation of specific sets of tau isoforms explains different neurodegeneration processes. *FEBS Lett.* **433**, 201–204.

Mailliot, C., Bussière, T., Caillet-Boudin, M. L., Delacourte, A., and Buée, L. (1998b). Alzheimer-specific epitope of AT100 in transfected cell lines with tau: Toward an efficient cell model of tau abnormal phosphorylation. *Neurosci. Lett.* **255**, 13–16.

Mailliot, C., Podevin-Dimster, V., Fiskum, G., Delacourte, A., and Buée, L. (1998c). Differential phosphorylation of the cerebral tau isoform in a canine model of cerebral ischemia/reperfusion. *Neurobiol. Aging* **19**, S56.

Mandelkow, E. M., and Mandelkow, E. (1998). Tau in Alzheimer's disease. *Trends Cell Biol.* **8**, 425–427.

Mann, D. M. A., Prinja, D., Davies, C. A., Ihara, Y., Delacourte, A., Defossez, A., Mayer, R. J., and Landon, M. (1989). Immunocytochemical profile of neurofibrillary tangles in Downs syndrome patients of different ages. *J. Neurol. Sci.* **92**, 247–260.

Marder, K., Tang, M. X., Cote, L., Stern, Y., and Mayeux, R. (1995). The frequency and associated risk factors for dementia in patients with Parkinson's disease. *Arch. Neurol. (Chicago)* **52**, 695–701.

Matsuo, E. S., Shin, R. W., Billingsley, M. L., Vandevoorde, A., O'Connor, M., Trojanowski, J. Q., and Lee, V. M. Y. (1994). Biopsy-derived adult human brain Tau is phosphorylated at many of the same sites as Alzheimer's disease paired helical filament tau. *Neuron* **13**, 989–1002.

Mawal-Dewan, M., Henley, J., Vandevoorde, A., Trojanowski, J. Q., and Lee, V. M. Y. (1994). The phosphorylation state of Tau in the developing rat brain is regulated by phosphoprotein phosphatases. *J. Biol. Chem.* **269**, 30981–30987.

Mawal-Dewan, M., Schmidt, M. L., Balin, B., Perl, D. P., Lee, V. M. Y., and Trojanowski, J. Q. (1996) Identification of phosphorylation sites in PHF-Tau from patients with Guam amyotrophic lateral sclerosis/parkinsonism-dementia complex. *J. Neuropathol. Exp. Neurol.* **55**, 1051–1059.

Mohit, A. A., Martin, J. H., and Miller, C. A. (1995). $p49^{3F12}$ kinase: A novel MAP kinase expressed in a subset of neuron in the human nervous system. *Neuron* **14**, 67–78.

Morishima-Kawashima, M., Hasegawa, M., Takio, K., Suzuki, M., Yoshida, H., Titani, K., and Ihara, Y. (1995). Proline-directed and non-proline-directed phosphorylation of PHF-tau. *J. Biol. Chem.* **270**, 823–829.

Morrison, J. H., and Hof, P. R. (1997). Life and death of neurons in the aging brain. *Science* **278**, 412–419.

Mulot, S. F. C., Hughes, K., Woodgett, J. R., Anderton, B. H., and Hanger, D. P. (1994). PHF-Tau from Alzheimer's brain comprises four species on SDS-PAGE which can be mimicked by an in vitro phosphorylation of human brain tau by glycogen synthase kinase-3β. *FEBS Lett.* **349**, 359–364.

Murrell, J., Zolo, P., Spillantini, M. G., Crowther, R. A., Goedert, M., Redi, F., Pietrini, P., Guazelli, M., and Ghetti, B. (1999). A mutation at codon 389 (G389R) in exon 13 of the tau gene causes frontotemporal dementia with numerous Pick bodies. *Clin. Neuropathol.* **18**, 145.

Ono, S., Inoue, K., Mannen, T., Kanda, F., Jinnai, K., and Takahashi, K. (1987). Neuropathological changes of the brain in myotonic dystrophy. Some new observations. *J. Neurol. Sci.* **81**, 301–320.

Papasozomenos, S. C. (1996). Heat shock induces rapid dephosphorylation of Tau in both female and male rats followed by hyperphosphorylation only in female rats: Implications for Alzheimer's disease. *J. Neurochem.* **66**, 1140–1149.

Paudel, H. K. (1997). The regulatory Ser262 of microtubule-associated protein tau is phosphorylated by phosphorylase kinase. *J. Biol. Chem.* **272**, 1777–1785.

Pérez-Tur, J., Buée, L., Morris, H., Waring, S., Onstead, L., Wavrant-De Vrièze, F., Crook, R., Buée-Scherrer, V., Hof, P. R., Perl, D. P., Petersen, R., McGeer, P., Delacourte, A., Hutton, M., Siddique, T., Ahlskog, E. J., Hardy, J., and Steele, J. (1999). Absence of mutations in the tau gene in amyotrophic lateral sclerosis/parkinsonism dementia complex of Guam. *Neurology* **53**, 411–413.

Pillon, B., Gouiderkhouja, N., Deweer, B., Vidailhet, M., Malapani, C., Dubois, B., and Agid, Y. (1995). Neuropsychological pattern of striatonigral degeneration: Comparison with Parkinson's disease and progressive supranuclear palsy. *J. Neurol. Neurosurg. Psychiatry* **58**, 174–179.

Pollock, N. J., Mirra, S. S., Binder, L. I., Hansen, L. A., abd Wood, J. G. (1986). Filamentous aggregates in Pick's disease, progressive supranuclear palsy, and Alzheimer's disease share antigenic determinants with microtubule-associated protein, tau. *Lancet* **22**, 1211.

Poorkaj, P., Bird, T. D., Wijernan, E., Nemens, E., Garruto, R. M., Anderson, L., Andreadis, A., Winderholt, W. C., Raskind, M., and Schellenberg, G. D. (1998). Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Ann. Neurol.* **43**, 815–825.

Pope, W., Enam, S. A., Bawa, N., Miller, B. E., Ghanbari, H., A. and Klein, W. L. (1993). Phosphorylated tau epitope of Alzheimer's disease is coupled to axon development in the avian central nervous system. *Exp. Neurol.* **120**, 106–113.

Probst, A., Tolnay, M., Langui, D., Goedert, M., and Spillantini, M. G. (1996). Pick's disease: Hyperphosphorylated tau protein segregates to the somatoaxonal compartment. *Acta Neuropathol.* **92**, 588–596.

Rebhan, M., Vacun, G., and Rosner, H. (1995). Complementary distribution of Tau proteins in different phosphorylation states within growing axons. *NeuroReport* **6**, 429–432.

Reynolds, C. H., Utton, M. A., Gibb, G. M., Yates, A., and Anderton, B. H. (1997a). Stress-activated protein kinase/c-jun N-terminal kinase phosphorylates tau protein. *J. Neurochem.* **68**, 1736–1744.

Reynolds, C. H., Nebreda, A. R., Gibb, G. M., Utton, M. A., and Anderton, B. H. (1997b). Reactivating kinase/p38 phosphorylates tau protein in vitro. *J. Neurochem.* **69**, 191–198.

Rizzu, P., Van Swieten, J. C., Joose, M., Hasegawa, M., Stevens, M., Tibben, A., Niermeijer, M. F., Hillebrandt, M., Ravid, R., Oostra, B. A., Goedert, M., Van Duijn, C. M., and Heutink, P. (1999). High prevalence of mutations in the microtubule-associated protein tau in a population study of fronto-temporal dementia in the Netherlands. *Am. J. Hum. Genet.* **64**, 414–421.

Roder, H. M., Fracasso, R. P., Hoffman, F. J., Witowsky, J. A., Davis, G., and Pellegrino, C. B. (1997). Phosphorylation-dependent monoclonal Tau antibodies do not reliably report phosphorylation by extracellular signal-regulated kinase 2 at specific sites. *J. Biol. Chem.* **272**, 4509–4515.

Rosenthal, R. E., Williams, R., Bogaert, Y. E., Getson, P. R., and Fiskum, G. (1992). Prevention of postischemic canine neurological injury through potentiation of brain energy metabolism by acetyl-L carnitine. *Stroke* **23**, 1312–1318.

Rösner, H., Rebhan, M., Vacun, G., and Vanmechelen, E. (1995). Developmental expression of Tau proteins in the chicken and rat brain: Rapid down-regulation of a paired helical filament epitope in the rat cerebral cortex coincides with the transition from immature to adult Tau isoforms. *Int. J. Dev. Neurosci.* **13**, 607–617.

Saito, T., Ishiguro, K., Uchida, T., Miyamoto, E., Kishimoto, T., and Hisanaga, S. (1995). In situ dephosphorylation of Tau by protein

phosphatase 2A and 2B in fetal rat primary cultured neurons. *FEBS Lett*. **376**, 238–242.

Schmidt, M. L., Huang, R., Martin, J. A., Henley, J., Mawal-Dewan, M., Hurtig, H. I., Lee, V. M., and Trojanowski, J. Q. (1996). Neurofibrillary tangles in progressive supranuclear palsy contain the same tau epitopes identified in Alzheimer's disease PHFtau. *J. Neuropathol. Exp. Neurol*. **l55**, 534–539.

Schneider, A., Biernat, J., von Bergen, M., Mandelkow, E., and Mandelkow, E. M. (1999), Phosphorylation that detaches tau protein from microtubules (Ser262, Ser214) also protects it against aggregation into Alzheimer paired helical filaments. *Biochemistry* **38**, 3549–3558.

Schweers, O., Mandelkow, E. M., Biernat, J., and Mandelkow, E. (1995). Oxidation of cysteine-322 in the repeat domain of microtubule-associated protein Tau controls the in vitro assembly of paired helical filaments. *Proc. Natl. Acad. Sci. U.S.A*. **92**, 8463–8467.

Sergeant, N., Bussière, T., Vermersch, P., Lejeune, J. P., and Delacourte, A. (1995). Isoelectric point differentiates PHF-tau from biopsy-derived human brain Tau proteins. *NeuroReport* **6**, 2217–2220.

Sergeant, N., David, J. P., Goedert, M., Jakes, R., Vermersch, P., Buée, L., Lefranc, D., Wattez, A., and Delacourte, A. (1997a). Two dimensional characterization of PHF-Tau from Alzheimer's disease: Demonstration of an additional 74 kDa component and age-related biochemical modifications. *J. Neurochem.* **69**, 834–844.

Sergeant, N., David, J. P., Lefranc, D., Vermersch, P., Wattez, A., and Delacourte, A. (1997b). Different distribution of phosphorylated tau protein isoforms in Alzheimer's and Pick's diseases. *FEBS Lett*. **412**, 578–582.

Sergeant, N., Wattez, A., and Delacourte, A. (1999). Neurofibrillary degeneration in progressive supranuclear palsy and corticobasal degeneration: Tau pathologies with exclusively ''exon 10'' isoforms. *J. Neurochem.* **72**, 1243–1249.

Seubert, P., Mawal-Dewan, M., Barbour, R., Jakes, R., Goedert, M., Johnson, G. V. W., Litersky, J. M., Schenk, D., Lieberburg, I., Trojanowski, J. Q., and Lee, V. M.-Y. (1995). Detection of phosphorylated Ser(262) in fetal tau, adult tau, and paired helical filament tau. *J. Biol. Chem*. **270**, 18917–18922.

Shackelford, D. A., and Nelson, K. E. (1996). Changes in phosphorylation of tau during ischemia and reperfusion in the rabbit spinal cord. *J. Neurochem.* **66**, 286–295.

Singh, T. J., Zaidi, T., Grundke-Iqbal., and Iqbal, K. (1996). Non-proline-dependent protein kinases phosphorylate several sites found in Tau from Alzheimer disease brain. *Mol. Cell. Biochem*. **154**, 143–151.

Sontag, E., Nunbhakdi-Craig, V., Lee, G., Bloom, G. S., and Mumby, M. C. (1996). Regulation of the phosphorylation state and microtubule-binding activity of Tau by protein phosphatase 2A. *Neuron* **17**, 1201–1207.

Soulié, C., Lepagnol, J., Delacourte, A., and Caillet-Boudin M. L. (1996). Dephosphorylation studies of SKNSH-SY 5Y cell Tau proteins by endogenous phosphatase activity. *Neurosci. Lett*. **206**, 189–192.

Spillantini, M. G., Bird, T. D., and Ghetti, B. (1998a). Frontotemporal dementia and Parkinsonism linked to chromosome 17: A new group of tauopathies. *Brain Pathol.* **8**, 387–402.

Spillantini, M. G., Murrell, J. R., Goedert, M., Farlow, M. R., Klug, A., and Ghetti, B. (1998b). Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. *Proc. Natl. Acad. Sci. U.S.A*. **95**, 7737–7741.

Steele, J. C., Richardson, J. C., and Olzewski, J (1964). Progressive supranuclear palsy. A heterogeneous degeneration involving brain stem, basal ganglia and cerebellum with vertical gaze ans pseudobulbar palsy, nuchal dystonia and dementia. *Arch. Neurol. (Chicago)* **10**, 333–359.

Szendrei, G. I., Lee, V. M.-Y., and Otvos, L. Jr. (1993). Recognition of the minimal epitope of monoclonal antibody Tau-1 depends upon the presence of a phosphate group but not its location. *J. Neurosci. Res*. **34**, 243–249.

Taylor, A. E., Saint-Cyr, J. A., and Lang, A. E. (1986). Frontal lobe dysfunction in Parkinson's disease. The cortical focus of neostriatal outflow. *Brain* **109**, 845–883.

Trojanowski, J. Q., and Lee, V. M. (1995). Phosphorylation of paired helical filament tau in Alzheimer's disease neurofibrillary lesions: Focusing on phosphatases. *FASEB J*. **9**, 1570–1576.

Vandermeeren, M., Mercken, M., Vanmechelen, E., Six, J., van de Voorde, A., Martin, J. J., and Cras, P. (1993). Detection of tau proteins in normal and Alzheimer's disease cerebrospinal fluid with a sensitive sandwich enzyme-linked immunosorbent assay. *J. Neurochem.* **61**, 1828–1834.

Vermersch, P., Frigard, B., and Delacourte, A. (1992a). Mapping of neurofibrillary degeneration in Alzheimer's disease: Evaluation of heterogeneity using the quantification of abnormal tau proteins. *Acta Neuropathol*. **85**, 48–54.

Vermersch, P., Frigard, B., David, J. P., Fallet-Bianco, C., and Delacourte, A. (1992b). Presence of abnormally phosphorylated tau proteins in the entorhinal cortex of aged non-demented subjects. *Neurosci. Lett*. **144**, 143–146.

Vermersch, P., Delacourte, A., Javoy-Agid, F., Hauw, J. J., and Agid, Y. (1993). Dementia in Parkinson's disease: Biochemical evidence for cortical involvement using the immunodetection of abnormal tau proteins. *Ann. Neurol*. **33**, 445–450.

Vermersch, P., Robitaille, Y., Bernier, L., Wattez, A., Gauvreau, D., and Delacourte, A. (1994). Biochemical mapping of neurofibrillary degeneration in a case of progressive supranuclear palsy: Evidence for general cortical involvement. *Acta Neuropathol*. **87**, 572–577.

Vermersch, P., Sergeant, N., Ruchoux, M. M., Hofmann-Radvanyi, H., Petit, H., Dewailly, P., and Delacourte, A. (1996). Specific Tau variants in the brain from patients with myotonic dystrophy. *Neurology* **47**, 711–717.

Vermersch, P., Buée-Scherrer, V., Buée, L., David, J.-P., Wattez, A., Sergeant, N., Hof, P. R., Agid, Y., Perl, D. P., Olanow, C. W., Robitaille, Y., Gauveau, D., Petit, H., and Delacourte, A. (1997). Cortical mapping of pathological tau proteins in several neurodegenerative disorders. *In* ''Connections, Cognition and Alzheimer's Disease'' (B. T. Hyman, C. Duyckaerts, and Y. Christen, eds.), pp. 41–52. Springer-Verlag, Berlin.

Vincent, I., Rosado, M., and Davies, P. (1996). Mitotic mechanisms in Alzheimer's disease? *J Cell Biol*. **132**, 413–425.

Weingarten, M. D., Lockwood, A. H., Hwo, S.-Y., and Kirschner, M. W. (1975). A protein factor essential for microtubule assembly. *Proc. Natl. Acad. Sci. U.S.A*. **72**, 1858–1862.

Yamagishi, M., and Ishizuka, Y. (1994). Abnormal tau protein expression in biopsied human olfactory mucosa. *Nippon Jibiinkoka Gakkai Kaiho* **97**, 466–472.

Yan, S. D., Yan, S. F., Chen, X., Fu, J., Chen, M., Kuppusamy, P., Smith, M. A., Perry, G., Godman, G. C., Nawroth, P., Zweiter, J. L., and Stern, D. (1995). Non-enzymatically glycated Tau in Alzheimer's disease induces neuronal oxidant stress resulting in cytokine gene expression and release of amyloid beta-peptide. *Nat. Med.* **1**, 693–699.

Zemlan, F. P., Rosenberg, W. S., Luebbe, P. A., Campbell, T. A., Dean, G. E., Weiner, N. E., Cohen, J. A., Rudick, R. A., and Woo, D. (1999). Quantification of axonal damage in traumatic brain injury: Affinity purification and characterization of cerebrospinal fluid tau proteins. *J. Neurochem.* **72**, 741–750.

Zheng-Fischhöfer, Q., Biernat, J., Mandelkow, E. M., Illenberger, S., Godemann, R., and Mandelkow, E. (1998). Sequential phosphorylation of tau by glycogen synthase kinase-3β and protein kinase A at Thr212 and Ser214 generates the Alzheimer-specific epitope of antibody AT100 and requires a paired-helical-filament-like conformation. *Eur. J. Biochem.* **252**, 542–552.

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22

Etiology, Genetics, and Pathogenesis of Alzheimer's Disease

I. Amyloid Hypothesis

Alzheimer's disease (AD) is an age-related neurodegenerative disease that causes a global loss of cognitive function and behavioral deficits. Although cholinesterase inhibitors can improve cognitive function slightly (Knopman and Morris, 1997), truly efficacious drugs for AD treatment and/or prevention are not yet available. However, the etiology of AD is beginning to yield to scientific inquiry. Plausible strategies for treatment and/or prevention have been formulated, but await further research. In the United States, AD afflicts four million individuals and imposes an annual tab of $80–100 billion (Hoyert and Rosenberg, 1999). Thus, one can readily appreciate the importance of recent progress in AD research. This chapter summarizes the current understanding of AD etiology and pathogenesis.

AD cases can be classified as ''familial'' or ''sporadic.'' Familial AD occurs in both early-onset (before age 65) and late-onset kindreds. In 1990, a mutation in the amyloid precursor protein (APP) was reported to cause hereditary cerebral hemorrhage with amyloidosis Dutch type (Levy *et al*., 1990). This finding linked amyloid deposition to an APP mutation for the first time and enabled investigators to embrace the idea that plaques were an important cause of neurodegeneration, rather than merely end products of a neurodegenerative process. Later, APP mutations were found to cause AD in a small number of early-onset, autosomal-dominant pedigrees (see Table 22.1).

The neuropathological hallmark of AD is the presence of neuritic plaques in brain parenchyma and cerebral blood vessels. Neuritic plaques consist of a protein core, surrounded by degenerating neurites, astrocytes, and activated macrophages. Alzheimer brain is also characterized by the presence of neurofibrillary tangles (NFT; accumulations of paired helical filaments within neuronal cell bodies), the loss of synapses and neurons, and reduced neurotransmitter concentrations. Cholinergic neurons are especially vulnerable to cell death; these neurons arise in the basal forebrain and terminate in the hippocampus and cerebral cortex. Neurofibrillary tangles consist primarily of ubiquitin and tau, a microtubule-associated protein.

The core protein of plaques is Aβ, a peptide derived from the amyloid precursor protein (APP), which is 39–43 amino acids long (Glenner and Wong, 1984). Plaques also contain numerous other components, including apolipoprotein E (Namba *et al*., 1991), α_1-antichymotrypsin (Abraham *et al*., 1988), serum amyloid P (Coria *et al*., 1988), interleukin-1 (Griffin *et al*., 1995), basic fibroblast growth factor (Gomez-Pinilla *et al*., 1990), α_2-macroglobulin (Bauer *et al*., 1991), low-density lipoprotein-related protein (LRP) (Tooyama *et al*., 1993), and perlecan (a heparin sulfate proteoglycan). Evidence for an inflammatory contribution to AD is provided by the presence of approximately 40 proteins known to play a role in inflammation (McGeer *et al*., 1996). Plaques are enriched in the small molecules, zinc, copper, and iron (Lovell *et al*., 1998).

Within plaques, the primary form of Aβ is Aβ_{42}, a highly-insoluble peptide that readily adopts a β-pleated sheet conformation (Iwatsubo *et al*., 1994, 1995; Fukumoto *et al*., 1996). Aβ molecules assemble into fibrils, which then pack into a highly ordered, crystalline-like lattice known as amyloid. The term ''amyloid'' can be applied to deposits derived from any protein in which a similar arrangement of molecules occurs. In addition to a core protein, all amyloid deposits are marked by the presence of heparan sulfate proteoglycans. ''Diffuse'' plaques are those in which Aβ molecules have not assembled into fibrils. Generally speaking, diffuse plaques are not surrounded by dystrophic neurites, activated microglia, or astrocytes. They occur in greater numbers than neuritic plaques and may be neuritic plaque precursors (Mackenzie, 1994). Neuritic plaque formation also occurs in Down syndrome and, to a lesser extent, in normal aging (Selkoe, 1991).

The ''amyloid hypothesis'' refers to the proposition that events leading to the manifestation of AD originate with the deposition of Aβ in amyloid deposits. Although flawed (or incomplete), this hypothesis is now supported by a large body of experimental work. It is worth noting that amyloid deposition occurs in other disorders such as Down syndrome,

TABLE 22.1 Amyloid Precursor Protein Mutations Associated with AD or Stroke

Pathogenic mutation	Reference
K/M 670/671 N/L (Swedish)	Mullan *et al.* (1992)
A682G (Flemish)	Hendricks *et al.* (1992)
E693Q (Dutch)	APP mutation implicated in hereditary cerebral hemorrhage with amyloidosis-Dutch (Levy *et al.*, 1990)
V715M (French)	Ancolio *et al.* (1999)
I716V	Eckman *et al.* (1997)
V717I (London)	Goate *et al.* (1991)
V717F	Murrell *et al.* (1991)
V717G	Chartier-Harlin *et al.* (1991)

Creutzfeldt–Jacob disease, Gertsmann–Sträussler–Scheinker disease, type II diabetes, familial amyloid polyneuropathy, and multiple myeloma. Aberrant protein deposition is a common theme in neurodegenerative disease (Kaytor and Warren, 2000). In Parkinson's disease, two mutations in the α-synuclein gene that cause early onset disease also accelerate α-synuclein aggregation (Narhi *et al.*, 1999).

Clearly, both environmental and genetic factors contribute to the risk of AD. Evidence from twin studies supports a role for environmental variables in AD (Breitner *et al.*, 1995; Nee and Lippa, 1999). Among the factors suggested to modify the risk for AD are head injury (Molgaard *et al.*, 1990; Roberts *et al.*, 1994), educational attainment (Stern *et al.*, 1992; Cobb *et al.*, 1995; Callahan *et al.*, 1996; Geerlings *et al.*, 1999), depression (Kokmen *et al*, 1996; Chen *et al.*, 1999), smoking (Hebert *et al.*, 1992; Lee, 1994; Hillier and Salib, 1997; Merchant *et al.*, 1999), vitamin E consumption (Vatassery *et al.*, 1999), diabetes (Leibson *et al.*, 1997) and hypertension (Skoog *et al.*, 1998; Behl, 1999). A review of the literature cited earlier will reveal that the legitimacy of many so-called environmental risk factors is open to debate. Stronger, but not conclusive, evidence indicates that estrogen replacement (Birge, 1997; Haskell *et al.*, 1997) and the chronic use of nonsteroidal anti-inflammatory drugs may reduce the risk of AD (McGeer *et al.*, 1996). AD and peripheral vascular disease share several risk factors in common; this indicates that impaired cholesterol metabolism may play a role in AD etiology (McKeon-O'Malley *et al.*, 1998). It remains to be determined whether any environmental factor is sufficient to cause AD in the absence of a permissive genotype.

On the genetic front, the identification of several AD-linked genes has yielded important insights into the etiology of AD, and steady progress continues to be made in this area. Female gender is a risk factor for AD, even when the longer life span of females is taken into account (Letenneur *et al.*, 1999). Almost all individuals with Down syndrome (trisomy 21) develop AD in midlife (Katzmann, 1986), presumably due to the overexpression of the APP gene on chromosome 21. The linkage of specific genes to AD risk is discussed in the following section.

II. Genetic Contributions to the Etiology of AD

To date, seven of the genes linked to AD include: the amyloid precursor protein (Chartier-Harlin *et al.*, 1991; Goate *et al.*, 1991; Murrell *et al.*, 1991), presenilin 1 (PS1) (Sherrington *et al.*, 1995), presenilin 2 (PS2) (Levy-Lahad *et al.*, 1995b), apolipoprotein E (Corder *et al.*, 1993), α_2-macroglobulin (Blacker *et al.*, 1998; Liao *et al.*, 1998), LRP (also known as the α2M receptor) (Kang *et al.*, 1997; Wavrant-DeVriéze *et al.*, 1997; Hollenbach *et al.*, 1998), and tau (Lilius *et al.*, 1999; Bullido *et al.*, 2000). Importantly, four of these proteins are related to each other: APP, ApoE, and α_2-macroglobulin are ligands for LRP.

Preliminary evidence has been presented for the involvement of other genes with AD (see Table 22.2). However, data do not permit firm conclusions to be drawn about other genes at this time. Deterministic mutations (i.e., mutations that can cause AD with 100% penetrance) occur in the genes for the amyloid precursor protein, presenilin 1 and presenilin 2, but these mutations account for only a small percentage of total AD cases. Mutations in APP and the presenilins cause AD by increasing the extracellular load of $A\beta_{42}$ (Scheuner *et al.*, 1996). Polymorphisms in the genes for apolipoprotein

TABLE 22.2 Genes in Which Mutations or Polymorphisms May Modify the Risk of AD[a]

Gene	Reference
Angiotensin 1-converting enzyme	Alvarez *et al.* (1999)
α_1-Antichymotrypsin	Haines *et al.* (1996); Talbot *et al.* (1996)
Bleomycin hydrolase	Farrer *et al.* (1998); Montoya *et al.* (1998)
Butyrylcholinesterase K	Brindle *et al.* (1998); Crawford *et al.* (1998); Hiltunen *et al.* (1998); Singleton *et al.* (1998); Tilley *et al.* (1999)
Cathepsin D	Papassotiropoulos *et al.* (1999)
Dihydrolipoyl succinyltransferase (DLST)	Sheu *et al.* (1999)
Estrogen receptor α gene	Brandi *et al.* (1999)
HLA	Ballerini *et al.* (1999)
Lipoprotein lipase	Baum *et al.* (1999)
Mitochondrial genome	Davis *et al.* (1997); Egensperger *et al.* (1997); Chagnon *et al.* (1999)
Myeloperoxidase	Reynolds *et al.* (1999)
Neurotropin-3	Kunugi *et al.* (1998)
Nitric oxide synthase 3	Dahiyat *et al.* (1999)
Serotonin transporter gene	Oliveira *et al.* (1998, 1999); Li *et al.* (1999)
Very low density lipoprotein receptor	Okuizumi *et al.* (1995, 1996); Pritchard *et al.* (1996)

[a]The genes may be associated with AD. Cited references include articles that report the association of a particular gene with AD, or a lack of such an association.

E, α_2-macroglobulin, LRP, and tau are known to increase the risk of AD. A brief description of each of these genes is given.

A. Amyloid Precursor Protein

As indicated earlier, the core protein of neuritic plaques, Aβ, is derived from APP. The APP gene is located at the boundary of 21.q.3 and 21.q22.1 (Kang *et al*., 1987) and is widely expressed in human tissues (Tanzi *et al*., 1988). Ten transcripts can be produced by alternative splicing of 19 exons (Wisniewski *et al*., 1994). However, Aβ cannot be generated by alternative splicing (Lemaire *et al*., 1989). Three major transcripts contain the Aβ sequence, and these encode proteins with 695, 771, or 770 amino acids (APP_{695}, APP_{751}, APP_{770}). The expression of APP_{695} is confined to the brain (Sola *et al*., 1993). APP_{751} and APP_{770} contain a region with 50% homology to a Kunitz protease inhibitor domain.

The APP gene lacks a TATA box and has a high GC content, features characteristic of a housekeeping gene (Salbaum *et al*., 1988). Gene expression produces a protein with the following domains: a signal peptide for transport of APP into the endoplasmic reticulum, a cysteine-rich sequence, a sequence including many negatively charged residues (glutamic acid and aspartic acid), and an uninterrupted stretch of seven threonine residues (Kang *et al*., 987) (see Fig. 22.1). A zinc II-binding site is located between the cysteine-rich domain and the negatively charged region (Bush *et al*., 1993). Two consensus sequences for N-linked glycosylation are located at amino acids 467–469 and 496–498. APP also contains a potential heparin-binding site (Small, 1994). APP is modified by post-translational mechanisms including N- and O-glycosylation (Weidemann *et al*., 1989; Pahlsson *et al*., 1992), sulfation of tyrosine residues (Weidemann *et al*., 1989), and phosphorylation (Oltersdorf *et al*., 1990). APP matures in the endoplasmic reticulum and the Golgi and then becomes inserted into the plasma membrane (Weidemann *et al*., 1989).

APP is a member of a family of highly conserved proteins. Other members of this family include amyloid precursor protein-like protein (APLP1) and APLP2 (Sprecher *et al*., 1993; Wasco *et al*., 1993), but the two latter proteins do not encode Aβ. APP plays a role in many normal functions, including wound healing (Smith *et al*., 1990; Van Nostrand *et al*., 1990), proliferation (Saitoh *et al*., 1989; Ninomiya *et al*., 1993), adhesion (Schubert *et al*., 1989; Breen *et al*., 1991; Chen and Yankner, 1991; Ghiso *et al*., 1992), neurite extension (Araki *et al*., 1991; Milward *et al*., 1992; Small, 1994), survival under stress (Mattson *et al*., 1993; Yamamoto *et al*., 1994), and synaptic plasticity (Mattson, 1994). Processes involved in the production of Aβ from APP are discussed under Section III.

B. Apolipoprotein E

Apolipoprotein E is a 34 kDa (299 residues) protein known primarily for its role in lipid transport (Mahley and Huang, 1999). The gene for ApoE has been mapped to chromosome 19 (Das *et al*., 1985). Three polymorphic alleles, ϵ2, ϵ3, and ϵ4 (corresponding to the proteins ApoE2, ApoE3, and ApoE4), occur at frequencies of 8, 75, and 15%, respectively. ApoE3 has Cys^{112} and Arg^{152}; in ApoE2, Arg^{158} is replaced by cysteine, and in ApoE4 , Cys^{112} is replaced by arginine. In the periphery, ApoE is synthesized primarily in the liver. ApoE cannot penetrate the blood–brain barrier, but is manufactured within the brain by astrocytes (Pitas *et al*., 1987). Apolipoprotein E is a ligand for three cell surface receptors: the low-density lipoprotein (LDL) receptor, the low-density lipoprotein-related protein, and the very-low-density lipoprotein (VLDL) receptor.

The ϵ4 allele of apolipoprotein E increases the risk of AD in a dose-dependent manner and lowers the age at onset (Corder *et al*., 1993). This finding has been confirmed repeatedly in a host of ethnic groups around the world. However, it is clear that apolipoprotein E alone does not cause AD because a proportion of elderly homozygotes are unaffected (Roses *et al*., 1994). Interestingly, the ϵ4 allele has also been shown to increase the risk of cardiovascular disease as well as AD (Lambert *et al*., 2000). Mutations occurring in noncoding regions of the ApoE gene are also associated with AD (see Table 22.3). In

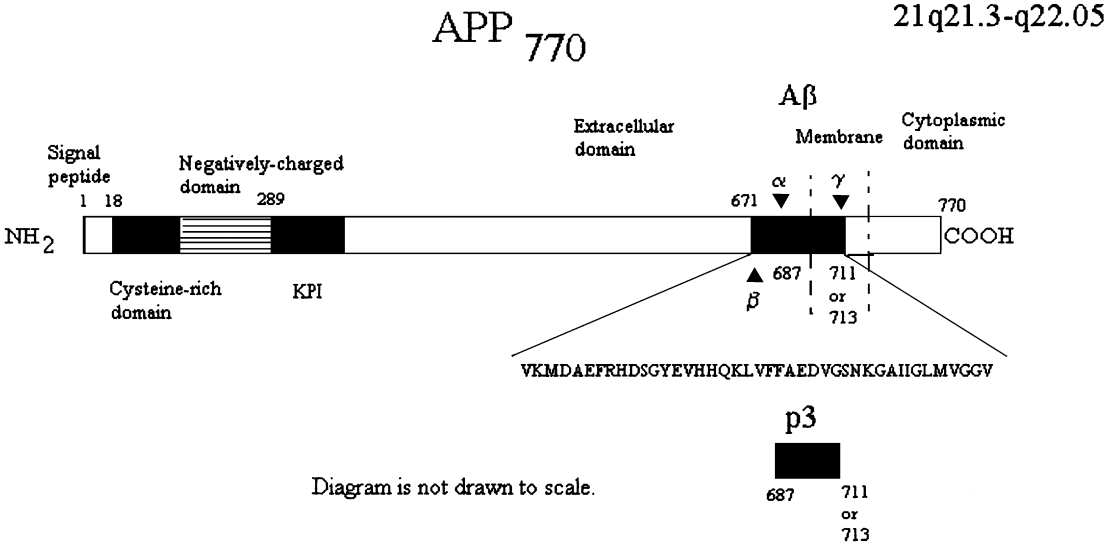

FIG. 22.1. Schematic diagram of APP (not drawn to scale).

TABLE 22.3 Apolipoprotein E Polymorphisms Associated with AD

Polymorphism	Reference
$\epsilon 2/\epsilon 3/\epsilon 4$ polymorphism	The $\epsilon 4$ allele is associated with sporadic and late-onset AD. This finding has been confirmed in more that 100 studies (Corder *et al*., 1993)
APOE*4 Pittsburgh (APOE*4P) (this is a missense mutation, L28P, caused by a T → C substitution in exon 3)	All APOE*4P carriers identified thus far also carry $\epsilon 4$. However, the risk of AD is five-fold greater in $\epsilon 4$ carriers who also carry APOE*4P than in those who bear the $\epsilon 4$ allele alone (Kamboh *et al*., 1999)
Th1/E47 cs polymorphism: G to mutation occurs at −186 bp in relation to the TATA box; this polymorphism is located in a consensus sequence for the transcription factor Th1E47	The presence of at least one T allele increases the risk of AD (Lambert *et al*., 1998)

contrast to the $\epsilon 4$ allele, the $\epsilon 2$ allele decreases the risk of AD (Corder *et al*., 1994; Talbot *et al*., 1994).

C. Presenilin 1 and Presenilin 2

Mutations occurring within the homologous, transmembrane proteins PS1 and PS2 cause early-onset familial AD with 100% penetrance. About 70 such mutations have been identified in PS1, whereas only 5 have been observed in PS2 (see Tables 22.4 and 22.5; Table 22.4 includes only a partial list of all PS1 mutations). Remarkably, mutations in PS1 and PS2 increase the $A\beta_{42}/A\beta_{40}$ ratio, or total $A\beta$ both *in vitro* and *in vivo* (Borchelt *et al*., 1996; Lemere *et al*., 1996; Mann *et al*., 1996; Scheuner *et al*., 1996; Citron *et al*., 1997, 1998; Tomita *et al*., 1997). The presenilins may be γ-secretases, the enzymes that generate final cleavage in the release of $A\beta$ from APP (Wolfe *et al*., 1999).

PS1 and PS2 share a similar pattern of gene expression. Both proteins are expressed primarily within neurons and are localized to the endoplasmic reticulum and Golgi (Kovacs *et al*., 1996; Blanchard *et al*., 1997).

The gene for PS1 is located on chromosome 14q24.3 and encodes a 43 to 45 kDa protein, 467 amino acids long (George-Hyslop *et al*., 1992; Schellenberg *et al*., 1992). The PS2 gene has been mapped to chromosome 1q42.1 and encodes a 53 to 55 kDa protein, 448 amino acids long (Levy-Lahad *et al*., 1995a,b; Takano *et al*., 1997). The presenilins are predicted to contain six (Lehmann *et al*., 1997), seven (Dewji and Singer, 1997), or eight (Li and Greenwald, 1998) transmembrane domains and a large hydrophilic loop. The N-terminal, C-terminal, and the large hydrophilic loop of PS1 protrude from the endoplasmic reticulum membrane into the cytoplasm (Doan *et al*., 1996).

The presenilins are cleaved endoproteolytically to produce N-terminal fragments and C-terminal fragments, with approximate molecular masses of 30 and 20 kDa, respectively (Thinakaran *et al*., 1996). This cleavage occurs at a site within the large hydrophilic loop. N- and C-terminal fragment associate with one another in a 1:1 ratio, forming a stable complex (Capell *et al*., 1998). Measurable quantities of the full-length proteins are difficult to detect by conventional methods, strongly suggesting that the cleavage products are the physiologically relevant entities.

Potential roles for the presenilins have been suggested in protein processing, Notch signaling and development, and apoptosis (Mattson *et al*., 1997, 1998; Guo *et al*., 1998a,b). Mice homozygous for a null mutation in the murine homologue of PS1 (i.e., PS1 knockout mice) die within minutes of birth (Shen *et al*., 1997). These animals exhibit gross skeletal defects, cerebral hemorrhage, and massive neuronal loss. The lethal phenotype of these animals indicates that PS1 plays an essential role in normal development.

D. α_2-Macroglobulin

α_2-Macroglobulin (α2M) is a ''pan-protease'' inhibitor involved in the clearance of proteins from the blood via endocytosis (Borth, 1992). α2M is composed of four identical subunits (180 kDa), each of which contains a 25 residue ''bait region,'' a cytokine-binding domain, and a receptor-binding domain. The bait region contains an internal cyclic thiol ester that is cleaved when a protease binds to α2M. This cleavage provokes a conformational change in α2M that permits it to enclose or ''capture'' the protease and to make its cytokine-binding and receptor-binding domains accessible for ligand binding. Activated α2M binds to its plasma membrane receptor, LRP, to deliver its captured protease. Ligand–LRP complexes are internalized via clathrin-coated pits and are then directed into an endosomal/lysosomal compartment (Kowal *et al*., 1989). There, ligands are released from LRP and degraded; LRP is recycled to the plasma membrane.

The gene for α2M is located on chromosome 12 (Fukushima *et al*., 1988). An AD-linked polymorphism is located in the 5′ splice site of exon 18. Two polymorphisms, occurring in this locale, α2M-2 and α2M-1, specify the presence or absence of a pentanucleotide deletion. Inheritance of a single α2M-2 allele increases the risk of AD three- to fourfold, but does not change the age at onset (Blacker *et al*., 1998). To date, it is not known whether the risk of AD increases with α2M-2 dosage. AD is also associated with a second polymorphic site, Val1000(GTC)/Ile1000(ATC), located near the cyclic thiol ester (Poller *et al*., 1992). AD risk is increased by the presence of a valine residue at this site (Liao *et al*., 1998). The risks conferred by the Val1000(GTC) allele of α2M, and the $\epsilon 4$ allele of ApoE, are independent and additive.

E. Lipoprotein-Related Protein

As described previously, LRP is a plasma membrane receptor for APP, ApoE, and α2M. Importantly, an $A\beta$-serine

TABLE 22.4 A Partial List of Presenilin 1 Mutations Associated with AD

Mutation	Reference	Mutation	Reference
A79V	Cruts *et al*. (1998)	A231V	Cruts *et al*. (1998)
V82L	Campion *et al*. (1995)	M233L	Aldudo *et al*. (1999)
V96F	Kamino *et al*. (1996)	M233T	Kwok *et al*. (1997)
F105L	Finckh *et al*. (2000)	L235P	Campion *et al*. (1996)
Y115H	Campion *et al*. (1995)	A246E	Sherrington *et al*. (1995)
Y115C	Cruts *et al*. (1998)	L250S	Harvey *et al*. (1998)
T116N	Romero *et al*. (1999)	A260V	Rogaev *et al*. (1995)
P117L	Wisniewski *et al*. (1998)	L262F	Forsell *et al*. (1997)
E120D	Poorkaj *et al*. (1998b)	C263R	Wasco *et al*. (1995)
Q120D	Reznik-Wolf *et al*. (1996)	P264L	Campion *et al*. (1995)
E120K	Reznik-Wolf *et al*. (1998)	P267S	Alzheimer's Disease Collaborative Group (1995)
E123K	Yasuda *et al*. (1999)	R269G	Perez-Tur *et al*. (1996)
N135D	Crook *et al*. (1997)	R269H	Gomez-Isla *et al*. (1997)
M139K	Dumanchin *et al*. (1998)	E273A	Kamimura *et al*. (1998)
M139T	Campion *et al*. (1995)	R278T	Kwok *et al*. (1997)
M139I	Boteva *et al*. (1996)	E280A	Alzheimer's Disease Collaborative Group (1995)
I143F	Rossor *et al*. (1996)	E280G	Alzheimer's Disease Collaborative Group (1995)
I143T	Cruts *et al*. (1995)	L282R	Aldudo *et al*. (1998a)
M146I	Jorgensen *et al*. (1996)	A285V	Aoki *et al*. (1997)
M146L	Sherrington *et al*. (1995)	L286V	Sherrington *et al*. (1995)
M146V	Alzheimer's Disease Collaborative Group (1995)	E318G	Reznik-Wolf *et al*. (1998); Aldudo *et al*. (1998b); Mattila *et al*. (1998)
T147I	Campion *et al*. (1999)	G378E	Besançon *et al*. (1998)
H163R	Sherrington *et al*. (1995)	G384A	Cruts *et al*. (1995)
H163Y	Perez-Tur *et al*. (1995); Axelman *et al*. (1998)	S390I	Campion *et al*. (1999)
W165C	Campion *et al*. (1999)	C410Y	Sherrington *et al*. (1995)
S169P	Ezquerra *et al*. (1999)	L424R	Kowalska *et al*. (1999)
S169L	Taddei *et al*. (1998)	A426P	Poorkaj *et al*. (1998b)
L171P	Ramirez-Duenas *et al*. (1998)	P436Q	Taddei *et al*. (1998)
L173W	Campion *et al*. (1999)	To to G at intron 9	Nishiwaki *et al*. (1997)
E184D	Yasuda *et al*. (1997)	Intronic polymorphism located 3' to exon 8	Wragg *et al*. (1996); but see Cai *et al*. (1997), Tysoe *et al*. (1997), Sorbi *et al*. (1997)
G209R	Sugiyama *et al*. (1999)	Deletion Delta9Finn	Crook *et al*. (1998); Prihar *et al*. (1999)
G209V	Poorkaj *et al*. (1998b)	58304G>A Delta9	Sato *et al*. (1998)
I213T	Kamino *et al*. (1996)	58304G>T Delta9	Perez-Tur *et al*. (1995)
A231T	Campion *et al*. (1995)	A deletion of G from the intron four splice donor consensus sequence	Tysoe *et al*. (1998); PS1 truncating mutation

TABLE 22.5 Presenilin 2 Mutations Associated with AD

Pathogenic mutation	Reference
T122P	Finckh *et al.* (2000)
N141I	Levy-Lahad *et al.* (1995a)
M239I	Finckh *et al.* (2000)
M239V	Rogaev *et al.* (1995)
Splice variant: mRNA lacks exon 5	Sato *et al.* (1999)

protease-α2M pathway is one of several mechanisms by which Aβ is cleared (Qiu *et al.*, 1999). LRP belongs to a family of proteins that includes the LDL receptor, megalin (also known as gp330), the VLDL receptor, and the vitellogenin receptor (Krieger and Herz, 1994).

F. Tau

The diagnosis of AD in postmortem brain requires the presence of both NFT and neuritic plaques. Unlike plaques, NFT are not specific to AD; these entities occur in many neurodegenerative disorders, including supranuclear palsy, dementia pugilistica, corticobasal degeneration, and fronto-temporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) (Goedert *et al.*, 1997). However, NFT density correlates well with dementia severity, whereas plaque density does not (Braak and Braak, 1991).

The primary protein component of NFT is tau (45–60 kDa), a protein that promotes the stabilization of microtubules (Weingarten *et al.*, 1975). In the central nervous system, alternative splicing of the tau gene results in the expression of six isoforms primarily (see Fig. 22.2). A seventh isoform containing a "big tau insert" is expressed prominently in the peripheral nervous system and to a much lesser extent in the central nervous system (Georgieff *et al.*, 1993). Each isoform possesses either three or four microtubule-binding domains (repeat units of 31 amino acids, encoded by exon 10) and 0, 1, or 2 different amino-terminal inserts. Differential expression of tau isoforms occurs during development (Couchie *et al.*, 1988; Kosik *et al.*, 1989). Within NFT, tau is hyperphosphorylated (Grundke-Iqbal *et al.*, 1986), which prevents its binding to microtubules (Bramblett *et al.*, 1993).

The finding made in 1991 that mutations in the amyloid precursor protein gene can cause AD with 100% penetrance focused attention on the role of Aβ in AD. Lately, there has been a resurgence of interest in tau. Tau mutations have been linked to frontotemporal dementia with parkinsonism (FTDP-17; previously known as Pick disease) (Poorkaj *et al.*, 1998a; Hutton *et al.*, 1998; Spillantini *et al.*, 1998; Iijima *et al.*, 1999) and to supranuclear palsy (Chambers *et al.*, 1999). Because plaque deposits are largely absent in FTDP-17 tauopathies, the latter findings indicate that tau aggregation is sufficient for neurodegeneration (Ghetti *et al.*, 1999).

Several groups have searched for AD-linked mutations in the tau gene without success (Crawford *et al.*, 1999; Roks *et al.*, 1999). However, two reports indicate that polymorphisms in the tau gene may increase the risk for AD when found in combination with the ApoE ϵ4 allele (Lilius *et al.*, 1999; Bullido *et al.*, 2000).

III. Pathogenesis

A. Fundamental Questions in AD Research

Most of the current research in AD is designed to address one of the following questions:

What factors regulate the cleavage of Aβ from APP, and Aβ assembly into fibrils?

How does Aβ, alone or in combination with other plaque components, cause neuronal cell death?

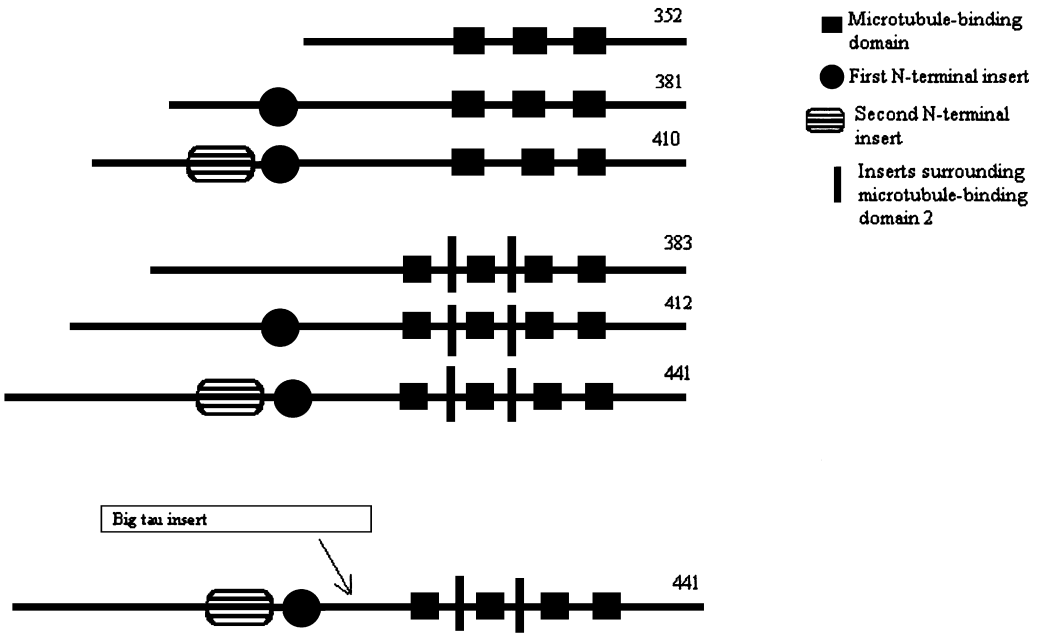

FIG. 22.2. Schematic diagram of tau protein isoforms.

What is the role of inflammation in the maturation of neuritic plaques?

What is the role of tau in AD?

A thorough discussion of last three questions is beyond the scope of this chapter (see Tolnay and Probst, 1999, for a review of the role of tau in AD.) This chapter concentrates, how Aβ is produced from APP, and on Aβ fibrillogenesis.

B. Amyloid, Neuritic Plaques, and Paired Helical Filaments

Within specific brain locale, certain conditions (as yet undefined) permit soluble Aβ molecules to adopt a high degree of β-pleated sheet conformation; this alteration facilitates the rearrangement of Aβ molecules into fibrils. Fibrils pack in cross-β conformation to form amyloid, a highly ordered arrangement of protein molecules that can be detected using Congo Red or Thioflavin S (Kirschner *et al*., 1987). Neuritic plaques, but not diffuse plaques, are visualized using these stains. Fibril stability is critically dependent on the primary structure of the peptide. This is exemplified by the fact that an APP mutation associated with early-onset FAD, $A\beta_{E22Q}$, increases the stability of Aβ fibrils dramatically (Fraser *et al*., 1992).

Within plaques, Aβ molecules differ in length, exhibiting heterogeneity at both amino (Tekirian *et al*., 1998) and carboxyl termini (Jarrett *et al*., 1993a). Plaques contain a mixture of Aβ molecules; $A\beta_{42}$ and $A\beta_{40}$ are the predominant species in extracellular neuritic plaques, whereas the vascular amyloid consists primarily of $A\beta_{39–40}$ (Dickson, 1997). As indicated earlier, a highly amyloidogenic form of Aβ, $A\beta_{42}$, is the initial Aβ species deposited in neuritic plaques (Iwatsubo *et al*., 1995). Diffuse plaques contain $A\beta_{42}$, but not $A\beta_{40}$ (Cummings *et al*., 1996). Plaque enlargement requires a nidus for further protein deposition; $A\beta_{42}$ may serve as a ''seed,'' which permits the deposition of $A\beta_{40}$ (Jarrett *et al*., 1993b). $A\beta_{40}$ constitutes the bulk of the Aβ produced by normal metabolism (Haass *et al*., 1992; Seubert *et al*., 1992).

Neuritic plaques can be surrounded by dystrophic neurites or by neurites containing paired helical filaments (PHF-type neurites). [PHF occurring within neurites are essentially equivalent to those found in neuronal cell bodies. In AD, PHF occur in three locations: within plaques, neurites, or neuronal cell bodies (Braak *et al*., 1986). PHF-type neurites found in the absence of plaques are known as ''neuropil threads'' (Dickson, 1997).] Among dystrophic neurites, diverse neuronal types are represented (Struble *et al*., 1987). In contrast, there is a hierarchy of neuronal types that are susceptible to PHF formation (Price *et al*., 1991). Both dystrophic neurites and PHF-type neurites are recognized by ubiquitin-specific antibodies, but only the latter are recognized by the Alz-50 antibody.

C. Aβ Formation, Aggregation, and Clearance

1. APP Cleavage Sites

APP is cleaved within Aβ at Lys^{687}-Leu^{688} (APP_{770} numbering), by one or more ''α-secretases.'' This cleavage generates a soluble N-terminal fragment ($sAPP_\alpha$) and a membrane-associated C-terminal fragment. Thus, α-secretase-mediated cleavage of APP precludes the production of Aβ. The amino and carboxy termini of Aβ are produced by the actions of ''β-secretase'' and ''γ-secretase,'' respectively. As in the case of α-secretase, β-secretase-mediated cleavage of APP produces an amino-terminal fragment ($sAPP_\beta$) and a membrane-associated C-terminal fragment. The ''p3'' peptide (3 kDa) is created when the C-terminal fragment produced by α-secretase cleavage becomes a substrate for γ-secretase (Haass *et al*., 1993). The p3 sequence may be amyloidogenic (Lalowski *et al*., 1996; Dickson, 1997). The cleavage of APP by γ-secretase is unusual in that it occurs within the transmembrane domain of APP. Aβ and p3 are normal constituents of biological fluids (Shoji *et al*., 1992). Secreted $sAPP_\alpha$ protects cells from toxic insults (Goodman and Mattson, 1994; Furukawa *et al*., 1996). In this regard, it may be much more effective than $sAPP_\beta$ (Barger and Mattson, 1996).

After its synthesis on ribosomes, APP is directed into the endoplasmic reticulum by its signal peptide (Kang *et al*., 1987). During its transit through the constitutive secretory pathway (endoplasmic reticulum, Golgi, and *trans*-Golgi network), APP is phosphorylated on its ectodomain. A small percentage of total holoprotein is inserted into the plasma membrane, where it is subject to cleavage by α- or β-secretases (Selkoe, 1998). Uncleaved holoprotein and the C-terminal fragments remaining in the plasma membrane after α-secretase or β-secretase cleavage (C83 and C99, respectively) are reinternalized via clathrin-coated vesicles. The latter molecules can be recycled to the cell surface or enter an endosomal/lysosomal pathway.

2. α-Secretase-Mediated Cleavage of APP

APP has a hydrophobic sequence near its carboxyl terminus (about 23 residues long), which directs its insertion into the plasma membrane and internal membranes of the endoplasmic reticulum, Golgi, and *trans*-Golgi network (Selkoe, 1998). α-Secretase-mediated cleavage of APP occurs both at the cell surface and within the constitutive secretory pathway. This cleavage does not depend on a specific sequence of amino acids; rather, it cuts APP at a specific distance from the plasma membrane (Maruyama *et al*., 1991).

Total α-secretase activity can be divided into PKC-independent and PKC-dependent components (Buxbaum *et al*., 1994), which represent basal and stimulated α-secretase activity, respectively. PKC-independent regulation of α- secretase activity involves the elevation of intracellular calcium (Buxbaum *et al*., 1994). Several metalloproteases belonging to the ADAM family have been shown to possess α-secretase-like activity; among these are TACE (tumor necrosis $factor_\alpha$ converting enyme, also known as ADAM-17), MDC9, and ADAM-10. ADAM metalloproteases are membrane-anchored proteins that contain a catalytic domain, an autoinhibitory domain, a disintegrin-like domain, a cysteine-rich sequence, and epidermal growth factor-like sequence. TACE, MDC9, and ADAM-10 also possess a consensus sequence (HEXXH) for a zinc-binding domain. TACE mediates most cellular PKC-dependent α-secretase activity (Buxbaum *et al*., 1998; Lammich *et al*., 1999). MDC9 mediates both basal and PKC-induced cleavage of APP_{695} at the α-secretase site, and inhibition of MDC9 increases β-secretase cleavage (Koike *et al*., 1999).

Overexpression of ADAM-10 in HEK 293 cells stimulates both basal and PKC-dependent α-secretase activity (Lammich *et al*., 1999).

3. Cleavage of Aβ from APP

There are several pathways for the production of Aβ_{42} and Aβ_{40}. Aβ_{40} is generated in recycling endosomes following reinternalization from the cell surface (Koo and Squazzo, 1994). Both peptides are produced in the secretory pathway (Chyung *et al*., 1997; Wild-Bode *et al*., 1997), but the primary site for Aβ_{42} production is the endoplasmic reticulum, whereas the primary site for Aβ_{40} production is the *trans*-Golgi network (Hartmann *et al*., 1997). Intracellular production of Aβ may be unique to neurons because nonneuronal cells produce significant amounts of Aβ_{42} and Aβ_{40} only at the cell surface (Hartmann *et al*., 1997).

Four groups independently identified an unusual membrane-bound aspartyl protease as the elusive β-secretase (Hussain *et al*., 1999; Sinha *et al*., 1999; Vassar *et al*., 1999; Yan *et al*., 1999). This enzyme has been named BACE (β-site APP-cleaving enzyme) by one group and Asp2 by another group. Although other proteases, such as cathepsin D, can cleave APP at the β-secretase site, BACE meets all the requirements of a true β-secretase. BACE is located within the Golgi and endosomes and has a pH optimum of 5–5.5.

To date, γ-secretase has not been identified. Because inhibition of γ-secretase activity is a prime therapeutic major therapeutic target, the identity of this enzyme may be known by the time this chapter is published. Clearly, presenilin 1 is implicated in γ-secretase cleavage, either as an essential cofactor or as the enzyme itself (Wolfe *et al*., 1999).

4. Regulation of APP Processing

The regulation of APP processing is extremely complex. It varies across species and also differs between neuronal and nonneuronal cell types. In nonneuronal cell lines or nonhuman cell lines, acetylcholine binding to muscarinic receptor subtypes concurrently increases $sAPP_\alpha$ production and *inhibits* Aβ production (Buxbaum *et al*., 1992; Hung *et al*., 1993; Jacobsen *et al*., 1994). This effect can be mimicked by phorbol ester, indicating the involvement of PKC in signal transduction. However, in cultures of primary human cerebral neurons, PKC activation increases the rate of $sAPP_\alpha$ release and *increases* the production of Aβ (LeBlanc *et al*., 1998). The latter finding, in conjunction with others, indicates that distinct pathways exist for α- and β-secretase-mediated cleavage of APP (Dyrks *et al*., 1994). PKC-induced α-secretase cleavage is regulated by protein phosphorylation, but does not depend on the phosphorylation of APP (Jacobsen *et al*., 1994). The *trans*-Golgi network is the site of regulated, intracellular α-secretase cleavage (Skovronsky *et al*., 2000).

5. Aβ Aggregation

Aβ aggregation is dependent on concentration, pH, and the length of incubation in aqueous media (Burdick *et al*., 1992). Aβ exists in a random conformation at low pH, a β-pleated sheet conformation at pH 4–7, and a random conformation at high pH (Barrow and Zagorski, 1991; Fraser *et al*., 1992). The effect of pH on Aβ conformation indicates that Aβ aggregation is influenced by the ionization state of key residues. Histidine-aspartic acid/glutamic acid salt bridges stabilize β-pleated sheets, facilitating fibril assembly (Fraser *et al*., 1991). Due to the presence of hydrophobic residues at its carboxyl terminus, Aβ_{42} is very insoluble in water at pH 7.4 (Burdick *et al*., 1992).

Aqueous solutions of Aβ exhibit kinetic rather than thermodynamic solubility (Jarrett and Lansbury, 1993). In other words, Aβ will precipitate from apparently soluble solutions, given sufficient time. The rate-limiting step in the formation of amyloid is nucleation, i.e., the formation of a certain sized Aβ oligomer, which can serve as a scaffold for further aggregation (Jarrett and Lansbury, 1992). Lag time, i.e., the time until a solution exhibiting kinetic solubility precipitates, is directly proportional to the size of the Aβ oligomer required for nucleation and inversely proportional to peptide concentration.

Biometals can induce Aβ aggregation *in vitro* (Bush *et al*., 1994). In AD, concentrations of zinc, copper, and iron are likely to be particularly important (for a review, see Atwood *et al*., 1999). Submicromolar copper induces the aggregation of Aβ_{40} at mildly acidic pH values, similar to those that might be encountered during mild acidosis (Atwood *et al*., 1998). Under acidic conditions, nanomolar concentrations of Aβ_{40} form aggregates, which can subsequently be dissociated by chelation or alkalinization. Aβ_{42}, but not Aβ_{40}, is precipitated by copper at pH 7.4.

In vitro, low micromolar concentrations of zinc induce the aggregation of Aβ_{40} at pH 7.4 (Huang *et al*., 1997). This reaction is mediated by dimeric Aβ, potentiated by α-helical-promoting solvents, inhibited by multimeric forms of Aβ, and requires NaCl. At pH 7.4, zinc-induced aggregation of Aβ_{40} is reversible by chelation over the course of several precipitation/solubilization cycles. Aβ aggregation also occurs in acidic solution (pH 5.5), but aggregates formed in this manner cannot be resolubilized by alkalinization (Huang *et al*., 1997). In canine CSF, half-maximal aggregation of endogenous Aβ is produced by zinc concentrations ranging from 120 to 140 μM (Brown *et al*., 1997). The ability of zinc to induce Aβ aggregation is dependent on the presence of a histidine residue at position 13 (Liu *et al*., 1999). Neuronal depolarization can trigger the massive release of zinc in response to pathological events (Howell *et al*., 1984), causing extracellular zinc concentrations to rise dramatically (Tonder *et al*., 1990; Koh *et al*., 1996). Thus, elevated zinc concentrations may facilitate Aβ aggregation *in vivo*.

Aβ aggregates formed in the presence of zinc are more dense and are solubilized less easily than those formed in the presence of copper (Moir *et al*., 1999). Apolipoprotein E inhibits zinc-induced Aβ aggregation, but enhances copper-induced aggregation. Furthermore, the extent to which metal-induced aggregation of Aβ occurs *in vitro* is altered by specific apolipoprotein E isoforms. Zinc- or copper-induced Aβ aggregation is greater in the presence of apolipoprotein E4 than apolipoprotein E3. This is consistent with the increased risk for AD conferred by the ϵ4 allele.

6. Aβ Clearance

Aβ clearance is not well understood, but may be critically important to AD pathogenesis. Pathways for Aβ clearance include, but are not limited to, the following:

In soluble fractions of human and rat brain, maximal clearance of Aβ occurs at pH 4–5 and is mediated by cathepsin D, an aspartyl protease (McDermott and Gibson, 1996, 1997; Hamazaki, 1996a). Because cathepsin D requires a low pH for catalytic activity, Aβ-degradation by cathepsin D must occur in an acidic intracellular compartment. Cathepsin D cleaves a wild-type Aβ sequence 20 times faster than it does a mutant Aβ sequence (a glycine for alanine substitution at position 21) associated with early-onset AD (Hamazaki, 1996b). This suggests that Aβ clearance by cathepsin D may be relevant to AD.

In vitro, Aβ proteolysis by insulin-degrading enzyme occurs at neutral pH (McDermott and Gibson, 1997).

In vivo, microglia cause Aβ degradation by releasing a protease thought to be a member of the disintegrin family (Mentlein *et al*., 1998).

Aβ can be cleared by a serine protease–α2M complex (Narita *et al*., 1997; Qiu *et al*., 1999). Because polymorphisms in the genes for both α2M and LRP are associated with AD, it is tempting to speculate that Aβ might compete with other molecules, such as cholesterol, for lysosomal clearance (Kowal *et al*., 1989).

IV. Therapeutic Strategies

Although AD treatment and prevention are still in the future, many potential therapeutic targets exist, each of which could be implemented via several routes. These include the following:

Inhibition of Aβ_{42} or Aβ_{40} secretion, with or without concomitant stimulation of sAPP$_\alpha$ secretion
Inhibition of Aβ aggregation or fibril formation
Resolubilization of plaques
Limitation of Aβ-induced toxicity
Stimulation of Aβ clearance
Prevention or limitation of brain inflammation
Administration of neurotrophic agents
Inhibition of neurofibrillary tangle formation
Inhibition of β-secretase or γ-secretase

For a fuller discussion of potential therapeutic strategies, see McKeon-O'Malley *et al*. (1998).

V. Summary

Tremendous progress has been made in the area of AD genetics. Several other genes, yet to be unidentified, may have a major impact on AD risk. However, it seems likely that many more genes will be discovered, each of which increases AD risk slightly. A poor combination of genetic risk factors may be sufficient to cause disease or to permit detrimental environmental factors to operate. The fact that four genes, which alter AD risk (APP, ApoE, α2M, and LRP), are related to each other indicates that cholesterol and other lipids may play a role in AD etiology. We speculate that AD may be caused by risk factors leading to sublethal vascular disease. If this speculation is substantiated by further research, it may be possible to implement lifestyle alterations for AD prevention. Presenilin research is likely to bring about many important findings in area of development, as well as in AD.

References

Abraham, C. R., Selkoe, D. J., and Potter, H. (1988). Immunochemical identification of the serine protease inhibitor, α1-antichymotrypsin in the brain amyloid deposits of Alzheimer's disease. *Cell (Cambridge, Mass.)* **52**, 487–501.

Aldudo, J., Bullido, M. J., Arbizu, T. *et al*. (1998a). Identification of a novel mutation (Leu282Arg) of the human presenilin 1 gene in Alzheimer's disease. *Neurosci. Lett*. **240**, 174–176.

Aldudo, J., Bullido, M. J., Frank, A. *et al*. (1998b). Missense mutation E318G of the presenilin-1 gene appears to be a nonpathogenic polymorphism *Ann. Neurol*. **44**, 985–986.

Aldudo, J., Bullido, M. J., and Valdivieso, F. (1999). DGGE method for the mutational analysis of the coding and proximal promoter regions of the Alzheimer's disease presenilin-1 gene: Two novel mutations. *Hum. Mutation* **14**, 433–439.

Alvarez, R., Alvarez, V., Lahoz, C. H. *et al*. (1999). Angiotensin converting enzyme and endothelial nitric oxide synthase DNA polymorphisms and late onset Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* **67**, 733–736.

Alzheimer's Disease Collaborative Group. (1995). The structure of the presenilin 1 (S182) gene and identification of six novel mutations in early onset AD families. *Nat. Genet*. **11**, 219–222.

Ancolio, K., Dumanchin, C., Barelli, H. *et al*. (1999). Unusual phenotypic alteration of beta amyloid precursor protein (betaAPP) maturation by a new Val-715 $\rightarrow$ Met βAPP-770 mutation responsible for probable early-onset Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A*. **96**, 4119–4124.

Aoki, M., Abe, K., Oda, N. *et al*. (1997). A presenilin-1 mutation in a Japanese family with Alzheimer's disease and distinctive abnormalities on cranial MRI. *Neurology* **48**, 1118–1120.

Araki, W., Kitaguchi, N., Tokushima, Y. *et al*. (1991). Trophic effect of β-amyloid precursor protein on cerebral cortical neurons in culture. *Biochem. Biophys. Res. Commun*. **181**, 265–271.

Atwood, C. S., Moir, R. D., Huang, X. *et al*. (1998). Dramatic aggregation of Alzheimer abeta by Cu(II) is induced by conditions representing physiological acidosis. *J. Biol. Chem*. **273**, 12817–12826.

Atwood, C. S., Huang, X., Moir, R. D. *et al*. (1999). Role of free radicals and metal ions in the pathogenesis of Alzheimer's disease. *Met. Ions Biol. Syst*. **36**, 309–364.

Axelman, K., Basun, H., and Lannfelt, L. (1998). Wide range of disease onset in a family with Alzheimer disease and a His163Tyr mutation in the presenilin-1 gene. *Arch. Neurol. (Chicago)* **55**, 698–702.

Ballerini, C., Nacmias, B., Rombola, G. *et al*. (1999). HLA A2 allele is associated with age at onset of Alzheimer's disease. *Ann. Neurol*. **45**, 397–400.

Barger, S. W., and Mattson, M. P. (1996). Induction of neuroprotective kappa B-dependent transcription by secreted forms of the Alzheimer's beta-amyloid precursor. *Mol. Brain Res*. **40**, 116–126.

Barrow, C. J., and Zagorski, M. G. (1991). Solution structures of β peptide and its constituent fragments: Relation to amyloid deposition. *Science* **253**, 179–182.

Bauer, J., Strauss, S., Schreiter-Gasser, U. *et al*. (1991). Interleukin-6 and alpha-2-macroglobulin indicate an acute-phase state in Alzheimer's disease cortices. *FEBS Lett*. **285**, 111–114.

Baum, L., Chen, L., Masliah, E. *et al*. (1999). Lipoprotein lipase mutations and Alzheimer's disease. *Am. J. Med. Genet*. **88**, 136–139.

Behl, C. (1999). Alzheimer's disease and oxidative stress: Implications for novel therapeutic approaches. *Prog. Neurobiol*. **57**, 301–323.

Besanon, R., Lorenzi, A., Cruts, M. *et al*. (1988). Missense mutation in exon 11 (Codon 378) of the presenilin-1 gene in a French family with early-onset Alzheimer's disease and transmission study by mismatch enhanced allele specific amplification. *Hum. Mutation* **11**, 481.

Birge, S. J. (1997). The role of estrogen in the treatment of Alzheimer's disease. *Neurology* **48**, S36–S41.

Blacker, D., Wilcox, M. A., Laird, N. M. *et al*. (1998). Alpha-2 macroglobulin is genetically associated with Alzheimer disease. *Nat. Genet*. **19**, 357–360.

Blanchard, V., Czech, C., Bonici, B. *et al*. (1997). Immunohistochemical analysis of presenilin 2 expression in the mouse brain: Distribution pattern and co-localization with presenilin 1 protein. *Brain Res*. **758**, 209–217.

Borchelt, D. R., Thinakaran, G., Eckman, C. *et al*. (1996). Familial Alzheimer's disease-linked presenilin 1 variants elevate Aβ1-42/1-40 ratio in vitro and in vivo. *Neuron* **17**, 1005–1013.

Borth, W. (1992). α2-Macroglobulin, a multifunctional binding protein with targeting characteristics. *FASEB J*. **6**, 3345–3353.

Boteva, K., Vitek, M., Mitsuda, H. *et al*. (1996). Mutation analysis of presenillin 1 gene in Alzheimer's disease *Lancet* **347**, 130–131.

Braak, H., and Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol*. **82**, 239–259.

Braak, H., Braak, E., Grundke-Iqbal, I. *et al*. (1986). Occurrence of neuropil threads in the senile human brain and in Alzheimer's disease: A third location of paired helical filaments outside of neurofibrillary tangles and neuritic plaques. *Neurosci. Lett*. **65**, 351–355.

Bramblett, G. T., Goedert, M., Jakes, R. *et al*. (1993). Abnormal tau phosphorylation at Ser396 in Alzheimer's disease recapitulates development and contributes to reduced microtubule binding. *Neuron* **10**, 1089–1099.

Brandi, M. L., Becherini, L., Gennari, L. *et al*. (1999). Association of the estrogen receptor alpha gene polymorphisms with sporadic Alzheimer's disease. *Biochem. Biophys. Res. Commun*. **265**, 335–338.

Breen, K. C., Bruce, M., and Anderton, B. H. (1991). Beta amyloid precursor protein mediates neuronal cell-cell and cell-surface adhesion. *J. Neurosci. Res*. **28**, 90–100.

Breitner, J. C., Welsh, K. A., Gau, B. A. *et al*. (1995). Alzheimer's disease in the National Academy of Sciences-National Research Council Registry of Aging Twin Veterans. III. Detection of cases, longitudinal results, and observations on twin concordance. *Arch. Neurol. (Chicago)* **52**, 763–771.

Brindle, N., Song, Y., Rogaeva, E. *et al*. (1998). Analysis of the butyrylcholinesterase gene and nearby chromosome 3 markers in Alzheimer disease. *Hum. Mol. Genet*. **7**, 933–935.

Brown, A. M., Tummolo, D. M., Rhodes, K. J. *et al*. (1997). Selective aggregation of endogenous beta-amyloid peptide and soluble amyloid precursor protein in cerebrospinal fluid by zinc. *J. Neurochem*. **69**, 1204–1212.

Bullido, M. J., Aldudo, J., Frank, A. *et al*. (2000). A polymorphism in the tau gene associated with risk for Alzheimer's disease. *Neurosci. Lett*. **278**, 49–52.

Burdick, D., Soreghan, B., Kwon, M. *et al*. (1992). Assembly and aggregation properties of synthetic Alzheimer's A4/beta amyloid peptide analogs. *J. Biol. Chem*. **267**, 546–554.

Bush, A. I., Multhaup, G., Moir, R. D. *et al*. (1993). A novel zinc(II) binding site modulates the function of the βA4 amyloid protein precursor of Alzheimer's disease. *J. Biol. Chem*. **268**, 16109–16112.

Bush, A. I., Pettingell, W. H., Multhaup, G. *et al*. (1994). Rapid induction of Alzheimer Aβ amyloid formation by zinc. *Science* **265**, 1464–1467.

Buxbaum, J. D., Oishi, M., Chen, H. I. *et al*. (1992). Cholinergic agonists and interleukin 1 regulate processing and secretion of the Alzheimer beta/A4 amyloid protein precursor. *Proc. Natl. Acad. Sci. U.S.A*. **89**, 10075–10078.

Buxbaum, J. D., Ruefli, A. A., Parker, C. A. *et al*. (1994). Calcium regulates processing of the Alzheimer amyloid protein precursor in a protein kinase C-independent manner. *Proc. Natl. Acad. Sci. U.S.A*. **91**, 4489–4493.

Buxbaum, J. D., Liu, K.-N., Luo, Y. *et al*. (1998). Evidence that tumor necrosis factor α converting enzyme is involved in regulated α-secretase cleavage of the Alzheimer amyloid protein precursor. *J. Biol. Chem*. **273**, 27765–27767.

Cai, X., Stanton, J., Fallin, D. *et al*. (1997). No association between the intronic presenilin-1 polymorphism and Alzheimer's disease in clinic and population-based samples. *Am. J. Med. Genet*. **74**, 202–203.

Callahan, C. M., Hall, K. S., Hui, S. L. *et al*. (1996). Relationship of age, education, and occupation with dementia among a community-based sample of African Americans. *Arch. Neurol. (Chicago)* **53**, 134–140.

Campion, D., Flaman, J. M., Brice, A. *et al*. (1995). Mutations of the presenilin I gene in families with early-onset Alzheimer's disease. *Hum. Mol. Genet*. **4**, 2373–2377.

Campion, D., Brice, A., Dumanchin, C. *et al*. (1996). A novel presenilin 1 mutation resulting in familial Alzheimer's disease with an onset age of 29 years. *NeuroReport* **7**, 1582–1584.

Campion, D., Dumanchin, C., Hannequin, D. *et al*. (1999). Early-onset autosomal dominant Alzheimer disease: Prevalence, genetic heterogeneity, and mutation spectrum. *Am. J. Hum. Genet*. **65**, 664–670.

Capell, A., Grunberg, J., Pesold, B. *et al*. (1998). The proteolytic fragments of the Alzheimer's disease-associated presenilin-1 form heterodimers and occur as a 100-150-kDa molecular mass complex. *J. Biol. Chem*. **273**, 3205–3211.

Chagnon, P., Gee, M., Filion, M. *et al*. (1999). Phylogenetic analysis of the mitochondrial genome indicates significant differences between patients with Alzheimer disease and controls in a French-Canadian founder population. *Am. J. Med. Genet*. **85**, 20–30.

Chambers, C. B., Lee, J. M., Troncoso, J. C. *et al*. (1999). Overexpression of four-repeat tau mRNA isoforms in progressive supranuclear palsy but not in Alzheimer's disease. *Ann. Neurol*. **46**, 325–332.

Chartier-Harlin, M. C., Crawford, F., and Houlden, H. (1991). Early-onset Alzheimer's disease caused by mutations at codon 717 of the beta-amyloid precursor protein gene. *Nature (London)* **353**, 844–846.

Chen, M., and Yankner, B. A. (1991). An antibody to the β amyloid and the amyloid precursor protein inhibits cell-substratum adhesion in many mammalian cell types. *Neurosci. Lett*. **125**, 223–226.

Chen, P., Ganguli, M., Mulsant, B. H. *et al*. (1999). The temporal relationship between depressive symptoms and dementia: A community-based prospective study. *Arch. Gen. Psychiatry* **56**, 261–266.

Chyung, A. S. C., Greenberg, B. D., Cook, D. G. *et al*. (1997). Novel β-secretase cleavage of β-amyloid precursor protein in the endoplasmic reticulum/intermediate compartment of NT2N cells. *J. Cell Biol*. **138**, 671–680.

Citron, M., Westaway, D., Xia, W. *et al*. (1997). Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid β-protein in both transfected cells and transgenic mice. *Nat. Med*. **3**, 67–72.

Citron, M., Eckman, C. B., Diehl, T. S. *et al*. (1998). Additive effects of PS1 and APP mutations on secretion of the 42- residue amyloid beta-protein. *Neurobiol. Dis*. **5**, 107–116.

Cobb, J. L., Wolf, P. A., Au, R. *et al*. (1995). The effect of education on the incidence of dementia and Alzheimer's disease in the Framingham Study. *Neurology* **45**, 1707–1712.

Corder, E. H., Saunders, A. M., Strittmatter, W. J. *et al*. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261**, 921–923.

Corder, E. H., Saunders, A. M., Risch, N. J. *et al*. (1994). Protective effect of apolipoprotein E type 2 for late-onset Alzheimer's disease. *Nat. Genet*. **7**, 184.

Coria, F., Castano, E., Larrondo-Lillo, M. *et al*. (1988). Isolation and characterization of amyloid P component from Alzheimer's disease and other types of cerebral amyloidosis. *Lab. Invest*. **58**, 454–458.

Couchie, D., Charrière-Bertrand, C., and Nuñez, J. (1988). Expression of the mRNA for tau proteins during brain development and in cultured neurons and astroglial cells. *J. Neurochem*. **50**, 1894–1899.

Crawford, F., Fallin, D., Suo, Z. *et al*. (1998). The butyrylcholinesterase gene is neither independently nor synergistically associated with late-onset AD in clinic- and community-based populations. *Neurosci. Lett*. **249**, 115–118.

Crawford, F., Freeman, M., Town, T. *et al*. (1999). No genetic association between polymorphisms in the Tau gene and Alzheimer's disease in clinic or population based samples. *Neurosci. Lett*. **266**, 193–196.

Crook, R., Ellis, R., Shanks, M. *et al*. (1997). Early-onset Alzheimer's disease with a presenilin-1 mutation at the site corresponding to the Volga German presenilin-2 mutation. *Ann. Neurol*. **42**, 124–128.

Crook, R., Verkkoniemi, A., Perez-Tur, J. *et al*. (1998). A variant of Alzheimer's disease with spastic paraparesis and unusual plaques due to deletion of exon 9 of presenillin 1. *Nat. Med*. **4**, 452–455.

Cruts, M., Martin, J. J., and van Broeckhoven, C. (1995). Molecular genetic analysis of familial early-onset Alzheimer's Disease linked to chromosome 14q24.3. *Hum. Mol. Genet*. **4**, 2363–2371.

Cruts, M., van Duijn, C. M., Backhovens, H. *et al*. (1998). Estimation of the genetic contribution of presenilin-1 and -2 mutations in a population-based study of presenile Alzheimer disease. *Hum. Mol. Genet*. **7**, 43–51.

Cummings, B. J., Satou, T., Head, E. *et al*. (1996). Diffuse plaques contain C-terminal Aβ42 and not Aβ40: Evidence from cats and dogs. *Neurobiol. Aging* **17**, 653–659.

Dahiyat, M., Cumming, A., Harrington, C. *et al*. (1999). Association between Alzheimer's disease and the NOS3 gene. *Ann. Neurol*. **46**, 664–667.

Das, H. K., McPherson, J., Bruns, G. A. *et al*. (1985). Isolation, characterization, and mapping to chromosome 19 of the human apolipoprotein E gene. *J. Biol. Chem*. **260**, 6240–6247.

Davis, R. E., Miller, S., Herrnstadt, C. *et al*. (1997). Mutations in mitochondrial cytochrome c oxidase genes segregate with late-onset Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A*. **94**, 4526–4531.

Dewji, N. N., and Singer, S. J. (1997). The seven-transmembrane spanning topography of the Alzheimer disease-related presenilin proteins in the plasma membranes of cultured cells. *Proc. Natl. Acad. Sci. U.S.A*. **94**, 14025–14030.

Dickson, D. W. (1997). The pathogenesis of senile plaques. *J. Neuropathol. Exp. Neurol*. **56**, 321–339.

Doan, A., Thinakaran, G., Borchelt, D. R. *et al*. (1996). Protein topology of presenilin 1. *Neuron* **17**, 1023–1030.

Dumanchin, C., Brice, A., Campion, D. *et al*. (1998). De novo presenilin 1 mutations are rare in clinically sporadic, early onset Alzheimer's disease cases. French Alzheimer's Disease Study Group. *J. Med. Genet*. **35**, 672–673.

Dyrks, T., Monning, U., Beyreuther, K. *et al*. (1994). Amyloid precursor protein secretion and βA4 amyloid generation are not mutually exclusive. *FEBS Lett*. **349**, 210–214.

Eckman, C. B., Mehta, N. D., Crook, R. *et al*. (1997). A new pathogenic mutation in the APP gene (I716V) increases the relative proportion of Aβ42(43). *Hum. Mol. Genet*. **6**, 2087–2089.

Egensperger, R., Kosel, S., Schnopp, N. M. *et al*. (1997). Association of the mitochondrial tRNA(A4336G) mutation with Alzheimer's and Parkinson's diseases. *Neuropathol. Appl. Neurobiol*. **23**, 315–321.

Ezquerra, M., Carnero, C., Blesa, R. *et al*. (1999). A presenilin 1 mutation (Ser169Pro) associated with early-onset AD and myoclonic seizures. *Neurology* **52**, 566–570.

Farrer, L. A., Abraham, C. R., Haines, J. L. *et al*. (1998). Association between bleomycin hydrolase and Alzheimer's disease in caucasians. *Ann. Neurol*. **44**, 808–811.

Finckh, U., ller-Thomsen, T., Mann, U. *et al*. (2000). High prevalence of pathogenic mutations in patients with early-onset dementia detected by sequence analyses of four different genes. *Am. J. Hum. Genet*. **66**, 110–117.

Forsell, C., Froelich, S., Axelman, K. *et al*. (1997). A novel pathogenic mutation (Leu262Phe) found in the presenilin 1 gene in early-onset Alzheimer's disease. *Neurosci. Lett*. **234**, 3–6.

Fraser, P. E., Nguyen, J. T., Surewicz, W. K. *et al*. (1991). pH-dependent structural transitions of Alzheimer amyloid peptides. *Biophys. J*. **60**, 1190–1201.

Fraser, P. E., Nguyen, J. T., Inouye, H. *et al*. (1992). Fibril formation by primate, rodent, and Dutch-hemorrhagic analogues of Alzheimer amyloid β-protein. *Biochemistry* **31**, 10716–10723.

Fukumoto, H., Asami-Odaka, A., Suzuki, N. *et al*. (1996). Amyloid beta protein deposition in normal aging has the same characteristics as that in Alzheimer's disease. Predominance of A beta 42(43) and association of A beta 40 with cored plaques. *Am. J. Pathol*. **148**, 259–265.

Fukushima, Y., Bell, G. I., and Shows, T. B. (1988). The polymorphic human alpha-2-macroglobulin gene (A2M) is located in chromosome region 12p12.3–p13.3. *Cytogenet. Cell Genet*. **48**, 58–59.

Furukawa, K., Sopher, B. L., Rydel, R. E. *et al*. (1996). Increased activity-regulating and neuroprotective efficacy of α-secretase-derived secreted amyloid precursor protein conferred by a C-terminal heparin-binding domain. *J. Neurochem*. **67**, 1882–1896.

Geerlings, M. I., Schmand, B., Jonker, C. *et al*. (1999). Education and incident Alzheimer's disease: A biased association due to selective attrition and use of a two-step diagnostic procedure? *Int. J. Epidemiol*. **28**, 492–497.

George-Hyslop, P., Haines, J., Rogaev, E. *et al*. (1992). Genetic evidence for a novel familial Alzheimer's disease locus on chromosome 14. *Nat. Genet*. **2**, 330–334.

Georgieff, I. S., Liem, R. K., Couchie, D. *et al*. (1993). Expression of high molecular weight tau in the central and peripheral nervous systems. *J. Cell Sci*. **105**(Pt. 3), 729–737.

Ghetti, B., Murrell, J., and Spillantini, M. G. (1999). Mutations in the Tau gene cause frontotemporal dementia. *Brain Res. Bull*. **50**, 471–472.

Ghiso, J., Rostagno, A., Gardella, J. E. *et al*. (1992). A 109-amino-acid C-terminal fragment of Alzheimer's-disease amyloid precursor protein contains a sequence, - RHDS-, that promotes cell adhesion. *Biochem. J*. **288**, 1053–1059.

Glenner, G. G., and Wong, C. W. (1984). Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun*. **120**, 885–890.

Goate, A., Chartier-Harlin, M. C., Mullan, M. *et al*. (1991). Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature (London)* **349**, 704–706.

Goedert, M., Trojanowski, J. Q., and Lee, V. M. Y. (1997). *In* ''Mechanisms of Dementia'' (W. Wasco, and R. E. Tanzi, ed.), p. 199. Humana Press, Totowa, NJ.

Gomez-Isla, T., Wasco, W., Pettingell, W. P. *et al*. (1997). A novel presenilin-1 mutation: Increased beta-amyloid and neurofibrillary changes. *Ann. Neurol*. **41**, 809–813.

Gomez-Pinilla, F., Cummings, B. J., and Cotman, C. W. (1990). Induction of basic fibroblast growth factor in Alzheimer's disease pathology. *NeuroReport* **1**, 211–214.

Goodman, Y., and Mattson, M. P. (1994). Secreted forms of beta-amyloid precursor protein protect hippocampal neurons against amyloid beta-peptide-induced oxidative injury. *Exp. Neurol*. **128**, 1–12.

Griffin, W. S. T., Sheng, J. G., Roberts, G. W. *et al*. (1995). Interleukin-1 expression in different plaques types in Alzheimer's disease: Significance in plaque evolution. *J. Neuropathol. Exp. Neurol*. **54**, 276–281.

Grundke-Iqbal, I., Iqbal, K., Tung, Y. *et al*. (1986). Abnormal phosphorylation of the microtubule-associated protein τ (tau) in Alzheimer cytoskeletal pathology. *Proc. Natl. Acad. Sci. U.S.A*. **83**, 4913–4917.

Guo, Q., Christakos, S., Robinson, N. *et al*. (1998a). Calbindin D28k blocks the proapoptotic actions of mutant presenilin 1: Reduced oxidative stress and preserved mitochondrial function. *Proc. Natl. Acad. Sci. U.S.A*. **95**, 3227–3232.

Guo, Q., Sopher, B., Furukawa, K. *et al*. (1998b). Alzheimer's presenilin mutation sensitizes neural cells to apoptosis induced by trophic factor withdrawal and amyloid β-peptide: Involvement of calcium and oxyradicals. *J. Neurosci*. **17**, 4212–4222.

Haass, C., Schlossmacher, M. G., Hung, A. Y. *et al*. (1992). Amyloid β-peptide is produced by cultured cells during normal metabolism. *Nature (London)* **359**, 322–325.

Haass, C., Hung, A. Y., Schlossmacher, M. G. *et al*. (1993). β-Amyloid peptide and a 3-kDa fragment are derived by distinct cellular mechanisms. *J. Biol. Chem.* **268**, 3021–3024.

Haines, J. L., Pritchard, M. L., Saunders, A. M. *et al*. (1996). No genetic effect of α1-antichymotrypsin in Alzheimer's disease. *Genomics* **33**, 53–56.

Hamazaki, H. (1996a). Cathepsin D is involved in the clearance of Alzheimer's beta-amyloid. *FEBS Lett*. **396**, 139–142.

Hamazaki, H. (1996b). A beta-amyloid peptide variant related with familial Alzheimer's disease and hereditary cerebral hemorrhage with amyloidosis is poorly eliminated by cathepsin D. *FEBS Lett*. **397**, 313–315.

Hartmann, T., Bieger, S. C., Bruhl, B. *et al*. (1997). Distinct sites of intracellular production for Alzheimer's disease Aβ40/42 amyloid peptides. *Nat. Med*. **3**, 1016–1020.

Harvey, R. J., Ellison, D., Hardy, J. *et al*. (1998). Chromosome 14 familial Alzheimer's disease: The clinical and neuropathological characteristics of a family with a leucine→serine (L250S) substitution at codon 250 of the presenilin 1 gene. *J. Neurol., Neurosurg. Psychiatry* **64**, 44–49.

Haskell, S. G., Richardson, E. D., and Horwitz, R. I. (1997). The effect of estrogen replacement therapy on cognitive function in women: A critical review of the literature. *J. Clin. Epidemiol*. **50**, 1249–1264.

Hebert, L. E., Scherr, P. A., Beckett, L. A. *et al*. (1992). Relation of smoking and alcohol consumption to incident Alzheimer's disease. *Am. J. Epidemiol*. **135**, 347–355.

Hendricks, L., van Duijn, C. M., Cras, P. *et al*. (1992). Presenile dementia and cerebral haemorrhage linked to a mutation at codon 692 of the beta-amyloid precursor protein gene. *Nat. Genet*. **1**, 218–221.

Hillier, V., and Salib, E. (1997). A case-control study of smoking and Alzheimer's disease. *Int. J. Geriatr. Psychiatry* **12**, 295–300.

Hiltunen, M., Mannermaa, A., Helisalmi, S. *et al*. (1998). Butyrylcholinesterase K variant and apolipoprotein E4 genes do not act in synergy in Finnish late-onset Alzheimer's disease patients. *Neurosci. Lett*. **250**, 69–71.

Hollenbach, E., Ackermann, S., Hyman, B. T. *et al*. (1998). Confirmation of an association between a polymorphism in exon 3 of the low-density lipoprotein receptor-related protein gene and Alzheimer's disease. *Neurology* **50**, 1905–1907.

Howell, G. A., Welch, M. G., and Frederickson, C. J. (1984). Stimulation-induced uptake and release of zinc in hippocampal slices. *Nature (London)* **308**, 736–738.

Hoyert, D. L., and Rosenberg, H. M. (1999). Mortality from Alzheimer's disease: An update. *Natl. Vital Stat. Rep*. **47**, 1–8.

Huang, X., Atwood, C. S., Moir, R. D. *et al*. (1997). Zinc-induced Alzheimer's Aβ1-40 aggregation is mediated by conformational factors. *J. Biol. Chem*. **272**, 26464–26470.

Hung, A. Y., Haass, C., Nitsch, R. M. *et al*. (1993). Activation of protein kinase C inhibits cellular production of the amyloid beta-protein. *J. Biol. Chem*. **268**, 22959–22962.

Hussain, I., Powell, D., Howlett, D. R. *et al*. (1999). Identification of a novel aspartic protease (Asp 2) as β-secretase. *Mol. Cell. Neurosci*. **14**, 419–427.

Hutton, M., Lendon, C. L., Rizzu, P. *et al*. (1998). Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature (London)* **393**, 702–705.

Iijima, M., Tabira, T., Poorkaj, P. *et al*. (1999). A distinct familial presenile dementia with a novel missense mutation in the tau gene. *NeuroReport* **10**, 497–501.

Iwatsubo, T., Odaka, A., Suzuki, N. *et al*. (1994). Visualization of Aβ42(43) and Aβ40 in senile plaques with end- specific Aβmonoclonals: Evidence that an initially deposited species is Aβ42(43). *Neuron* **13**, 45–53.

Iwatsubo, T., Mann, D. M., Odaka, A. *et al*. (1995). Amyloid beta protein (Aβ) deposition: Aβ42(43) precedes Aβ40 in Down syndrome. *Ann. Neurol*. **37**, 294–299.

Jacobsen, J. S., Spruyt, M. A., Brown, A. M. *et al*. (1994). The release of Alzheimer's disease β-amyloid is reduced by phorbol treatment. *J. Biol. Chem.* **269**, 8376–8382.

Jarrett, J. T., and Lansbury, P. T., Jr. (1992). Amyloid fibril formation requires a chemically discriminating nucleation event: Studies of an amyloidogenic sequence from the bacterial protein OsmB. *Biochemistry* **31**, 12345–12352.

Jarrett, J. T., and Lansbury, P. T., Jr. (1993). Seeding ''one-dimensional crystallization'' of amyloid: A pathogenic mechanism in Alzheimer's disease and scrapie? *Cell (Cambridge, Mass.)* **73**, 1055–1058.

Jarrett, J. T., Berger, E. P., and Lansbury, P. T. (1993a). The C-terminus of the β protein is critical in amyloidogenesis. *Ann. N. Y. Acad. Sci*. **695**, 144–148.

Jarrett, J. T., Berger, E. P., and Lansbury, P. T., Jr. (1993b). The carboxy terminus of the β amyloid protein is critical for the seeding of amyloid formation: Implications for the pathogenesis of Alzheimer's disease. *Biochemistry* **32**, 4693–4697.

Jorgensen, P., Bus, C., Pallisgaard, N. *et al*. (1996). Familial Alzheimer's disease co-segregates with a Met146Ile substitution in presenilin-1. *Clin. Genet*. **50**, 281–286.

Kamboh, M. I., Aston, C. E., Perez-Tur, J. *et al*. (1999). A novel mutation in the apolipoprotein E gene (APOE*4 Pittsburgh) is associated with the risk of late-onset Alzheimer's disease. *Neurosci. Lett*. **263**, 129–132.

Kamimura, K., Tanahashi, H., Yamanaka, H. *et al*. (1998). Familial Alzheimer's disease genes in Japanese. *J. Neurol. Sci*. **160**, 76–81.

Kamino, K., Sato, S., Sakaki, Y. *et al*. (1996). Three different mutations of presenilin 1 gene in early-onset Alzheimer's disease families. *Neurosci. Lett*. **208**, 195–198.

Kang, D. E., Saitoh, T., Chen, X. *et al*. (1997). Genetic association of the low density lipoprotein receptor-related protein gene (LRP), an apolipoprotein E receptor, with late-onset Alzheimer's disease. *Neurology* **49**, 56–61.

Kang, J., Lemaire, H. G., and Unterbeck, A. (1987). The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature (London)* **325**, 733–736.

Katzmann, R. (1986). Alzheimer's disease. *N. Engl. J. Med*. **314**, 964–973.

Kaytor, M. D., and Warren, S. T. (2000). Aberrant protein deposition and neurological disease. *J. Biol. Chem*. **274**, 37507–37510.

Kirschner, D. A., Inouye, H., Duffy, L. K. *et al*. (1987). Synthetic peptide homologous to beta protein from Alzheimer disease forms amyloid-like fibrils in vitro. *Proc. Natl. Acad. Sci. U.S.A*. **84**, 6953–6957.

Knopman, D. S., and Morris, J. C. (1997). An update on primary drug therapies for Alzheimer disease. *Arch. Neurol. (Chicago)* **54**, 1406–1409.

Koh, J. Y., Suh, S. W., Gwag, B. J. *et al*. (1996). The role of zinc in selective neuronal death after transient global cerebral ischemia. *Science* **272**, 1013–1016.

Koike, H., Tomioka, S., Sorimachi, H. *et al*. (1999). Membrane-anchored metalloprotease MDC9 has an α-secretase activity responsible for processing the amyloid precursor protein. *Biochem. J*. **343**(Pt. 2), 371–375.

Kokmen, E., Beard, C. M., O'Brien, P. C. *et al*. (1996). Epidemiology of dementia in Rochester, Minnesota. *Mayo Clin. Proc*. **71**, 275–282.

Koo, E. H., and Squazzo, S. L. (1994). Evidence that production and release of amyloid β-protein involves the endocytic pathway. *J. Biol. Chem.* **269**, 17386–17389.

Kosik, K. S., Orecchio, L. D., Bakalis, S. *et al*. (1989). Developmentally regulated expression of specific tau sequences. *Neuron* **2**, 1389–1397.

Kovacs, D. M., Fausett, H. J., Page, K. J. *et al*. (1996). Alzheimer-associated presenilins 1 and 2: Neuronal expression in brain and localization to intracellular membranes in mammalian cells. *Nat. Med*. **2**, 224–229.

Kowal, R. C., Herz, J., Goldstein, J. L. *et al*. (1989). Low density lipoprotein receptor-related protein mediates uptake of cholesteryl esters derived from apoprotein E-enriched lipoproteins. *Proc. Natl. Acad. Sci. U.S.A*. **86**, 5810–5814.

Kowalska, A., Forsell, C., Florczak, J. *et al*. (1999). A Polish pedigree with Alzheimer's disease determined by a novel mutation in exon 12 of the presenilin 1 gene: Clinical and molecular characterization. *Folia Neuropathol*. **37**, 57–61.

Krieger, M., and Herz, J. (1994). Structures and functions of multi-ligand lipoprotein receptors: Macrophage scavenger receptors and LDL receptor-related protein. *Annu. Rev. Biochem*. **63**, 601–637.

Kunugi, H., Hattori, M., Ueki, A. *et al*. (1998). Possible association of missense mutation (Gly[-63]Glu) of the neurotrophin-3 gene with Alzheimer's disease in Japanese. *Neurosci. Lett*. **241**, 65–67.

Kwok, J. B., Taddei, K., Hallupp, M. *et al*. (1997). Two novel (M233T and R278T) presenilin-1 mutations in early-onset Alzheimer's disease pedigrees and preliminary evidence for association of presenilin-1 mutations with a novel phenotype. *NeuroReport* **8**, 1537–1542.

Lalowski, M., Golabek, A., Lemere, C. A. *et al*. (1996). The ''nonamyloidogenic'' p3 fragment (amyloid β17–42) is a major constituent of Down's syndrome cerebellar preamyloid. *J. Biol. Chem*. **271**, 33623–33631.

Lambert, J. C., Pasquier, F., Cottel, D. *et al*. (1998). A new polymorphism in the APOE promoter associated with risk of developing Alzheimer's disease. *Hum. Mol. Genet*. **7**, 533–540.

Lambert, J. C., Brousseau, T., Defosse, V. *et al*. (2000). Independent association of an APOE gene promoter polymorphism with increased risk of myocardial infarction and decreased APOE plasma concentrations-the ECTIM Study. *Hum. Mol. Genet*. **9**, 57–61.

Lammich, S., Kojro, E., Postina, R. *et al*. (1999). Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proc. Natl. Acad. Sci. U.S.A*. **96**, 3922–3927.

LeBlanc, A. C., Koutroumanis, M., and Goodyer, C. G. (1998). Protein kinase C activation increases release of secreted amyloid precursor protein without decreasing Abeta production in human primary neuron cultures. *J. Neurosci*. **18**, 2907–2913.

Lee, P. N. (1994). Smoking and Alzheimer's disease: A review of the epidemiological evidence. *Neuroepidemiology* **13**, 131–144.

Lehmann, S., Chiesa, R., and Harris, D. A. (1997). Evidence for a six-transmembrane domain structure of presenilin 1. *J. Biol. Chem*. **272**, 12047–12051.

Leibson, C. L., Rocca, W. A., Hanson, V. A. *et al*. (1997). The risk of dementia among persons with diabetes mellitus. *Ann. N. Y. Acad. Sci*. **826**, 422–427.

Lemaire, H. G., Salbaum, J. M., Multhaup, G. *et al*. (1989). The PreA4 695 precursor protein of Alzheimer's disease A4 amyloid is encoded by 16 exons. *Nucleic Acids Res*. **17**, 517–522.

Lemere, C. A., Lopera, F., Kosik, K. S. *et al*. (1996). The E280A presenilin 1 Alzheimer mutation produces increased Aβ42 deposition and severe cerebellar pathology. *Nat. Med*. **2**, 1146–1150.

Letenneur, L., Gilleron, V., Commenges, D. *et al*. (1999). Are sex and educational level independent predictors of dementia and Alzheimer's disease? Incidence data from the PAQUID project. *J. Neurol. Neurosurg. Psychiatry* **66**, 177–183.

Levy, E., Carman, M. D., Fernandez-Madrid, I. J. *et al*. (1990). Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch-type. *Science* **248**, 1124–1126.

Levy-Lahad, E., Wijsman, E. M., Nemens, E. *et al*. (1995a). A familial Alzheimer's disease locus on chromosome 1. *Science* **269**, 970–973.

Levy-Lahad, E., Wasco, W., Poorkaj, P. *et al*. (1995b). Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science* **269**, 973–977.

Li, T., Holmes, C., Sham, P. C. *et al*. (1999). Allelic functional variation of serotonin transporter expression is a susceptibility factor for late onset Alzheimer's disease. *NeuroReport* **8**, 683–686.

Li, X., and Greenwald, I. (1998). Additional evidence for an eight-transmembrane-domain topology for *Caenorhabditis elegans* and human presenilins. *Proc. Natl. Acad. Sci. U.S.A*. **95**, 7109–7114.

Liao, A., Nitsch, R. M., Greenberg, S. M. *et al*. (1998). Genetic association of an alpha2-macroglobulin (Val1000Ile) polymorphism and Alzheimer's disease. *Hum. Mol. Genet*. **7**, 1953–1956.

Lilius, L., Froelich, Fabre S., Basun, H. *et al*. (1999). Tau gene polymorphisms and apolipoprotein E ϵ4 may interact to increase risk for Alzheimer's disease. *Neurosci. Lett*. **277**, 29–32.

Liu, S. T., Howlett, G., and Barrow, C. J. (1999). Histidine-13 is a crucial residue in the zinc ion-induced aggregation of the A beta peptide of Alzheimer's disease. *Biochemistry* **38**, 9373–9378.

Lovell, M. A., Robertson, J. D., Teesdale, W. J. *et al*. (1998). Copper, iron and zinc in Alzheimer's disease senile plaques. *J. Neurosci. Res*.

Mackenzie, I. R. A. (1994). Senile plaques do not progressively accumulate with normal aging. *Acta Neuropathol*. **87**, 520–525.

Mahley, R. W., and Huang, Y. (1999). Apolipoprotein E: From atherosclerosis to Alzheimer's disease and beyond. *Curr. Opin. Lipidol*. **10**, 207–217.

Mann, D. M., Iwatsubo, T., Cairns, N. J. *et al*. (1996). Amyloid beta protein (Abeta) deposition in chromosome 14-linked Alzheimer's disease: Predominance of Aβ42(43). *Ann. Neurol*. **40**, 149–156.

Maruyama, K., Kametani, F., Usami, M. *et al*. (1991). ''Secretase,'' Alzheimer amyloid protein precursor secreting enzyme is not sequence-specific. *Biochem. Biophys. Res. Commun*. **179**, 1670–1676.

Mattila, K. M., Forsell, C., Pirttila, T. *et al*. (1998). The Glu318Gly mutation of the presenilin-1 gene does not necessarily cause Alzheimer's disease. *Ann. Neurol*. **44**, 965–967.

Mattson, M. P. (1994). Secreted forms of β-amyloid precursor protein modulate dendrite outgrowth and calcium responses to glutamate in cultured embryonic hippocampal neurons. *J. Neurobiol*. **25**, 439–450.

Mattson, M. P., Cheng, B., Culwell, A. R. *et al*. (1993). Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of the β-amyloid precursor protein. *Neuron* **10**, 243–254.

Mattson, M. P., Robinson, N., and Guo, Q. (1997). Estrogens stabilize mitochondrial function and protect neural cells against the pro-apoptotic action of mutant presenilin-1. *NeuroReport* **8**, 3817–3821.

Mattson, M. P., Guo, Q., Furukawa, K. *et al*. (1998). Presenilins, the endoplasmic reticulum, and neuronal apoptosis in Alzheimer's disease. *J. Neurochem*. **70**, 1–14.

McDermott, J. R., and Gibson, A. M. (1996). Degradation of Alzheimer's beta-amyloid protein by human cathepsin D. *NeuroReport* **7**, 2163–2166.

McDermott, J. R., and Gibson, A. M. (1997). Degradation of Alzheimer's beta-amyloid protein by human and rat brain peptidases: Involvement of insulin-degrading enzyme. *Neurochem. Res*. **22**, 49–56.

McGeer, P. L., Schulzer, M., and McGeer, E. G. (1996). Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: A review of 17 epidemiologic studies. *Neurology* **47**, 425–432.

McKeon-O'Malley, C., Saunders, A. J., Bush, A. I. *et al*. (1998). Potential therapeutic targets for Alzheimer's disease. *Emerg. Ther. Targets* **2**, 157–179.

Mentlein, R., Ludwig, R., and Martensen, I. (1998). Proteolytic degradation of Alzheimer's disease amyloid β-peptide by a metalloproteinase from microglia cells. *J. Neurochem*. **70**, 721–726.

Merchant, C., Tang, M. X., Albert, S. *et al*. (1999). The influence of smoking on the risk of Alzheimer's disease. *Neurology* **52**, 1408–1412.

Milward, E. A., Papadopoulos, R., Fuller, S. J. *et al*. (1992). The amyloid protein precursor of Alzheimer's disease is a mediator of the effects of nerve growth factor on neurite outgrowth. *Neuron* **9**, 129–137.

Moir, R. D., Atwood, C. S., Romano, D. M. *et al*. (1999). Differential effects of apolipoprotein E isoforms on metal-induced aggregation of A beta using physiological concentrations. *Biochemistry* **38**, 4595–4603.

Molgaard, C. A., Stanford, E. P., Morton, D. J. *et al*. (1990). Epidemiology of head trauma and neurocognitive impairment in a multi-ethnic population. *Neuroepidemiology* **9**, 233–242.

Montoya, S. E., Aston, C. E., DeKosky, S. T. *et al*. (1998). Bleomycin hydrolase is associated with risk of sporadic Alzheimer's disease. *Nat. Genet*. **19**, 404.

Mullan, M., Crawford, F., Axelman, K. *et al*. (1992). A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of β-amyloid. *Nat. Genet*. **1**, 345–347.

Murrell, J., Farlow, M., and Ghetti, B. (1991). A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease. *Science* **254**, 97–98.

Namba, Y., Tomonaga, M., Kawasaki, H. *et al*. (1991). Apolipoprotein E immunoreactivity in cerebral deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease. *Brain Res*. **541**, 163–166.

Narhi, L., Wood, S. J., Steavenson, S. *et al*. (1999). Both familial Parkinson's disease mutations accelerate α-synuclein aggregation. *J. Biol. Chem*. **274**, 9843–9846.

Narita, M., Holtzman, D. M., Schwartz, A. L. *et al*. (1997). α2-macroglobulin complexes with and mediates the endocytosis of β-amyloid via cell surface low-density lipoprotein receptor-related protein. *J. Neurochem*. **69**, 1904–1911.

Nee, L. E., and Lippa, C. F. (1999). Alzheimer's disease in 22 twin pairs—13-year follow-up: Hormonal, infectious and traumatic factors. *Dementia Geriatr. Cogn. Disord*. **10**, 148–151.

Ninomiya, H., Roch, J., Sundsmo, M. P. *et al*. (1993). Amino acid sequence RERMS represents the active domain of amyloid β/A4 protein precursor that promotes fibroblast growth. *J. Cell Biol*. **121**(4), 879–886.

Nishiwaki, Y., Kamino, K., Yoshiiwa, A. *et al*. (1997). T/G polymorphism at intron 9 of presenilin 1 gene is associated with, but not responsible for sporadic late-onset Alzheimer's disease in Japanese population. *Neurosci. Lett*. **227**, 123–126.

Okuizumi, K., Onodera, O., Namba, Y. *et al*. (1995). Genetic association of the very low density lipoprotein (VLDL) receptor gene with sporadic Alzheimer's disease. *Nat. Genet*. **11**, 207–209.

Okuizumi, K., Onodera, O., Seki, K. *et al*. (1996). Lack of association of very low density lipoprotein receptor gene polymorphism with Caucasian Alzheimer's disease. *Ann. Neurol*. **40**, 251–254.

Oliveira, J. R., Gallindo, R. M., Maia, L. G. *et al*. (1998). The short variant of the polymorphism within the promoter region of the serotonin transporter gene is a risk factor for late onset Alzheimer's disease. *Mol. Psychiatry* **3**, 438–441.

Oliveira, J. R., Shimokomaki, C. M., Brito-Marques, P. R. *et al*. (1999). The association of the short variant of the 5-HTTPLR polymorphism and the ApoE epsilon4 allele does not increase the risk for late onset Alzheimer's disease. *Mol. Psychiatry* **4**, 19–20.

Oltersdorf, T., Ward, P. J., and Henriksson, T. (1990). The Alzheimer amyloid precursor protein. Identification of a stable intermediate in the biosynthetic/degradative pathway. *J. Biol. Chem*. **265**, 4492–4497.

Pahlsson, P., Shakin-Eshleman, S. H., and Spitalnik, S. L. (1992). N-linked glycosylation of β-amyloid precursor protein. *Biochem. Biophys. Res. Commun*. **189**, 1667–1673.

Papassotiropoulos, A., Bagli, M., Feder, O. *et al*. (1999). Genetic polymorphism of cathepsin D is strongly associated with the risk for developing sporadic Alzheimer's disease. *Neurosci. Lett*. **262**, 171–174.

Perez-Tur, J., Froelich, S., Prihar, G. *et al*. (1995). A mutation in Alzheimer's disease destroying a splice acceptor site in the presenilin-1 gene. *NeuroReport* **7**, 297–301.

Perez-Tur, J., Croxton, R., Wright, K. *et al*. (1996). A further presenilin 1 mutation in the exon 8 cluster in familial Alzheimer's disease. *NeuroDegeneration* **5**, 207–212.

Pitas, R. E., Boyles, J. K., Lee, S. H. *et al*. (1987). Astrocytes synthesize apolipoprotein E-containing lipoproteins. *Biochim. Biophys. Acta* **917**, 148–161.

Poller, W., Faber, J. P., Klobeck, G. *et al*. (1992). Cloning of the human alpha 2-macroglobulin gene and detection of mutations in two functional domains: The bait region and the thiolester site. *Hum. Genet*. **88**, 313–319.

Poorkaj, P., Bird, T. D., Wijsman, E. *et al*. (1998a). Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Ann. Neurol*. **43**, 815–825.

Poorkaj, P., Sharma, V., Anderson, L. *et al*. (1998b). Missense mutations in the chromosome 14 familial Alzheimer's disease presenilin 1 gene. *Hum. Mutation* **11**, 216–221.

Price, J. L., Davis, P. B., Morris, J. C. *et al*. (1991). The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. *Neurobiol. Aging* **12**, 295–312.

Prihar, G., Verkkoniem, A., Perez-Tur, J. *et al*. (1999). Alzheimer disease PS-1 exon 9 deletion defined. *Nat. Med*. **5**, 1090.

Pritchard, M. L., Saunders, A. M., Gaskell, P. C. *et al*. (1996). No association between very low density lipoprotein receptor (VLDL-R) and Alzheimer disease in American Caucasians. *Neurosci. Lett*. **209**, 105–108.

Qiu, Z., Strickland, D. K., Hyman, B. T. *et al*. (1999). Alpha2-macroglobulin enhances the clearance of endogenous soluble beta-amyloid peptide via low-density lipoprotein receptor-related protein in cortical neurons. *J. Neurochem*. **73**, 1393–1398.

Ramirez-Duenas, M. G., Rogaeva, E. A., Leal, C. A. *et al*. (1998). A novel Leu171Pro mutation in presenilin-1 gene in a Mexican family with early onset Alzheimer disease. *Ann. Genet*. **41**, 149–153.

Reynolds, W. F., Rhees, J., Maciejewski, D. *et al*. (1999). Myeloperoxidase polymorphism is associated with gender specific risk for Alzheimer's disease. *Exp. Neurol*. **155**, 31–41.

Reznik-Wolf, H., Treves, T. A., Davidson, M. *et al*. (1996). A novel mutation of presenilin 1 in familial Alzheimer's disease in Israel detected by denaturing gradient gel electrophoresis. *Hum. Genet*. **98**, 700–702.

Reznik-Wolf, H., Treves, T. A., Shabtai, H. *et al*. (1998). Germline mutational analysis of presenilin 1 and APP genes in Jewish-Israeli individuals with familial or early-onset Alzheimer disease using denaturing gradient gel electrophoresis (DGGE). *Eur. J. Hum. Genet*. **6**, 176–180.

Roberts, G. W., Gentleman, S. M., Lynch, A. *et al*. (1994). β amyloid protein deposition in the brain after severe head injury: Implications for the pathogenesis of Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* **57**, 419–425.

Rogaev, E. I., Sherrington, R., Rogaeva, E. A. *et al*. (1995). Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature (London)* **376**, 775–778.

Roks, G., Dermaut, B., Heutink, P. *et al*. (1999). Mutation screening of the tau gene in patients with early-onset Alzheimer's disease. *Neurosci. Lett*. **277**, 137–139.

Romero, I., Jorgensen, P., Bolwig, G. *et al*. (1999). A presenilin-1 Thr116Asn substitution in a family with early-onset Alzheimer's disease. *NeuroReport* **10**, 2255–2260.

Roses, A. D., Strittmatter, W. J., Pericak-Vance, M. A. *et al*. (1994). Clinical application of apolipoprotein E genotyping to Alzheimer's disease. *Lancet* **343**, 1564–1565.

Rossor, M. N., Fox, N. C., Beck, J. *et al*. (1996). Incomplete penetrance of familial Alzheimer's disease in a pedigree with a novel presenilin-1 gene mutation. *Lancet* **347**, 1560.

Saitoh, T., Sundsmo, M. P., Roch, J. *et al*. (1989). Secreted form of amyloid β protein precursor is involved in the growth regulation of fibroblasts. *Cell (Cambridge, Mass.)* **58**, 615–622.

Salbaum, J. M., Weidemann, A., Lemaire, H. G. *et al*. (1988). The promoter of Alzheimer's disease amyloid A4 precursor gene. *EMBO J*. **7**, 2807–2813.

Sato, N., Hori, O., Yamaguchi, A. *et al*. (1999). A novel presenilin-2 splice variant in human Alzheimer's disease brain tissue. *J. Neurochem*. **72**, 2498–2505.

Sato, S., Kamino, K., Miki, T. *et al*. (1998). Splicing mutation of presenilin-1 gene for early-onset familial Alzheimer's disease. *Hum. Mutation Suppl.* **1**, S91–S94.

Schellenberg, G. D., Bird, T. D., Wijsman, E. M. *et al*. (1992). Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome 14. *Science* **258**, 668–671.

Scheuner, D., Eckman, C., Jensen, M. *et al*. (1996). Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat. Med*. **2**, 864–870.

Schubert, D., Jin, L-W., Saitoh, T. *et al*. (1989). The regulation of amyloid β protein precursor secretion and its modulatory role in cell adhesion. *Neuron* **3**, 689–694.

Selkoe, D. J. (1991). The molecular pathology of Alzheimer's disease. *Neuron* **6**, 487–498.

Selkoe, D. J. (1998). The cell biology of beta-amyloid precursor protein and presenilin in Alzheimer's disease. *Trends Cell Biol*. **8**, 447–453.

Seubert, P., Vigo-Pelfrey, C., and Esch, F. S. (1992). Isolation and quantitation of soluble Alzheimer's β-peptide from biological fluids. *Nature (London)* **359**, 325–327.

Shen, J., Bronson, R. T., Chen, D. F. *et al*. (1997). Skeletal and CNS defects in presesilin-1-deficient mice. *Cell (Cambridge, Mass.)* **89**, 629–639.

Sherrington, R., Rogaev, E. I., Liang, Y. *et al*. (1995). Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature (London)* **375**, 754–760.

Sheu, K. F., Brown, A. M., Haroutunian, V. *et al*. (1999). Modulation by DLST of the genetic risk of Alzheimer's disease in a very elderly population. *Ann. Neurol*. **45**, 48–53.

Shoji, M., Golde, T., Ghiso, J. *et al*. (1992). Production of the Alzheimer amyloid β protein by normal proteolytic processing. *Science* **258**, 126–129.

Singleton, A. B., Smith, G., Gibson, A. M. *et al*. (1998). No association between the K variant of the butyrylcholinesterase gene and pathologically confirmed Alzheimer's disease. *Hum. Mol. Genet*. **7**, 937–939.

Sinha, S., Anderson, J. P., Barbour, R. *et al*. (1999). Purification and cloning of amyloid precursor protein beta-secretase from human brain. *Nature (London)* **402**, 537–540.

Skoog, I., Wallin, A., Fredman, P. *et al*. (1998). A population study on blood-brain barrier function in 85-year-olds: Relation to Alzheimer's disease and vascular dementia. *Neurology* **50**, 966–971.

Skovronsky, D. M., Moore, D. B., Milla, M. E. *et al*. (2000). Protein kinase C-dependent α-secretase competes with β-secretase for cleavage of amyloid-beta precursor protein in the trans-Golgi network. *J. Biol. Chem*. **275**, 2568–2575.

Small, D. H. (1994). A heparin binding domain in the amyloid protein precursor of Alzheimer's disease is involved in the regulation of neurite outgrowth. *J. Neurosci*. **14**, 2117–2127.

Smith, R. P., Higuchi, D. A., and Broze, G. J. (1990). Platelet coagulation factor XIa-inhibitor, a form of Alzheimer amyloid precursor protein. *Science* **248**, 1126–1128.

Sola, C., Mengod, G., Probst, A. *et al*. (1993). Differential regional and cellular distribution of β-amyloid precursor protein messenger RNAs containing and lacking the Kunitz protease inhibitor domain in the brain of the human, rat and mouse. *Neuroscience* **53**, 267–295.

Sorbi, S., Nacmias, B., Tedde, A. *et al*. (1997). Presenilin-1 gene intronic polymorphism in sporadic and familial Alzheimer's disease. *Neurosci. Lett*. **222**, 132–134.

Spillantini, M. G., Murrell, J. R., Goedert, M. *et al*. (1998). Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. *Proc. Natl. Acad. Sci. U.S.A*. **95**, 7737–7741.

Sprecher, C. A., Grant, F. J., Grimm, G. *et al*. (1993). Molecular cloning of the cDNA for a human amyloid precursor protein homolog: Evidence for a multigene family. *Biochemistry* **32**, 4481–4486.

Stern, Y., Alexander, G. E., Prohovnik, I. *et al*. (1992). Inverse relationship between education and parietotemporal perfusion deficit in Alzheimer's disease. *Ann. Neurol*. **32**, 371–375.

Struble, R. G., Powers, R. E., Casanova, M. F. *et al*. (1987). Neuropeptidergic systems in plaques of Alzheimer's disease. *J. Neuropathol. Exp. Neurol*. **46**, 567–584.

Sugiyama, N., SUZUKI, K., Matsumura, T. *et al*. (1999). A novel missense mutation (G209R) in exon 8 of the presenilin 1 gene in a Japanese family with presenile familial Alzheimer's disease. *Hum. Mutation* **14**, 90.

Taddei, K., Kwok, J. B., Kril, J. J. *et al*. (1998). Two novel presenilin-1 mutations (Ser169Leu and Pro436Gln) associated with very early onset Alzheimer's disease. *NeuroReport* **9**, 3335–3339.

Takano, T., Sahara, N., Yamanouchi, Y. *et al*. (1997). Assignment of Alzheimer's presenilin-2 (PS-2) gene to 1q42.1 by fluorescence in situ hybridization. *Neurosci. Lett*. **221**, 205–207.

Talbot, C. J., Lendon, C., Craddock, N. *et al*. (1994). Protection against Alzheimer's disease with APOE ϵ2. *Lancet* **343**, 1432–1433.

Talbot, C. J., Houlden, H., Craddock, N. *et al*. (1996). Polymorphism in AACT gene may lower age of onset of Alzheimer's disease. *NeuroReport* **7**, 534–536.

Tanzi, R. E., McClatchey, A. I., Lamperti, E. D. *et al*. (1988). Protease inhibitor domain encoded by an amyloid protein precursor mRNA associtated with Alzheimer disease. *Nature (London)* **331**, 528–530.

Tekirian, T. L., Saido, T. C., Markesbery, W. R. *et al*. (1998). N-terminal heterogeneity of parenchymal and cerebrovascular Abeta deposits. *J. Neuropathol. Exp. Neurol*. **57**, 76–94.

Thinakaran, G., Borchelt, D. R., Lee, M. K. *et al*. (1996). Endoproteolysis of presenilin 1 and accumulation of processed derivatives in vivo. *Neuron* **17**, 181–190.

Tilley, L., Morgan, K., Grainger, J. *et al*. (1999). Evaluation of polymorphisms in the presenilin-1 gene and the butyrylcholinesterase gene as risk factors in sporadic Alzheimer's disease. *Eur. J. Hum. Genet*. **7**, 659–663.

Tolnay, M., and Probst, A. (1999). Tau protein pathology in Alzheimer's disease and related disorders. *Neuropathol. Appl. Neurobiol*. **25**, 171–187.

Tomita, T., Maruyama, K., Saido, T. C. *et al*. (1997). The presenilin 2 mutation (N141I) linked to familial Alzheimer disease (Volga German families) increases the secretion of amyloid beta protein ending at the 42nd (or 43rd) residue. *Proc. Natl. Acad. Sci. U.S.A*. **94**, 2025–2030.

Tonder, N., Johansen, F. F., Frederickson, C. J. *et al*. (1990). Possible role of zinc in the selective degeneration of dentate hilar neurons after cerebral ischemia in the adult rat. *Neurosci. Lett*. **109**, 247–252.

Tooyama, I., Kawamata, T., Akiyama, H. *et al*. (1993). Immunohistochemical study of a α2-macroglobulin receptor in Alzheimer and control postmortem human brain. *Mol. Chem. Neuropathol*. **18**, 153–160.

Tysoe, C., Whittaker, J., Cairns, N. J. *et al*. (1997). Presenilin-1 intron 8 polymorphism is not associated with autopsy-confirmed late-onset Alzheimer's disease. *Neurosci. Lett*. **222**, 68–69.

Tysoe, C., Whittaker, J., Xuereb, J. *et al*. (1998). A presenilin-1 truncating mutation is present in two cases with autopsy-confirmed early-onset Alzheimer disease. *Am. J. Hum. Genet*. **62**, 70–76.

Van Nostrand, W. E., Schmaier, A. H., Farrow, J. S. *et al*. (1990). Protease Nexin-II (Amyloid β-Protein Precursor): A platelet α-granule protein. *Science* **248**, 745–748.

Vassar, R., Bennett, B. D., Babu-Khan, S. *et al*. (1999). β-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* **286**, 735–741.

Vatassery, G. T., Bauer, T., and Dysken, M. (1999). High doses of vitamin E in the treatment of disorders of the central nervous system in the aged. *Am. J. Clin. Nutr*. **70**, 793–801.

Wasco, W., Gurubhagavatula, S., Paradis, M. D. *et al*. (1993). Isolation and characterization of the human APLP2 gene, encoding a homologue of the Alzheimer's associated β protein precursor. *Nat. Genet*. **5**, 95–100.

Wasco, W., Pettingell, W. P., Jondro, P. D. *et al*. (1995). Familial Alzheimer's chromosome 14 mutations. *Nat. Med*. **1**, 848.

Wavrant-DeVrièze, F., Perez-Tur, J., Lambert, J. C. *et al*. (1997). Association between the low density lipoprotein receptor-

related protein (LRP) and Alzheimer's disease. *Neurosci. Lett*. **227**, 68–70.

Weidemann, A., Konig, G., Bunke, D. *et al*. (1989). Identification, biogenesis, and localization of precursors of Alzheimer's disease A4 protein. *Cell (Cambridge, Mass.)* **57**, 115–126.

Weingarten, M. D., Lockwood, A. H., Hwo, S. Y. *et al*. (1975). A protein factor essential for microtubule assembly. *Proc. Natl. Acad. Sci. U.S.A*. **72**, 1858–1862.

Wild-Bode, C., Yamazaki, T., Capell, A. *et al*. (1997). Intracellular generation and accumulation of amyloid beta-peptide terminating at amino acid 42. *J. Biol. Chem*. **272**, 16085–16088.

Wisniewski, T., Ghiso, J., and Frangione, B. (1994). Alzheimer's disease and soluble Aβ. *Neurobiol. Aging* **15**, 143–152.

Wisniewski, T., Dowjat, W. K., Buxbaum, J. D. *et al*. (1998). A novel Polish presenilin-1 mutation (P117L) is associated with familial Alzheimer's disease and leads to death as early as the age of 28 years. *NeuroReport* **9**, 217–221.

Wolfe, M. S., Xia, W., Ostaszewski, B. L. *et al*. (1999). Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. *Nature (London)* **398**, 513–517.

Wragg, M., Hutton, M., and Talbot, C. J. (1996). Genetic association between intronic polymorphism in presenilin-1 gene and late-onset Alzheimer's disease. Alzheimer's Disease Collaborative Group. *Lancet* **347**, 509–512.

Yamamoto, K., Miyoshi, T., Yae, T. *et al*. (1994). The survival of rat cerebral cortical neurons in the presence of trophic APP peptides. *J. Neurobiol*. **25**, 585–594.

Yan, R., Bienkowski, M. J., Shuck, M. E. *et al*. (1999). Membrane-anchored aspartyl protease with Alzheimer's disease β-secretase activity. *Nature (London)* **402**, 533–537.

Yasuda, M., Maeda, K., Ikejiri, Y. *et al*. (1997). A novel missense mutation in the presenilin-1 gene in a familial Alzheimer's disease pedigree with abundant amyloid angiopathy. *Neurosci. Lett*. **232**, 29–32.

Yasuda, M., Maeda, K., Hashimoto, M. *et al*. (1999). A pedigree with a novel presenilin 1 mutation at a residue that is not conserved in presenilin 2. *Arch. Neurol. (Chicago)* **56**, 65–69.

Mark P. Mattson

23

Inflammation, Free Radicals, Glycation, Metabolism and Apoptosis, and Heavy Metals

I. Roles of Cytokines and Inflammation in Alzheimer's Disease

Studies of the roles of the immune system in Alzheimer's disease (AD) have brought forth many intriguing findings. It is very clear that, at least in the end stages of AD, the profile of cellular and molecular alterations in and surrounding amyloid deposits and degenerating neurons resembles an inflammatory response. Microglia are activated, levels of several different inflammatory cytokines [e.g., tumor necrosis factor (TNF) and interleukin (IL)-1β] and proinflammatory cell adhesion proteins are increased, and the complement cascade is activated. Many of these changes are likely a response to neuronal injury and death and, as such, might either protect or endanger the remaining neurons. For example, microglia produce both neurotoxic compounds, such as nitric oxide and excitotoxins, and neuroprotective agents, such as TNF and transforming growth factor (TGF)-β. In addition, the inflammatory process appears to have quite complex effects on enzymatic processing of the amyloid precursor protein, and deposition and clearance of amyloid β-peptide (Aβ). The development of genetic mouse "models" of AD in which amyloid is deposited in the brain reveals some evidence of an inflammatory response, suggesting that amyloid may be an important trigger of inflammation in AD. Interestingly, studies of presenilin-1 mutant mice are revealing alterations in lymphocyte signaling, suggesting that immune alterations may occur prior to, and possibly contribute to, neuronal degeneration in AD. Initial epidemiological data and clinical trials suggested that increased intake of nonsteroidal anti-inflammatory drugs can decrease the risk for AD, although more recent findings suggest that the antioxidant activity of these drugs may underlie their beneficial effects. A very recent and potentially important finding related to the role of the immune system in AD is the observation that immunization of amyloid precursor protein (APP) mutant mice with aggregated Aβ can suppress amyloid deposition in the brain. Although speculative, the latter findings suggest that a vaccine for AD may be possible.

A. Studies of Brain Tissue from AD Patients Reveal Inflammation-like Alterations in the Brain

The first line of evidence suggesting that inflammation-like processes play a role in the pathogenesis of AD comes from analyses of postmortem brain tissue from AD patients. Histological analyses reveal the presence of several different inflammatory mediators, including proteins involved in the complement cascade and cytokines, in association with amyloid plaques and neurofibrillary tangles. Among the earliest evidence for an inflammatory process in association with amyloid deposition and neuronal degeneration in AD was the work of Eikelenboom and Stam (1982), who described the accumulation of IgG and κ and λ light chains in neuritic plaques. The latter study also documented the presence of the complement factors C1q, C3b, C3c, C3d, and C4 in plaques. Their subsequent studies used immunoenzymatic techniques, and specific antibodies against subunits of individual complement components and activated complement products, to demonstrate complement activation in amyloid plaques (Eikelenboom *et al*., 1989). Further analyses indicated that the cascade does not proceed beyond C3 (Veerhuis *et al*., 1995). Double labeling of brain sections from AD and control patients with thioflavin (which binds to amyloid fibrils) and an antibody against the complement protein C1q demonstrated localization of C1q in plaques containing fibrillar Aβ but not in diffuse plaques (Afagh *et al*., 1996). Many neurons in AD brain were C1q positive, whereas microglia and astrocytes associated with C1q-positive plaques were themselves not C1q-positive. Levels of several acute-phase proteins (α_1-antichymotrypsin, ceruloplasmin, and complement proteins), but not others (haptoglobin, transferrin, and C-reactive protein), were increased in serum of AD patients compared to control patients (Giometto *et al*., 1988). Another study revealed increased levels of several acute-phase proteins in amorphous plaques in cerebral cortex, but not in the cerebellum, of patients with AD (Rozemuller *et al*., 1990).

TNFα levels are elevated in microglia associated with amyloid plaques in the brains of AD patients (Dickson *et al*., 1993), and levels of circulating TNFα are also increased in AD patients (Fillit *et al*., 1991), suggesting a systemic acute-phase-like condition. Increased levels of TGF-β1 are associated with a subset of senile plaques in AD, suggesting that it may play a role in cellular responses to, and/or deposition of, Aβ (van der Wal *et al*., 1993). Levels of TGF-β2 are increased in large neurofibrillary tangle-bearing neurons and in astrocytes of patients with sporadic AD, as well as in patients with mutations in presenilin-1 (Flanders *et al*., 1995). Analyses of cerebral cortical tissue samples from AD patients and age-matched control patients documented significant increases in levels of interleukin-6 (IL-6) and α_2-macroglobulin (Bauer *et al*., 1991; Griffin *et al*., 1998), both of which are also increased in the acute-phase response. Levels of the serine protease inhibitor α_1-antichymotrypsin are increased in association with amyloid plaques in brain tissue from AD patients (Abraham *et al*., 1988).

Cyclooxygenase-2 (COX-2) catalyzes the production of prostaglandins from arachidonic acid and is an important target for anti-inflammatory drugs. COX-2 is upregulated in microglia following brain injury and exposure to lipopolysaccharide but, in contrast to peripheral macrophages, is not induced by TNFα, IL-1β, or IL-6 (Bauer *et al*., 1997). The latter findings suggest that microglia are an important source of prostaglandins and therefore likely play a central role in inflammatory responses in the brain. Levels of mRNA encoding COX-2 were reported to be decreased in brain tissue from AD patients (Chang *et al*., 1996). However, subsequent studies showed that levels of COX-2 immunoreactivity are increased in hippocampal pyramidal neurons of AD patients compared to age-matched controls, and the magnitude of the increase correlates with amyloid plaque density (Ho *et al*., 1999). The latter study also showed that cultured neurons from COX-2-overexpressing transgenic mice are more vulnerable to amyloid β-peptide toxicity than neurons from nontransgenic mice, suggesting that the increased levels of COX-2 in AD could contribute to the neurodegenerative process.

Levels of integrins, proteins upregulated in vascular endothelial cells, and other cell types following injury were shown to be associated with amyloid plaques (Eikelenboom *et al*., 1989, 1994). Immunohistochemical studies have shown that levels of the vitronectin receptor, an integrin, are greatly increased in reactive microglia in both gray and white matter in the brains of AD patients (Akiyama *et al*., 1991). The latter study also showed that levels of vitronectin are increased in neurofibrillary tangle-bearing neurons and senile plaques, suggesting a role for vitronectin in the phagocytosis of damaged cells by microglia in AD. In addition, levels of leukocyte adhesion molecules of the LFA-1 family are increased in microglial cells surrounding amyloid plaques (Rozemuller *et al*., 1989).

Finally, although the vast majority of data obtained in analyses of AD brain tissue suggest that inflammatory cascades involve cells intrinsic to the brain parenchyma, some data suggest a role for peripheral immune cells. For example, Itagaki *et al*. (1988) reported evidence for the presence of leucocyte common antigen positive cells and T-cytotoxic suppressor cells in AD brain tissue.

B. Experimental Studies That Elucidate the Cellular and Molecular Basis of Inflammatory Cascades in AD

By analogy with other tissues and studies of brain injury, it is very likely that a major component of the inflammation-like changes evident in analyses of AD brain tissue is the result of cellular responses to neuronal degeneration and death. Increased levels of oxidative stress (see Section II) may also induce glial activation and cytokine cascades. Experimental cell culture and animal models of AD have shown that Aβ can induce many inflammation-like alterations in glial cells and neurons and that inflammatory mediators can interact with Aβ, and other AD-relevant neurotoxic agents, to either promote or suppress neuronal degeneration. It has been shown that Aβ activates the classical complement pathway (Rogers *et al*., 1992) by interacting with a site within the collagen-like domain of C1q, with Aβ 1–42 being more effective than 1–40 (Jiang *et al*., 1994). The major site at which Aβ 1–42 binds to C1q is within the collagen-like amino acids 14–26 of C1q, a region of interaction of other activators of C1q. Coincubation of Aβ with C1q results in enhanced peptide aggregation (Webster *et al*., 1994). Aβ activates microglia and greatly potentiates microglial activation by interferon-γ (Meda *et al*., 1995). Microglial activation by Aβ may involve binding of receptors for advanced glycation end products expressed in microglia (Mattson and Rydel, 1996). Oxyradicals and excitotoxins produced by activated microglia may promote neuronal degeneration. Studies of transgenic mice expressing mutations in amyloid precursor protein linked to early-onset AD have provided evidence for microglial and astrocyte activation in association with amyloid deposits (Hsiao *et al*., 1996; Chen *et al*., 1998). Thus, data ranging from cell culture studies to animal models to AD patients strongly suggest that amyloid plays a major role in inducing glial activation and inflammatory cytokine cascades (Fig. 23.1).

However, several cytokines produced in inflammatory responses may serve neuroprotective functions. Primary hippocampal neurons treated with TNFα exhibit increased resistance to cell death induced by excitotoxic and oxidative insults, and exposure to Aβ (Cheng *et al*., 1994; Barger *et al*., 1995). Studies of mice lacking TNFα receptors suggest a similar neuroprotective role for TNFα *in vivo* (Bruce *et al*., 1996). The signal transduction pathway that mediates the antiapoptotic and antiexcitotoxic effects of TNFα involves activation of the transcription factor NF-κB, which induces the expression of several neuroprotective proteins, including manganese superoxide dismutase and Bcl-2 (Mattson *et al*., 1997a, 2000; Yu *et al*., 1999). Pretreatment of cultured cortical neurons with TGFβ1 confers resistance to excitotoxicity and Aβ toxicity (Prehn *et al*., 1993; Chao *et al*., 1994). Because of these *in vitro* findings and because TGFβ is localized to sites where neuronal degeneration is occurring in AD brains (see earlier discussion), it is possible that TGFβ serves an important neuroprotective function. However, results generated in transgenic mice clearly indicate that chronic overproduction of TGFβ1 may also promote amyloidogenesis and neurodegeneration through its stimulatory effects on the deposition of extracellular matrix proteins (Finch *et al*., 1993; Wyss-Coray *et al*., 1995).

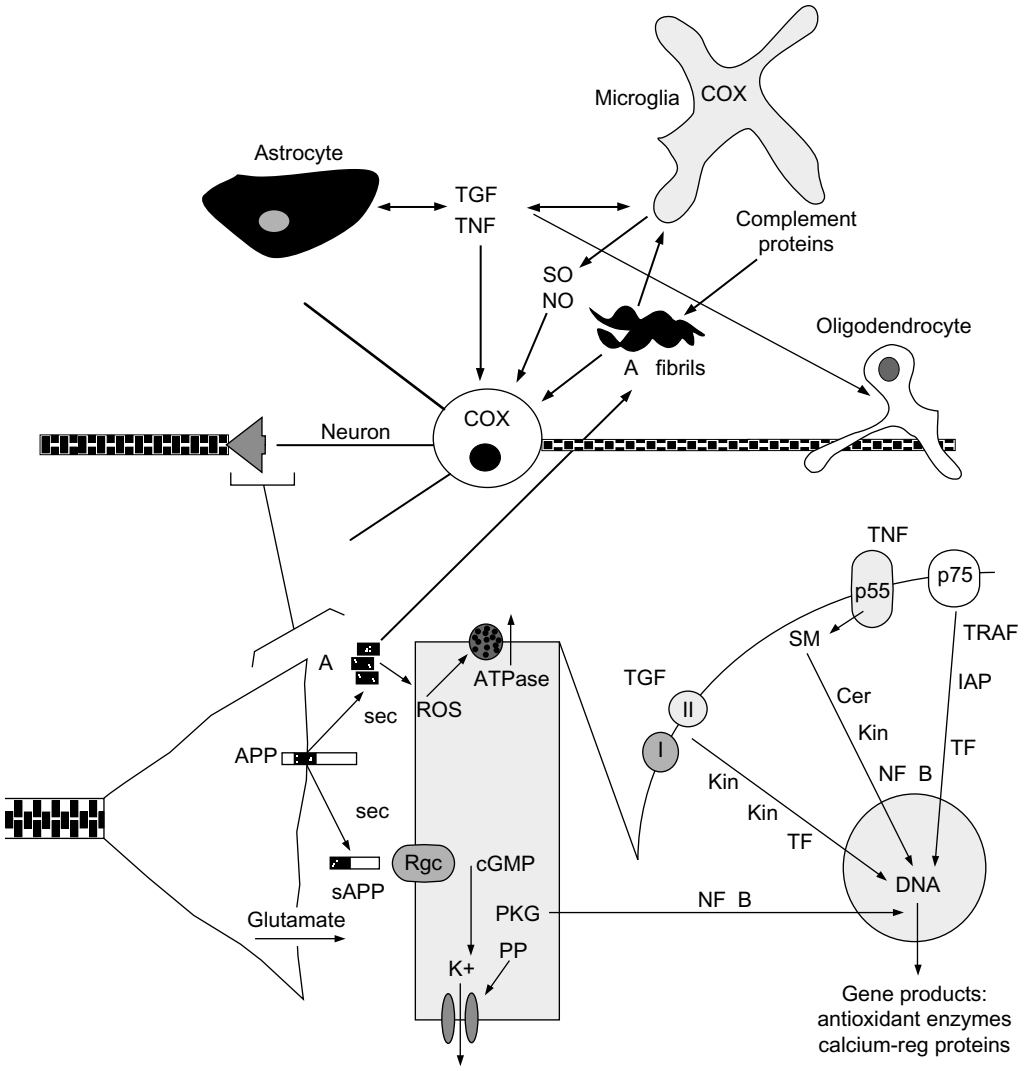

FIG. 23.1. Working model of inflammatory mechanisms that either promote or suppress neuronal degeneration. Neuronal injury and deposition of amyloid β-peptide (Aβ) are likely to trigger inflammatory cascades in AD. Aβ arises from the β-amyloid precursor protein (βAPP); βAPP is transported axonally and may accumulate in membranes of presynaptic terminals (left). Aβ can form aggregates, which induce oxidative stress in neurons and microglial cells, and activate microglia. Alternative enzymatic cleavages of βAPP by α-secretase result in the release of sAPPα from neurons. sAPPα activates a putative receptor guanylate cyclase (GC) in neurons, resulting in cGMP production and activation of cGMP-dependent protein kinase, which then promotes the activation of K^+ channels. cGMP-dependent protein kinase may also induce NF-κB activation, resulting in increased transcription of genes encoding neuroprotective proteins. Activated microglia produce superoxide (via activity of cyclooxygenase; COX) and nitric oxide, which may damage neurons. Cytokines such as TGFβ and TNFα are produced by activated microglia and astrocytes. TGFβ acts on astrocytes and microglia in an autocrine manner to inhibit activation and proliferation, whereas TNFα promotes activation and proliferation of both glial cell types. TGFβ inhibits the proliferation of oligodendrocytes, whereas TNFα may damage oligodendrocytes. Both TGFβ and TNFα increase resistance of neurons to oxidative and excitotoxic insults and may thereby serve a neuroprotective role in AD. TGFβ and TNFα activate specific cell surface receptors, resulting in an intracellular transduction cascade involving kinases (Kin) and transcription factors (TF).

A role for oxygenases in the neurodegenerative process in AD is suggested by studies showing that lipoxygenase inhibitors such as nordihydroguaiaretic acid can protect cultured hippocampal neurons against Aβ toxicity (Goodman *et al*., 1994) and that indomethacin (a cyclooxygenase inhibitor) can suppress microglial activation after infusion of Aβ into the brains of adult rats (Netland *et al*., 1998). However, one concern with such experimental studies, and with the epidemiological and clinical data described later, is that many of the oxygenase inhibitors possess intrinsic antioxidant activity above and beyond their ability to inhibit the oxygenases (Goodman *et al*., 1994). Thus, the beneficial effects of anti-inflammatory drugs may result from their antioxidant actions rather than (or in addition to) their ability to suppress prostanoid production.

Although it is very clear that inflammatory processes play an important role in the pathogenesis of AD after amyloid deposition and neuronal degeneration, it has been suggested that immune alterations may occur prior to amyloid deposition and

TABLE 23.1 Alterations in Lymphocyte Signaling in Presenilin-1 Mutant Knockin Mice

Parameter	Alteration in splenocytes from PS1 mutant mice
Calcium levels	Increased spontaneous elevation of $[Ca^{2+}]_i$
	Increased calcium responses to different stimuli
Mitochondrial function	Impaired mitochondrial function and oxyradical levels
Apoptosis	Enhanced spontaneous apoptosis
	Enhanced induced apoptosis
Proliferation	Decreased response to concanavalin A

neuronal degeneration. Mutations in the gene encoding presenilin-1 are responsible for many cases of early onset autosomal dominant AD (Mattson *et al*., 1998). Studies of presenilin-1 mutant knockin mice have shown that an important consequence of the mutations is that calcium regulation is altered in neurons; specifically, presenilin-1 mutations result in enhanced calcium release from ryanodine-sensitive endoplasmic reticulum calcium stores (Guo *et al*., 1999a,b,c). The perturbed calcium regulation endangers neurons such that they are more vulnerable to Aβ, excitotoxicity, and apoptosis. Earlier studies of lymphocytes from patients with sporadic AD and age-matched control patients revealed that calcium signaling is altered in these peripheral immune cells in AD (Eckert *et al*., 1996). Studies have shown that splenocytes from presenilin-1 mutant mice exhibit altered proliferative responses to mitogens, perturbed calcium homeostasis, and increased vulnerability to apoptosis (Table 23.1). The latter alterations were age dependent and occurred prior to any evidence for neurodegenerative changes in the brain. These findings suggest the possibility that alterations in the immune system precede, and possibly contribute to, the neurodegenerative process in AD.

Another type of alteration in AD patients that may contribute to neuronal degeneration and inflammatory processes is dysregulation of the hypothalamus–pituitary–adrenal axis, which manifests increased levels of glucocorticoids and an abnormal dexamethasone suppression response (Raskind *et al*., 1982). Interestingly, studies of amyloid precursor protein mutant transgenic mice have revealed perturbed stress responses associated with hypoglycemia (Pedersen *et al*., 1999). APP mutant mice exhibited severe hypoglycemia and death following food restriction and sustained elevations of plasma glucocorticoid levels and hypoglycemia following restraint stress. These abnormalities were evident in relatively young mice prior to the overt deposition of Aβ, but the presence of diffuse accumulations of Aβ in the hypothalamus suggests a role for soluble forms of the peptide in dysregulation of hypothalamus–pituitary–adrenal function. In light of the fact that inflammatory processes can be modified by the stress response (Elenkov *et al*., 1999), alterations in this neuroendocrine system might modify the disease process in AD patients.

C. Epidemiological and Clinical Data Supporting a Role for Inflammation in AD

Some of the first data implicating an inflammatory response in the pathogenesis of neuronal degeneration and cognitive dysfunction in AD came from epidemiological studies. The results of 15 different studies that examined the relationship of glucocoricoid and nonsteroidal anti-inflammatory drug use and onset or progression of AD have been reviewed (Breitner, 1996). Collectively, epidemiological data make quite a compelling case that the risk for AD is reduced in individuals with a history of chronic treatment with nonsteroidal anti-inflammatory drugs. The possibility that anti-inflammatory drugs might slow the progression of AD is supported by findings of a double-blind, placebo-controlled study in which 100–150 mg of indomethacin/day reduced cognitive decline in patients with mild to moderate AD (Rogers *et al*., 1993). However, a more recent trial of glucocorticoids proved negative (Alzheimer's Consortium Study). The latter result is perhaps not unexpected in light of evidence that glucocorticoids can endanger neurons and increase their vulnerability to Aβ toxicity (Goodman *et al*., 1996). Nevertheless, when taken together with the biochemical, histological, and experimental findings described earlier, epidemiological and clinical data suggest several obvious preventative and therapeutic strategies for AD. The use of nonsteroidal anti-inflammatory drugs, which also possess intrinsic antioxidant actions, seems particularly promising.

II. Free Radicals and the Pathogenesis of AD

Increased oxyradical-mediated damage to cellular proteins, lipids, and nucleic acids occurs in several different age-related neurodegenerative disorders, including AD. Studies of postmortem brain tissue from AD patients have documented increased levels of markers of oxidative stress in association with the two major histopathological lesions: neurofibrillary tangles and deposits of Aβ. Analyses of experimental cell culture and animal models of AD have provided evidence that Aβ induces oxidative stress in neurons, resulting in perturbed energy and ion homeostasis and increased vulnerability to apoptosis and excitotoxicity. Genetic mutations in the APP and presenilins may promote oxidative stress by altering APP processing and disrupting endoplasmic reticulum calcium homeostasis. Experimental and epidemiological data suggest that manipulations that reduce levels of oxidative stress in the brain, such as dietary restriction and administration of vitamin E and estrogen, may reduce risk for AD.

A. Cellular Oxidative Stress Is Increased in Brain Tissue from AD Patients

There is no question that, as in other age-related degenerative diseases (e.g., cardiovascular disease, type 2 diabetes, and cancer), increased levels of cellular oxidative stress play a major role in AD (Mattson, 1997; Markesbery, 1997). Analyses of postmortem tissue from AD patients have revealed increased levels of protein oxidation, membrane lipid peroxidation, and oxidative damage to DNA in association with neuritic plaques and neurofibrillary tangles (C. D. Smith *et al*., 1991; Moccoci *et al*., 1994; Good *et al*., 1996; M. A. Smith *et al*., 1997a; Fu *et al*., 1998). Levels of lipid peroxidation products, including the toxic aldehyde 4-hydroxynonenal, are increased in the cerebrospinal fluid of AD patients relative

to age-matched control patients (Lovell *et al*., 1997), suggesting a widespread increase in cellular oxidative stress in the brain. Further evidence of ongoing oxidative stress in AD brain comes from studies showing that levels of antioxidant enzymes such as catalase, Mn-SOD, and Cu/Zn-SOD are altered in vulnerable brain regions in AD patients compared to corresponding tissues from age-matched control patients (Bruce *et al*., 1997).

B. Experimental Evidence Linking Amyloid Deposition to Oxidative Stress and Neuronal Degeneration in AD

One line of evidence suggesting that increased oxidative stress is a relatively early and pivotal event in the neurodegenerative process in AD comes from analyses of the effects of APP metabolites on cultured neurons (Mattson, 1997, 1998). APP is an axonally transported integral membrane protein, the source of the 40–42 amino acid Aβ that is the major component of amyloid ''plaques'' in the brains of AD patients. Aβ is liberated from APP via two proteolytic cleavages: an initial cleavage at the N terminus of Aβ leaves behind a C-terminal APP fragment, which is then endocytosed and further cleaved at the C terminus of Aβ (Fig. 23.2). Aβ is then released from cells and accumulates in extracellular compartments, initially as diffuse nonfibrillary deposits, which may later transform into classic amyloid fibrils with a β-pleated sheet structure.

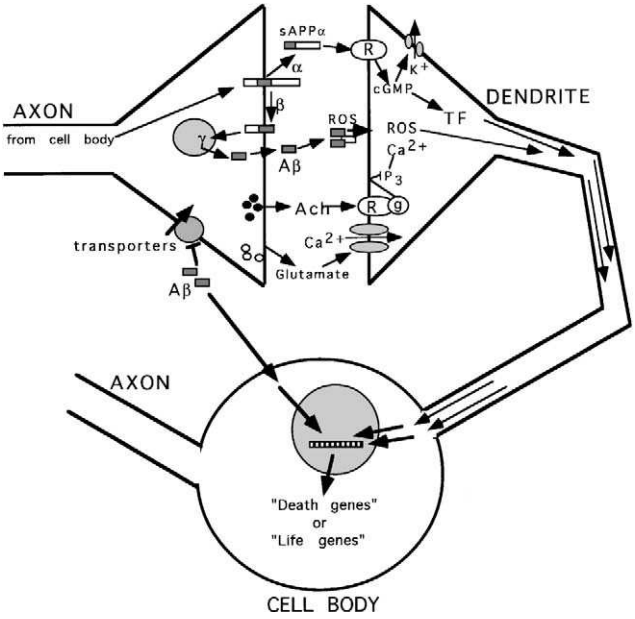

FIG. 23.2. Amyloid precursor protein metabolism and its involvement in synaptic dysfunction and neuronal degeneration in AD. APP is cleaved proteolytically at at least four different sites. α-Secretase cleaves between amino acids 612 and 613, which lies within the Aβ sequence (this cleavage releases sAPPα from the cell surface). β-Secretase cleaves at the N terminus of Aβ (between amino acids 596 and 597), releasing sAPPβ from the cell surface and leaving a C-terminal membrane-associated fragment containing intact Aβ. γ-Secretase cleaves at the C terminus of Aβ at at least two different sites, resulting in the release of intact Aβ1–40 or Aβ1–42. In AD, APP processing is altered in a manner that increases levels of neurotoxic forms of Aβ and decreases levels of neuroprotective sAPPα. See text and Mattson (1997) for further details.

In general, degenerated neurons are associated with fibrillar Aβ deposits, but not with diffuse deposits. Exposure of cultured hippocampal and cortical neurons to Aβ can increase their vulnerability to excitotoxic and metabolic insults (Mattson *et al*., 1992; Cheng and Mattson, 1992; Mark *et al*., 1995b) and can induce apoptosis (Mattson and Begley, 1998; and see Section IV). The mechanism whereby Aβ damages neurons involves, as an initial event, induction of oxidative stress, specifically membrane lipid peroxidation (Goodman and Mattson, 1994; Butterfield *et al*., 1994; Mark *et al*., 1997a). The chemistry underlying the ability of Aβ to induce membrane lipid peroxidation is not established, but appears to occur during the process of transition from soluble to aggregated form and may involve free radical generation by the peptide itself (Hensley *et al*., 1994), possibly catalyzed by metal (Fe^{2+} and or Cu^{2+}) ions (see Section V). Lipid peroxidation is necessary for the neurotoxic actions of Aβ because antioxidants that suppress membrane lipid peroxidation (e.g., vitamin E, propyl gallate, and uric acid) or detoxify 4-hydroxynonenal (e.g., glutathione) protect neurons against Aβ-induced apoptosis and excitotoxicity (Goodman and Mattson, 1994; Mark *et al*., 1995b, 1997a; Keller *et al*., 1998a).

Further studies have elucidated the events subsequent to membrane lipid peroxidation that promote neuronal degeneration (Fig. 23.3). Lipid peroxidation results in production of the aldehyde 4-hydroxynonenal, which, in turn, covalently modifies many different cellular proteins on cysteine, lysine, and histidine residues. Four proteins modified by 4-hydroxynonenal are plasma membrane transporters: the Na^+/K^+-ATPase, the Ca^{2+}-ATPase, the glucose transporter GLUT-3, and the glutamate transporter GLT-1 (Keller *et al*., 1997a; Mark *et al*., 1997a,b; Blanc *et al*., 1998). These transporters play critical roles in the maintenance of cellular ion and energy homeostasis. Accordingly, their modification by 4-hydroxynonenal promotes membrane depolarization and energy (ATP) depletion, thereby rendering neurons vulnerable to excitotoxic and apoptotic cell death. This oxidative stress-mediated cascade of events occurring at the level of the plasma membrane ultimately leads to cellular calcium overload, mitochondrial oxyradical production and dysfunction, and activation of proteases (e.g., caspases and calpains) that contribute to the final destruction of the neuron (Guo *et al*., 1997, 1999a; Keller *et al*., 1998a; Chan and Mattson, 1999). It should also be noted that subtoxic levels of oxidative stress induced by Aβ can disrupt neuronal signaling pathways and may thereby contribute to cognitive dysfunction. For example, Aβ can impair coupling of muscarinic acetylcholine receptors to the GTP-binding protein Gq11 by a mechanism involving membrane lipid peroxidation and 4-hydroxynonenal production (Kelly *et al*., 1996; Blanc *et al*., 1997).

A decrease in the levels of neurotrophic factors and/or impairment of neurotrophic factor signaling pathways has been proposed to contribute to the neurodegenerative process in AD and related disorders (Mattson and Lindvall, 1997). Cell culture studies have directly demonstrated that several different neurotrophic factors, including basic fibroblast growth factor, brain-derived neurotrophic factor, and activity-dependent neurotrophic factor, can protect neurons against oxidative insults, including exposure to Aβ (Mattson *et al*., 1993c, 1995). Interestingly, the secreted form of APP (sAPPα; produced

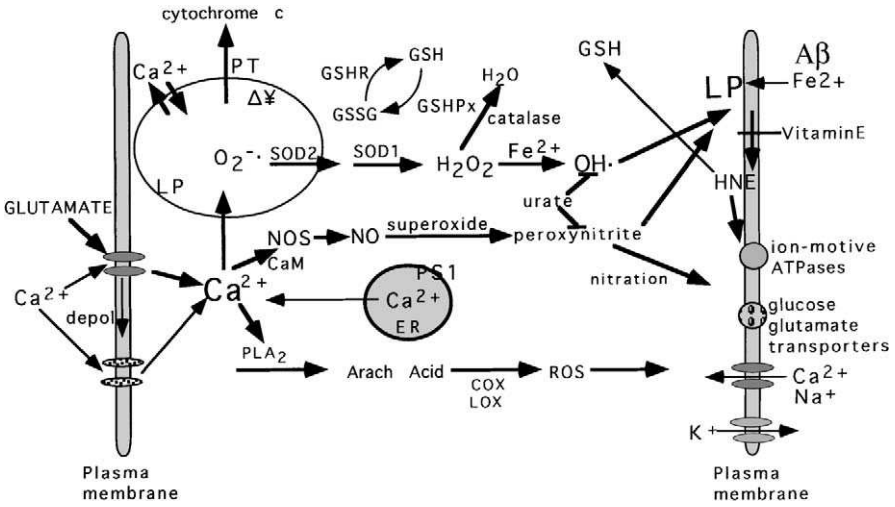

FIG. 23.3. Sources of reactive oxygen species in the brain, mechanisms for their detoxification, and mechanisms of oxidative disruption of neuronal ion homeostasis. The preeminent subcellular source of oxyradicals is mitochondria, wherein $O_2^-\cdot$ is generated during the electron transport process. Superoxide dismutases (SOD) convert $O_2^-\cdot$ to H_2O_2, which, in the presence of Fe^{2+}, generates $OH\cdot$. $O_2^-\cdot$ can also interact with nitric oxide (NO) to form peroxynitrite. Both $OH\cdot$ and peroxynitrite induce membrane lipid peroxidation (LP), which may occur in the plasma membrane, mitochondrial membranes, and endoplasmic reticulum (ER) membranes. Additionally, exogenous agents such as amyloid β-peptide (Aβ) can induce LP. LP liberates 4-hydroxynonenal (HNE), which binds to membrane transporters and ion channels, thereby altering their activities. Impairment of the Na^+/K^+-ATPase, glucose transporter, and glutamate transporters results in membrane depolarization and excessive activation of glutamate receptors, resulting in excitotoxicity. LP also perturbs ion homeostasis in ER and mitochondria, thereby compromising their important Ca^{2+} sequestration functions. It should be noted that not only does MLP lead to an elevation of $[Ca^{2+}]_i$ but, conversely, elevation of $[Ca^{2+}]_i$ promotes MLP by inducing NO and $O_2^-\cdot$ production, as well as by activation of phospholipases, resulting in production of arachidonic acid, which is then acted on by cyclooxygenases (COX) and lipoxygenases (LOX) with resulant generation of reactive oxygen species (ROS). PS1, presenilin-1. Modified from Mattson (1998a).

via anzymatic cleavage of APP in the middle of Aβ) potently protects neurons against Aβ toxicity by a mechanism involving reduced levels of oxidative stress (Goodman and Mattson, 1994; Furukawa *et al*., 1996). Levels of sAPPα have been reported to be decreased in the cerebrospinal fluid of AD patients (Palmert *et al*., 1990) and in neurons expressing presenilin-1 mutations (Ancolio *et al*., 1997). Thus, a decrease in the trophic support of sAPPα may contribute to the increased oxidative stress documented in the brains of AD patients.

C. Role of Oxidative Stress in the Pathogenic Actions of Genetic Aberrancies Linked to Early-Onset AD

Some cases of AD are inherited in an autosomal dominant manner and have an early age of onset (30–50 years of age). Identification of mutations in APP and presenilin-1 as being causally linked to some such cases of familial AD has proven invaluable in producing novel experimental models of AD and in elucidating the molecular and biochemical underpinnings of the neurodegenerative process. Patients with APP and presenilin mutations exhibit brain pathology essentially indistinguishable from that of patients with sporadic AD. Although not yet confirmed, one would presume that (as is the case in sporadic AD patients) oxidative cellular damage is also increased in the brains of familial AD patients. Studies of transgenic mice expressing AD-linked APP and presenilin mutations support a central role for oxidative stress in the pathogenesis of AD. For example, increased levels of membrane lipid peroxidation and protein oxidation occur in association with age-related amyloid accumulation in the brains of APP mutant transgenic mice (Smith *et al*., 1998a). Analyses of cortical tissue from knockin mice expressing the M146V presenile-1 mutation have shown that levels of protein oxidation are increased in older presenile-1 mutant mice (18 months old) compared to age-matched control mice (Fig. 23.4) The mechanism whereby APP mutations lead to increased oxidative stress most likely

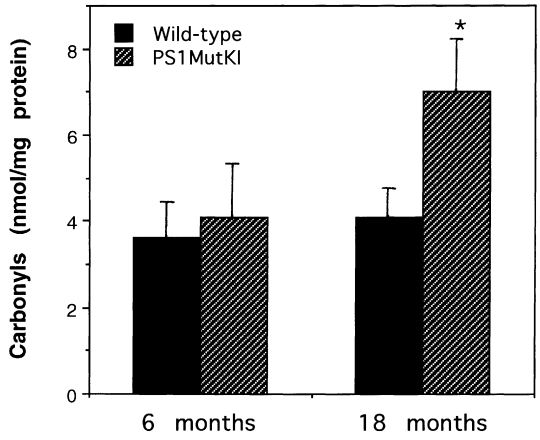

FIG. 23.4. Evidence that levels of protein oxidation are increased in aged PS1 mutant knockin mice. Levels of protein carbonyls were quantified in homogenates of cerebral cortex from 6- and 18-month-old wild-type and PS1 mutant knockin mice. Values are the mean $\pm$SD (four mice/group). $^*p < 0.01$ compared to value for 18-month-old wild-type mice.

involves the altered proteolytic processing scenario described earlier, which results in increased production of neurotoxic forms of Aβ and decreased production of neuroprotective sAPPα (Mattson, 1997).

A series of studies of cultured neurons have elucidated the mechanism whereby presenilin-1 mutations promote oxidative stress and neuronal degeneration. PC12 cells overexpressing mutant presenilin-1 exhibit increased vulnerability to apoptosis, which is associated with an increased production of hydrogen peroxide following exposure to Aβ (Guo *et al*., 1996, 1997). The latter studies further showed that antioxidants can counteract the proapoptotic action of the presenilin-1 mutations. PC12 cells expressing mutant presenilin-1 also exhibit increased mitochondrial oxyradical production following exposure to mitochondrial toxins (Keller *et al*., 1998b). The enhanced oxyradical production in cells expressing mutant presenilin-1 results from a disturbance in cellular calcium homeostasis. Specifically, presenilin-1 mutations result in enhanced calcium release from IP_3- and ryanodine-sensitive endoplasmic reticulum stores (Guo *et al*., 1996, 1997). Accordingly, treatment of neurons expressing mutant presenilin-1 with dantrolene, an agent that blocks calcium release from the endoplasmic reticulum, protects them against the endangering effect of the mutation (Guo *et al*., 1997, 1999a). Hippocampal and cortical neurons in presenilin-1 mutant knockin mice exhibit increased vulnerabilty to excitotoxic and ischemic injury, two insults that damage neurons, in part, via free radical production (Guo *et al*., 1999a; Mattson *et al*., 2000). Analyses of calcium regulation and free radical metabolism in hippocampal neurons from presenilin-1 mutant mice have shown that perturbed calcium homeostasis in the endoplasmic reticulum plays a central role in promoting oxidative stress (Guo *et al*., 1999a,b,c).

Apolipoprotein E, well known for its role in cholesterol metabolism and the pathogenesis of cardiovascular disease, also plays a role in modifying risk for AD. Thus, individuals with the ApoE4 isoform of apolipoprotein E are at increased risk for AD (Strittmatter and Roses, 1996) and exhibit increased levels of markers of oxidative stress (e.g., levels of 4-hydroxynonenal associated with neurofibrillary tangles) (Montine *et al*., 1997) than individuals expressing a different isoform (ApoE2 or ApoE3). Studies have identified a mechanism whereby ApoE4 promotes, whereas ApoE3 and ApoE2 suppress oxidative damage to neurons. Thus, the ApoE isoforms differ in the amount of 4-hydroxynonenal they can bind with ApoE2 binding more than ApoE3, which, in turn, binds more than ApoE4 (Pedersen *et al*., 2000). The latter study further showed that ApoE2 and ApoE3 were much more effective in protecting hippocampal neurons against Aβ toxicity than ApoE4. Thus, the apolipoprotein E genotype appears to affect risk for AD by modifying the antioxidant capacity of the protein.

D. Epidemiological and Experimental Data Suggest That Dietary Restriction and Antioxidants May Reduce Risk for AD

Epidemiological and clinical data support the oxidative stress hypothesis of AD. Increased dietary supplementation with vitamins E and C are associated with reduced risk for AD (Morris *et al*., 1998). In addition, estrogen replacement therapy in postmenopausal women decreases their risk for AD greatly (Tang *et al*., 1996). Experimental studies have shown that estrogen exhibits antioxidant activity and that its ability to suppress membrane lipid peroxidation accounts for its ability to protect neurons against AD-relevant insults, including exposure to Aβ and Fe^{2+} (Goodman *et al*., 1996; Keller and Mattson, 1997). A clinical trial of vitamin E in patients with mild AD resulted in a significant slowing of the progression of the disease (Sano *et al*., 1997). Moreover, the benefit of anti-inflammatory drugs in AD patients may result from their well-established antioxidant activity (see Section I).

Reducing the calorie intake of laboratory rats and mice increases their life span dramatically and reduces the development of age-related cancers and deficits in immune function (Sohal and Weindruch, 1996). Although benefits of dietary restriction on the cardiovascular, immune, and endocrine systems have been demonstrated, its effects on the nervous system are largely unknown. Data suggest that dietary restriction may slow age-related molecular changes in the brain and attenuate age-related deficits in learning and memory ability and motor function in rodents (Finch and Morgan, 1997). In a prospective study of a multiethnic cohort in New York City, it was found that those with the lowest daily calorie intakes had the lowest risk for AD (Mayeux *et al*., 1999). A similarly designed study showed that persons with a low calorie intake have reduced risk for Parkinson's disease (Logroscino *et al*., 1996).

In experimental studies relevant to AD, maintenance of rats on dietary restriction for 2–4 months resulted in resistance of hippocampal neurons to kainate-induced degeneration and associated deficits in learning and memory (Bruce-Keller *et al*., 1999). Interestingly, maintenance of presenilin-1 mutant knockin mice on a calorie-restricted diet (a manipulation known to reduce levels of age-related oxidative stress) results in decreased vulnerability of hippocampal neurons to excitotoxic injury (Zhu *et al*., 1999). In addition, maintenance of mice on dietary restriction increases the resistance of dopaminergic neurons in their substantia nigra to MPTP toxicity in a model of Parkinson's disease (Duan and Mattson, 1999), suggesting that dietary restriction may be of benefit in several different age-related neurodegenerative disorders. The mechanism whereby dietary restriction protects neurons in the brain against aging and disease may involve reduced levels of oxidative stress and a preconditioning response (Lee *et al*., 1999; Yu and Mattson, 1999).

III. Glycation in Aging and AD

Glycation is a process in which monosaccharides such as glucose modify N-terminal amino acid groups, and arginine and lysine residues, of proteins. Glycation is promoted by transition metals and oxyradicals, and glycation, in turn, induces oxidative stress. There is a progressive increase in glycation of many different proteins during aging, and an apparent acceleration of glycation of proteins in the brains of patients with AD. Both the microtubule-associated protein tau (the major component of neurofibrillary tangles) and Aβ (the major component of plaques) have been reported to be heavily glycated. Experimental studies have shown that glycated tau is neuro-

toxic, that glycation enhances amyloid β-peptide induced neuronal damage, and that Aβ can bind to cell surface receptors for advanced glycation end products located on microglia, thereby inducing microglia to produce neurotoxic substances. Protein glycation may contribute to the increased levels of oxidative stress, and synaptic and neuronal degeneration in AD, and therapeutic agents that suppress glycation are therefore being evaluated.

A. Glycation Chemistry

One well-recognized marker of aging is the modification of proteins by sugars, a process called glycation (Brownlee, 1995). The process of protein glycation, which is called the "Maillard reaction," involves the reaction of monosaccharides (e.g., glucose, fructose and hexose-phosphates) with N-terminal amino groups (and side chains of arginine and lysine residues) of proteins (Fig. 23.5). The initial reaction involves reversible formation of Schiff–base adducts, which then form Amadori products. Subsequent oxidation of the Amadori products as catalyzed by Fe^{2+}, Cu^{2+}, or hydroxyl radicals, for example, results in the formation (via reactive intermediates) of protein-bound advanced glycation end products (AGE). AGE may consist of one or more of several cross-links, including pyralline and pentosidine. Importantly, AGE formation is largely irreversible and causes cross-linking of proteins such that they form insoluble deposits. The formation of AGE has been studied extensively as a process that occurs in extracellular matrix proteins such as collagen and has been linked to several age-related diseases, including atherosclerosis and type-2 diabetes (Mullarkey *et al*., 1990; Ceriello, 1999; Sano *et al*., 1999). The following findings suggest an important role for AGE in the cross-linking and deposition of amyloid and cytoskeletal proteins in the brain in AD.

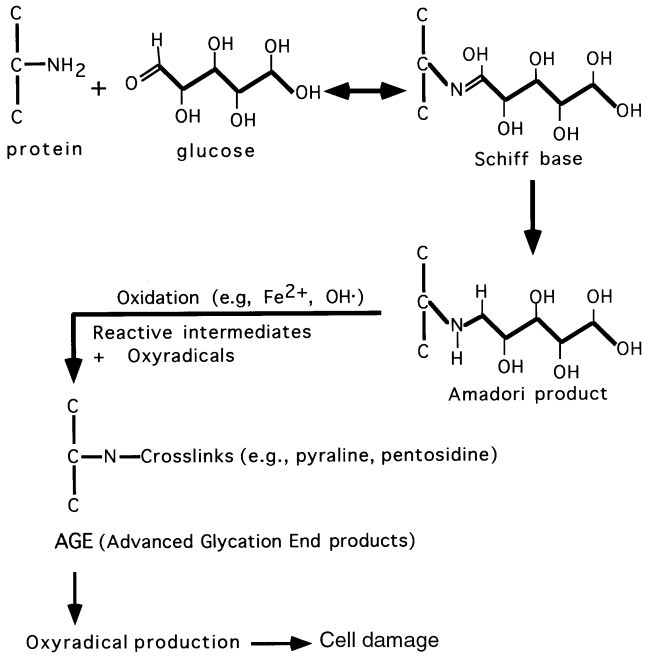

FIG. 23.5. Chemistry of protein glycation.

B. Evidence for Increased Protein Glycation in the Brain in AD

Several findings suggest that protein glycation may contribute to the pathogenesis of AD. As in other organ systems, levels of AGE increase in the brain during aging. Histochemical analyses indicate that AGE products accumulate to particularly high levels in pyramidal neurons of the cerebral cortex and hippocampus (Li *et al*., 1995; Kimura *et al*., 1996), neuronal populations that are selectively vulnerable in AD. Advanced glycation end products are associated with both amyloid plaques and neurofibrillary tangles in postmortem brain tissue from AD patients (Smith *et al*., 1994; Vitek *et al*., 1994). Immunostaining with antibodies that selectively recognize AGE-modified proteins reveals staining associated with extracellular amyloid deposits (both diffuse and senile plaques), as well as in astrocytes (Kimura *et al*., 1995). Ko *et al*. (1999) generated antibodies that selectively recognize Nϵ-carboxymethyl-lysine, a stable AGE known to accumulate during aging. The antibodies labeled PHF-tau on immunoblots, and immunoelectron microscopy demonstrated immunoreactivity with PHF. The patterns of AGE in AD brain are roughly similar to the patterns of oxidative stress (see Section II), suggesting that increased oxidative stress plays a role in AGE formation. The latter possibility would be consistent with the evidence that increased levels of oxidative stress occur at a relatively early stage in the neurodegenerative process. Because AGE may promote further oxidative stress, an amplifying cascade of free radical production and protein glycation and cross-linking may occur.

C. Experimental Evidence Implicating AGE in the Pathogenesis of AD

Two proteins central to the pathogenic process in AD that are modified by AGE are Aβ (Vitek *et al*., 1994), the main component of plaques, and the microtubule-associated protein tau (Yan *et al*., 1995), the major component of neurofibrillary tangles. In light of the well-established ability of glycation to promote protein cross-linking, it seems likely that the modification of amyloid β-peptide and tau by AGE may accelerate their cross-linking and fibril formation. In the case of tau, amino acids within the microtubule-binding domain are glycated (Ledesma *et al*., 1995), whereas amino acids modified in Aβ are unclear. Nonglycated Aβ generates free radicals and induces oxidative stress in neurons (Hensley *et al*., 1994; Goodman and Mattson, 1994), and this process is enhanced by Fe^{2+} (Goodman and Mattson, 1994). Such Aβ-associated free radical production appears to play a key role in peptide aggregation and fibril formation (Dyrks *et al*., 1992; Hensley *et al*., 1994). It is therefore likely that in the presence of Fe^{2+}, radicals arising from Aβ contribute to both glycation of the amyloid and neurotoxicity. Indeed, Aβ induces membrane lipid peroxidation and promotes apoptosis in neurons (Mark *et al*., 1995b; Kruman *et al*., 1997), and glycation of Aβ may enhance its neurotoxic effects (Yan *et al*., 1996). Experimental studies have provided evidence that glycated tau is also capable of inducing oxidative stress in cultured neuronal cells (Yan *et al*., 1995). Thus, protein glycation may contribute to oxidative stress occurring at the cell surface (Aβ) and within neurons (tau).

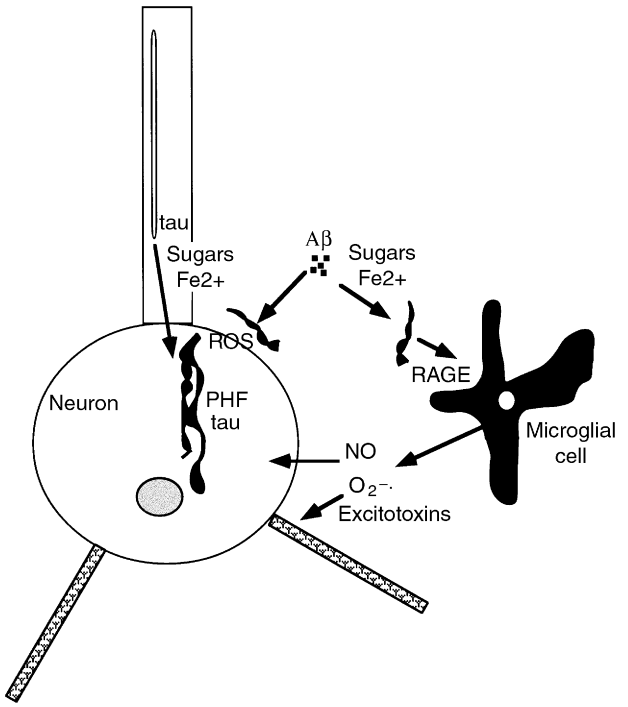

FIG. 23.6. Possible mechanisms whereby glycation contributes to aggregation of amyloid and tau and neuronal degeneration in AD.

One established mechanism whereby AGE can induce oxidative stress is by activating microglia (Vlassara *et al*., 1986, 1988). Microglia express several receptors that can bind glycated proteins, including the scavenger receptor and the (receptor for advanced glycation end products (RAGE) (Schmidt *et al*., 1994; Araki *et al*., 1995). Activation of the receptors induces cytokine production and generation of reactive oxygen species (superoxide and nitric oxide) and excitotoxins, which may have adverse effects on neurons (Fig. 23.6). A role for microglial activation by AGE in the pathogenesis of AD is suggested by the presence of activated microglia in association with amyloid plaques (see Section I) and by the ability of Aβ to activate microglia via a RAGE-mediated mechanism (Yan *et al*., 1996). An indirect, microglia-mediated mechanism of AGE-induced neuronal injury in AD is further suggested by the lack of AGE receptors in neurons. Thus, it is unlikely that RAGE mediates direct neurotoxic effects of glycated proteins. The cytokine cascade induced by glycated proteins may play an important role in inducing an inflammation-like process involving microglial activation. In this regard, increased levels of activation of the transcription factor NF-κB have been documented in cells associated with amyloid deposits in AD brain (Kaltschmidt *et al*., 1997). Whereas activation of NF-κB in neurons can protect them against apoptosis and excitotoxicity (Barger *et al*., 1995; Mattson *et al*., 1997; Yu *et al*., 1999), activation of NF-κB in microglia induces cell activation and cytokine production. However, NF-κB also induces expression of neuroprotective cytokines such as TGF-β, and neurotrophic factors, such as nerve growth factor (NGF) (Heese *et al*., 1998). Therefore, the relative contributions of production of neurotoxic versus neuroprotective agents by microglia remain an important area for future investigations.

Beyond glycation of proteins linked to AD, per se, glycation of other proteins may contribute to AD and related disorders. There is a general increase in protein glycation throughout the body during usual aging, and there are several examples in the literature of proteins subject to glycation that are relevant to the neurodegenerative cascade in AD. Thus, it was shown that glycation of the glucose transporter protein results in impaired glucose transport in erythrocytes (Bilan and Klip, 1990), and impaired glucose transport is believed to be an important early event in the neurodegenerative process in AD (Mark *et al*., 1997b). Similarly, the plasma membrane Na^+/K^+-ATPase can be glycated on residues within its catalytic site, resulting in impaired ATPase activity (Katori *et al*., 1999); experimental findings relevant to AD have shown that Aβ and oxidative stress can impair Na^+/K^+-ATPase activity in hippocampal neurons (Mark *et al*., 1995b).

D. Therapeutic Approaches That Target Glycation

Efforts to develop compounds that inhibit or even reverse protein glycation are being actively pursued. Munch *et al*. (1997) reported that formation of AGE-modified Aβ could be prevented by the AGE inhibitors aminoguanidine, carnosine, and tenilsetam. Other compounds reported to inhibit glycation include aryl and heterocyclic ureido, and aryl and heterocyclic carboxamidophenoxyisobutyric acids (Rahbar *et al*., 1999).

IV. Signaling and Apoptosis in AD

The extended period of time during which neurons die and the morphological features of the cell death in AD are consistent with the process of apoptosis. Analyses of postmortem brain tissue from AD patients have also revealed biochemical changes suggestive of apoptosis, including increased production of ''death proteins'' such as Par-4 and Bax, activation of cysteine proteases called caspases, and release from mitochondria of apoptotic factors such as cytochrome c. Experimental studies have shown that insults relevant to AD (e.g., exposure of neurons to Aβ and oxidative and metabolic insults) induce neuronal apoptosis. Data from analyses of brain tissue from AD patients and experimental models suggest that oxidative stress and disruption of cellular calcium homeostasis are important triggers of neuronal apoptosis in AD. In addition, studies of mice expressing mutant forms of presenilin-1 linked to early-onset inherited AD strongly implicate apoptosis in the neurodegenerative process. Interestingly, findings have shown that apoptotic biochemical cascades can be activated locally in synaptic terminals and neurites of neurons and that degeneration of these regions of neurons (and resultant synaptic dysfunction) may occur prior to cell death. Research is also revealing signaling mechanisms that may normally prevent neuronal apoptosis, and possibly AD. For example, several different neurotrophic factors have been shown to prevent neuronal apoptosis in experimental models of AD. Moreover, antioxidant administration and caloric restriction can protect neurons against insults relevant to AD, suggesting that dietary manipulations may prove effective in preventing and treating AD.

A. Signaling Mechanisms That Regulate Neuronal Survival: Alterations in AD

During brain development, neuroblasts initially proliferate and then cease dividing and differentiate into neurons. Differentiation involves a variety of morphological and biochemical changes, including formation of axons and dendrites, and expression of neurotransmitter receptors and ion channels. During the period of synapse formation, many neurons die by a process called apoptosis or ''programmed cell death.'' Studies of such developmental neuronal death have revealed that whether or not a neuron dies depends on the state of activation of different signal transduction pathways by intercellular signals such as neurotrophic factors and neurotransmitters. Neurotrophic factors are a diverse group of proteins that are produced by neurons and glial cells and act on neurons to promote their survival, growth, and plasticity. Activation of cell surface receptors by neurotrophic factors induces signaling cascades involving multiple kinases, which ultimately result in changes in gene expression and/or protein function. For example, nerve growth factor and brain-derived neurotrophic factor can induce the expression of antioxidant enzymes and can modulate ion channel function in cultured hippocampal neurons (Cheng *et al*., 1993; Mattson *et al*., 1995). Neurotransmitters also influence neuronal survival, growth, and plasticity. One prominent example is the excitatory neurotransmitter glutamate, which has been shown to regulate neurite outgrowth and synaptogenesis in the developing hippocampus (Mattson, 1988) on the one hand, while making a major contribution to neuronal calcium overload and cell death in experimental models of neurodegenerative disorders on the other (Mattson *et al*., 1993a; Doble, 1999). Additional types of intercellular signals that play important roles in regulating neuronal survival and plasticity are cytokines such as TNF and TGF-β (Mattson *et al*., 1996).

Evidence that signaling systems that normally regulate neuronal survival and plasticity are altered in AD is accumulating. Alterations in levels of neurotrophic factors and/or their receptors have been found in studies of postmortem brain tissue from AD patients. For example, NGF levels increase in many of the brain regions innervated by basal forebrain cholinergic neurons, but decrease in the nucleus basalis, suggesting the occurrence of perturbations of NGF signaling (Scott *et al*., 1995). Moreover, there appears to be a defect in retrograde transport of NGF in basal forebrain cholinergic neurons in AD associated with a decrease in expression of the high-affinity NGF receptor (trkA) by the same neurons (Mufson and Kordower, 1997). Thus, the reduced ability to respond to NGF could contribute to the demise of cholinergic neurons in the brains of AD patients. Levels of brain-derived neurotrophic factor were decreased significantly in the hippocampus of AD patients (Phillips *et al*., 1991), whereas levels of the secreted form of APP were decreased in cerebrospinal fluid from AD patients (Palmert *et al*., 1990). It is therefore possible that deficiencies of these factors could contribute to the degeneration of neurons in AD.

Levels of some neurotrophic factors (e.g., basic fibroblast growth factor) and cytokines (e.g., TGF-β and IL-1) are elevated in the vicinity of neuritic plaques (Cummings *et al*., 1993; van der Wal *et al*., 1993; Griffin *et al*., 1995). The influence of these factors on neurons in those locations is not known, although studies of experimental models of AD suggest that neurotrophic factor signaling can prevent neuronal cell death. Thus, several different neurotrophic factors and cytokines, including basic fibroblast growth factor, brain-derived neurotrophic factor, activity-dependent neurotrophic factor, the secreted form of APP, and TNF can protect cultured hippocampal neurons against cell death induced by amyloid β-peptide and oxidative and metabolic insults relevant to the pathogenesis of AD (Cheng and Mattson, 1991; Mattson *et al*., 1993c; Goodman and Mattson, 1994; Guo *et al*., 1999c). Neurotrophic factors and cytokines promote neuron survival by modulating gene expression in a manner that suppresses oxyradical production and stabilizes cellular calcium homeostasis (Mattson and Lindvall, 1997).

A contribution of overactivation of glutamate receptors to the pathogenesis of AD is suggested by studies showing that glutamate receptor activation can induce alterations in the cytoskeleton and the microtubule-associated protein tau similar to those seen in neurofibrillary tangles (Mattson, 1990; Stein-Behrens *et al*., 1994) and that Aβ and oxidative stress render neruons vulnerable to excitotoxicity (Mattson *et al*., 1992). In addition, deficits in neurotrophic factors, such as brain-derived neurotrophic factor and the secreted form of APP, may promote excitotoxic neuronal degeneration in AD because these factors can protect neurons against excitotoxicity in cell culture and *in vivo* (Mattson *et al*., 1993d; Cheng and Mattson, 1994; Smith-Swintosky *et al*., 1994). Moreover, hippocampal and cortical neurons in mice expressing mutations in the presenilin-1 gene linked to early-onset, autosomal-dominant AD exhibit increased vulnerability to excitotoxicity and ischemia (Guo *et al*., 1999a; Mattson *et al*., 2000a). Excessive glutamate receptor activation can induce apoptosis in neurons. Dysregulation of other neurotransmitter systems has also been documented in studies of AD patients. For example, alterations in cholinergic signaling in AD are well established and may contribute to both cognitive dysfunction and neuronal death (Mattson and Pedersen, 1998). Exposure of cultured cortical neurons to Aβ and oxidative insults impairs muscarinic cholinergic signaling in a manner similar to that seen in brain tissue from AD patients (Kelly *et al*., 1996; Blanc *et al*., 1997). Finally, GABA agonists can protect neurons against excitotoxicity (Mattson and Kater, 1989) and Aβ toxicity (Mark *et al*., 1995a), and studies have shown that transgenic mice overexpressing a presenilin-1 mutation exhibit altered hippocampal synaptic plasticity that can be restored to normal by treatment with agents that activate GABA receptors. Thus, altered inhibitory transmission might also contribute to the neurodegenerative process in AD.

B. Aberrant Signaling and Neuronal Apoptosis in AD

Exposure to aggregating forms of Aβ, insufficient neurotrophic factor support, overactivation of glutamate receptors, and metabolic and oxidative stress can each induce neuronal apoptosis in experimental models relevant to AD (Mattson and Furukawa, 1996; Mattson, 1997). Before describing the evidence supporting a role for neuronal apoptosis in AD, several morphological, biochemical, and molecular features that are currently being used to classify the cell death process as

TABLE 23.2 Examples of Criteria Used to Distinguish Apoptosis and Necrosis

Apoptosis	Necrosis
Cell shrinkage	Cell swelling
Membrane blebbing	Loss of membrane integrity
Membrane integrity maintained	Organellar disruption
Exposure of phosphatidylserine on cell surface	
Nuclear DNA condensation and fragmentation	
Maintenance of ATP levels	Decrease in ATP levels
Partial maintenance of ion homeostasis	Loss of ion homeostasis
Mitochondrial membrane permeability transition	
Release of cytochrome c	
Production of Par-4, Bax, and other "death proteins"	Cessation of protein synthesis
Caspase activation	

apoptosis will be reviewed briefly (Table 23.2). Morphological characteristics of neuronal apoptosis include cell body shrinkage, formation of "blebs" on the cell surface, nuclear condensation and fragmentation, and neurite fragmentation. Biochemical changes that typify neurons undergoing apoptosis include mitochondrial reactive oxygen species production and membrane depolarization (Keller *et al*., 1998a), activation of cysteine proteases of the caspase family (Nicholson and Thornberry, 1997), and loss of plasma membrane phospholipid asymmetry (Kruman *et al*., 1997). Molecular changes associated with apoptosis include induction of the expression of proapoptotic proteins such as prostate apoptosis response-4 (Par-4) (Guo *et al*., 1998a; Chan *et al*., 1999b) and certain members of the Bcl-2 family of proteins such as Bad and Bax (Chan and Mattson, 1999). In addition to such criteria, it is important to establish that the death of neurons in a particular experimental paradigm can be prevented by manipulations that block key steps in the apoptotic cascade. Examples include administration of caspase inhibitors, suppression of Par-4 expression and function using molecular approaches, and use of agents such as cyclosporin A that block mitochondrial membrane permeability transition (Guo *et al*., 1998a; Keller *et al*., 1998a).

Data obtained in studies of postmortem brain tissue from AD patients are consistent with apoptosis playing a role in the neurodegenerative process. Morphological analyses reveal evidence for nuclear DNA fragmentation in degenerating neurons (Smale *et al*., 1995; Su *et al*., 1994) and lack of morphological evidence of classic necrotic cell death (e.g., cell swelling and organelle damage). In addition, immunohistochemical data indicate increased caspase activation (Masliah *et al*., 1998; Chan *et al*., 1999a) and increased expression of apoptosis-related genes such as Bax and GADD45 (Torp *et al*., 1998; Tortosa *et al*., 1998) in neurons in brain tissue from AD patients compared to age-matched control patients. Analyses have also documented increased levels of Par-4 mRNA and protein in vulnerable regions of AD brain (e.g., hippocampus and inferior parietal cortex) compared to the same regions of age-matched control patients and to less vulnerable brain regions of AD patients (Guo *et al*., 1998a). Double-labeled immunohistochemical analyses in the latter study further showed that Par-4 levels are increased in many neurofibrillary tangle-bearing neurons.

Perhaps the strongest evidence supporting a major role for apoptosis in the pathogenesis of AD comes from experimental studies in animal and cell culture models. Exposure of cultured hippocampal and cortical cultures to Aβ induces neuronal apoptosis (Loo *et al*., 1993; Mark *et al*., 1995b; Kruman *et al*., 1997), which is mediated by Par-4 induction and caspase activation (Guo *et al*., 1998a; Chan *et al*., 1999a). Mutations in APP that are linked causally to inherited forms of AD increase production of the apoptosis-inducing Aβ, while decreasing production of the antiapoptotic secreted form of APP (Furukawa *et al*., 1996; Mattson, 1997; Guo *et al*., 1998b). Further support for a role for apoptosis in AD comes from studies of cultured neurons and transgenic mice expressing presenilin-1 mutations linked to early-onset autosomal dominant AD. Overexpression of presenilin-1 mutations in cultured PC12 cells increases their vulnerabilty to apoptosis induced by amyloid β-peptide and trophic factor withdrawal (Guo *et al*., 1996, 1997). The proapoptotic effect of presenilin-1 mutations appears to result primarily from an alteration of calcium regulation in the endoplasmic reticulum, such that levels of calcium release are increased when neurons are exposed to various insults (Guo *et al*., 1997, 1998c, 1999a). The disturbed cellular calcium homeostasis, in turn, endangers neurons by promoting Par-4 production (Chan *et al*., 1999b), mitochondrial dysfunction, and caspase activation (Guo *et al*., 1998a, 1999b; Chan *et al*., 1999a). Moreover, treatment of primary neurons expressing mutant presenilin-1 with neurotrophic factors, including basic fibroblast growth factor and activity-dependent neurotrophic factor, counteracted the proapoptotic action of the presenilin mutation (Guo *et al*., 1999c).

C. Involvement of Apoptotic Cascades in Dysfunction and Degeneration of Synapses

Synapses are believed to be sites where the neurodegenerative process begins in AD (Terry, 1994; DeKosky *et al*., 1996). The reason may be that both proapoptotic (e.g., glutamate receptors) and antiapoptotic (e.g., neurotrophic factor receptors) signaling pathways are highly concentrated in synaptic terminals. Moreover, it appears to be the case that Aβ is preferentially deposited in synaptic regions, most likely because the APP is axonally transported and therefore is present at high levels in presynaptic terminals (Mattson, 1997). Studies of cortical synaptosomes and cultured hippocampal neurons have provided evidence that insults relevant to AD can induce apoptotic biochemical cascades in synaptic compartments (Mattson and Duan, 1999). Exposure of rat cortical synaptosomes to apoptotic insults, including Aβ, results in caspase activation, loss of plasma membrane phospholipid asymmetry, and mitochondrial dysfunction (calcium uptake, membrane depolarization, and oxyradical production) (Mattson *et al*., 1998b). Synaptic degeneration in AD likely involves membrane lipid peroxidation and impairment of ion-motive

ATPases and glucose transporters (Mark *et al*., 1995b; Keller *et al*., 1997a). Par-4 levels are increased rapidly in cortical synaptosomes and in dendrites of hippocampal neurons in culture following exposure to Fe^{2+} and 4-hydroxynonenal, two insults relevant to the pathogenesis of AD (Duan *et al*., 1999). Treatment of synaptosomes with the protein synthesis inhibitor cycloheximide or Par-4 antisense oligonucleotides attenuated insult-induced mitochondrial dysfunction and caspase activation in synaptosomes and prevented death of cultured hippocampal neurons following exposure to excitotoxic and apoptotic insults, indicating a necessary role for Par-4 expression in synaptically driven apoptotic cascades. Additional support for a role for synaptic apoptosis in AD comes from studies in which cortical synaptosomes from transgenic mice expressing AD-linked presenilin-1 mutations exhibit enhanced elevations of cytoplasmic calcium levels following exposure to depolarizing agents, Aβ, and a mitochondrial toxin compared to synaptosomes from nontransgenic mice and mice overexpressing wild-type presenilin-1 (Begley *et al*., 1999). Consistent with apoptosis, it was shown that mitochondrial dysfunction and caspase activation after exposure to amyloid β-peptide were exacerbed in synaptosomes from presenilin-1 mutant mice. Treatment of synaptosomes with agents that buffer cytoplasmic calcium or prevent calcium release from the endoplasmic reticulum protected against the adverse effects of the presenilin-1 mutations.

That evidence that apoptotic cascades can be activated locally in pre- and postsynaptic compartments has important implications for the pathogenesis of AD. As described earlier, overactivation of glutamate receptors is implicated in the neurodegenerative process in AD. Influx of calcium into postsynaptic spines resulting from glutamate receptor activation is likely to be an important initial trigger for local activation of apoptotic cascades involving Par-4 induction, caspase activation, and mitochondrial dysfunction (Fig. 23.7). This would result in degeneration of postsynaptic spines, followed by dendrite degeneration and ultimately cell death. However, deficits in neurotrophic factor signaling might initially result in dysfunction and degeneration of presynaptic terminals, the compartments where receptors for neurotrophic factors are most concentrated. It is therefore likely that many neurons are functionally disconnected from their afferent input cells in the early stages of AD. The latter possibility suggests that the blockade of propogation of apoptotic signals from synapses to the cell body may prevent neuronal death and provide the opportunity for formation of new synapses.

D. Implications of Apoptotic Signaling for Prevention and Treatment of AD

What are the implications of evidence for neuronal apoptosis in AD for prevention and treatment of this disorder? One could inhibit the apoptotic process by preventing initiation of the cascade, by blocking a specific step in the cascade, or by stimulating antiapoptotic signaling pathways. Altered processing of APP appears to be an early event in AD, and drugs that modulate APP processing in a manner that decreases Aβ production are therefore being developed. Oxidative stress also appears to be a relatively early event in the neurodegenerative process in AD. Studies of experimental models of AD have

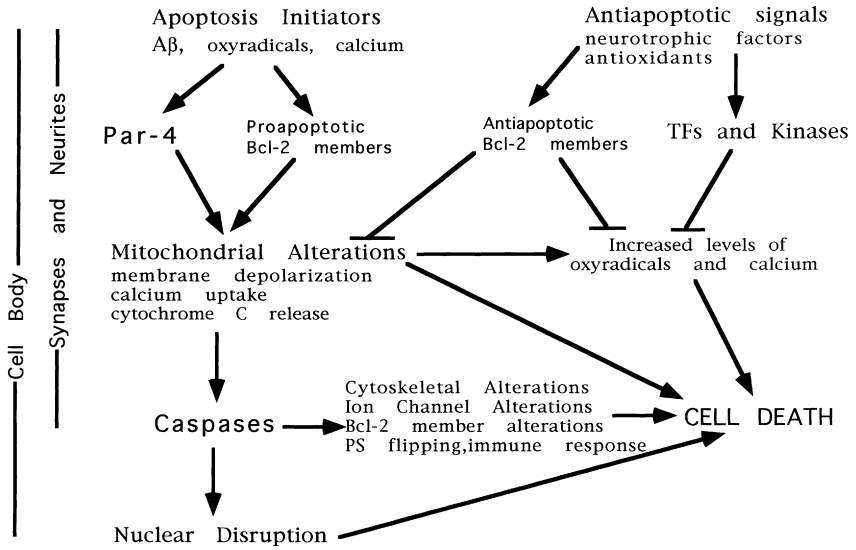

FIG. 23.7. General view of the biochemical pathways involved in neuronal apoptosis and neuroprotection. Apoptotic and antiapoptotic signals can be activated locally in synapses and neurites. Initiating signals, such as amyloid β-peptide, oxyradicals, and calcium, lead to induction of Par-4 and Bcl-2 family members. Par-4 and proapoptotic Bcl-2 family members induce mitochondrial alterations, which, in turn, lead to caspase activation and alterations in cellular calcium homeostasis and free radical metabolism. Caspases cleave substrates that modulate synaptic plasticity. Caspases also mediate proteolysis of substrates that lead to nuclear chromatin condensation and fragmentation. Antiapoptotic pathways can be activated by neurotrophic factors and other signals. Such pathways lead to activation of transcription factors (TF) that induce expression of neuroprotective proteins such as antioxidant enzymes and calcium-regulating proteins. In addition, antiapoptotic signals may modulate cell death pathways by activating kinases that phosphorylate substrates such as ion channels and Bcl-2 family members that influence the cell death process.

shown that the efficacy of several antioxidants is effective in preventing neuronal apoptosis, including vitamin E, uric acid, and estrogens (Goodman and Mattson, 1994; Goodman *et al*., 1996; Mattson *et al*., 1997; Keller *et al*., 1998a). Epidemiological and clinical trial data suggest that vitamin E and estrogens may indeed be effective in reducing risk for, and slowing the course of, AD (Sano *et al*., 1997; Tang *et al*., 1996). Looking further down the apoptotic cascade, one finds that calcium-stabilizing agents, such as dantrolene and nifedipine, are effective in experimental models of AD (Guo *et al*., 1997, 1999a). Caspase inhibitors and agents that stabilize mitochondrial function (cyclosporin A) are additional approaches warranted by experimental data. Another approach is the administration of neurotrophic factors or compounds that enhance the production of neurotrophic factors (Mufson and Kordower, 1997). The potential utility of agents that target specific proteins in the apoptotic cascade, such as caspase inhibitors and Par-4 antagonists, awaits further studies in animal models of AD. Perhaps the most clear information that has arisen from studies of the role of neuronal apoptosis in AD is that the neuronal cell death process involves increased levels of oxidative stress and disruption of calcium homeostasis, processes that appear to be convergence points in both apoptotic and nonapoptotic neuronal death.

Perhaps the approach that, at the present time, is most likely to be effective in preventing age-related neuronal apoptosis and AD is dietary restriction. Epidemiological studies (Mayeux *et al*., 1999) and experimental data (Bruce-Keller *et al*., 1999; Zhu *et al*., 1999) suggest that dietary restriction increases the resistance of neurons to age-related neurodegenerative disorders, including AD. Dietary restriction may exert its beneficial effects by reducing levels of oxidative stress and by enhancing the expression of neuroprotective proteins such as heat-shock proteins and neurotrophic factors (Duan and Mattson, 1999; Lee *et al*., 1999; Yu and Mattson, 1999). Although preventative approaches for AD appear promising, treatment of AD patients is problematic because by the time patients manifest symptoms, considerable neuronal loss has already occurred. Nevertheless, therapies based on antioxidants, calcium-modulating agents, and neurotrophic factors may prove effective in slowing the course of the disease process and should therefore continue to be pursued. Whether therapies that target specific apoptotic proteins such as Par-4 and caspases will prove effective remains to be determined.

V. Metals and the Pathophysiology of AD

Perhaps no area of research on AD has received more coverage in the lay press than investigations of possible roles of heavy metals. Indeed, statements such as ''don't use aluminum pots or antiperspirants containing aluminum'' or ''have the fillings in your teeth replaced—they contain mercury which may cause AD'' have been aired. Unfortunately, available data indicate that aluminum and mercury exposure are not major risk factors for AD. However, a building body of evidence based on sound chemistry and cell biology does suggest important roles for iron, copper, and zinc in the pathogenesis of neuronal degeneration in AD. Levels of iron, copper, and zinc are increased in neurofibrillary tangle-bearing neurons and neuritic plaques. Iron (Fe^{2+}) plays a particularly prominent role in free radical biochemistry as it catalyzes the conversion of hydrogen peroxide (present in all brain cells) to hydroxyl radical, a highly destructive radical that induces membrane lipid peroxidation. Iron, copper, and zinc have been shown to potentiate aggregation and neurotoxicity of Aβ and may thereby render neurons vulnerable to excitotoxicity and apoptosis. Copper and zinc also interact with APP in ways that may alter its metabolism so as to impair its normal functions and increase amyloid production. Zinc (Zn^{2+}) exhibits several actions that may modify the neurodegenerative process in AD, including its ability to modify neurotransmitter release and excitatory amino acid receptor activation. Finally, molecular genetic data suggest that aberrancies in genes that regulate iron and copper metabolism may contribute to some cases of AD (Fig. 23.8).

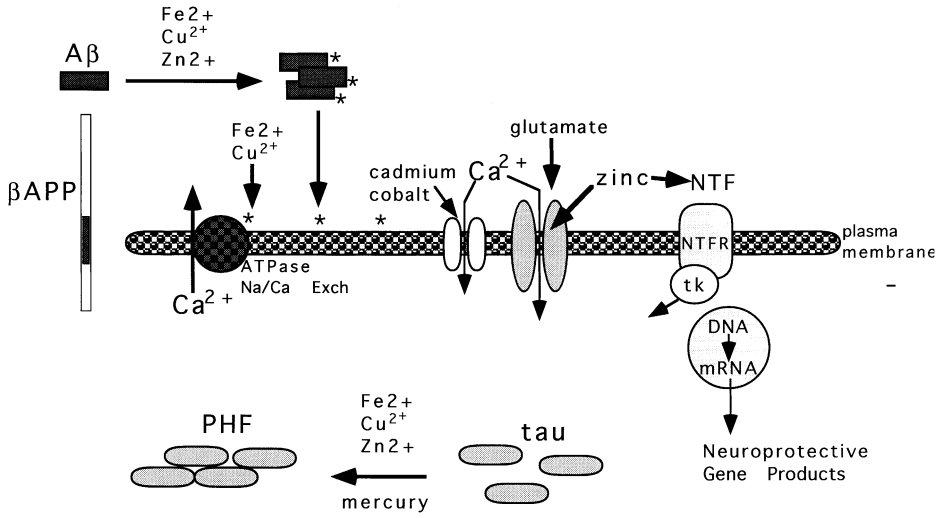

FIG. 23.8. Mechanisms whereby metals may promote neuronal degeneration in AD. Asterisks denote sites of free radical production. Aβ, amyloid β-peptide; βAPP, β-amyloid precursor protein; NTF, neurotrophic factor; NTFR, neurotrophic factor receptor; tk, tyrosine kinase domain.

A. Metal Neurochemistry

The literature on AD is filled with studies that have examined the possible involvement of heavy metals such as aluminum, iron, zinc, and mercury in the disease process. Although considerable controversy has surrounded this particular area of research, even a brief consideration of the chemistry of metals and experimental demonstrations of their abilities to damage cells makes them a reasonable focus of research in the field of neurodegenerative disorders. Metals are obtained in the diet and are generally bound to proteins in the blood and in cells. Metal-binding proteins include the iron-binding proteins ferritin and transferrin, the copper-binding protein ceruloplasmin, and the metallothionein family of metal-binding proteins that are responsible for sequestering iron, copper, and zinc in various tissues, including the brain (Aschner *et al*., 1997). Metals can be moved between cellular compartments via specific membrane transporters, whereas in ionic form some metals can move through ion channels. Metal-binding sites play important roles in a variety of biochemical reactions in brain cells (Prohaska, 1987). For example, iron plays a central role in the electron transport process that ultimately results in ATP production. Activation of soluble guanylate cyclase by nitric oxide requires interaction of nitric oxide with the iron-binding heme domain of the cyclase. The activity of other enzymes, such as copper-zinc superoxide dismutase, requires their binding two different metals. A final example of the diverse functions of iron in the brain is its role in the concentration of amine transmitters, including dopamine and serotonin. Before examining the possible roles of heavy metals in AD, it is important to understand at least the fundamentals of the neurochemistry of these metals.

The chemistry of iron in biological systems has been described in detail in previous review articles (Koeppen, 1995; Boldt, 1999). Iron concentration is quite high in the brain, reaching millimolar concentrations in basal ganglia structures and 200–600 μM in the hippocampus and cerebral cortex. By comparison, plasma iron concentrations are 10- to 50-fold lower. Several iron-binding proteins play important roles in controlling the amount of free iron in the brain. Transferrin is the major iron-binding protein in plasma. Specific receptors for transferrin are located in cells that comprise the blood–brain barrier and are largely responsible for the transport of iron into the brain, and neurons also possess high-affinity transferrin receptors, which may play a major role in uptake of this metal by neurons (Bradbury, 1997). Plasma membranes of endothelial cells and neurons also contain transferrin-independent transporters. In order for iron to be transported across membranes by either type of transporter it must first be reduced from Fe^{3+} to Fe^{2+} via a ferrireductase activity. Iron is stored within cells bound mainly to ferritin, a large multisubunit protein that can bind at least 4000 atoms of iron. In the brain, ferritin is present at highest levels in microglia and oligodendrocytes.

Previous review articles have covered the field of copper biochemistry nicely (Linder and Hazegh-Azam, 1996; Malmstrom and Leckner, 1998). Concentrations of copper are approximately five times higher in the brain than in plasma, and within the brain are higher in gray matter as compared to white matter. Copper is essential for the function of many different enzymes in the brain, including cytochrome c oxidase, dopamine β-monoxygenase, and lysyl oxidase. Within neurons, copper concentrates in nerve terminals and mitochondria. In contrast to the blood, wherein the majority of copper is bound to ceruloplasmin, the majority of exchangeable copper in the brain is in the form of low molecular weight amino acid complexes. The uptake of copper into the brain is mediated by a carrier-mediated facilitated diffusion process similar to that of neutral amino acids. Cellular copper storage and detoxification are mediated largely by metallothioneins and glutathione, the latter being the most abundant nonprotein thiol in brain cells. As with zinc (see later), copper can be released at synapses in a calcium-dependent manner, and synapses contain a low-affinity copper uptake system.

Of particular importance from the perspective of neurodegenerative disorders is the ability of Fe^{2+} and Cu^{2+} to promote membrane lipid peroxidation. This is accomplished via the Fenton reaction, which involves hyroxyl radical production as the result of the interaction of Fe^{2+} and Cu^{2+} with hydogen peroxide. Indeed, the Fenton reaction appears to play a prominent role in the pathogenesis of several different neurodegenerative disorders, including Parkinson's disease (Jellinger, 1999), stroke (Gutteridge, 1994), and AD (Markesbery and Carney, 1999). Whereas Cu^{2+} can also catalyze the Fenton reaction, available data suggest that the concentration of free Cu^{2+} is normally so low in cells (approximately one Cu^{2+}/cell) that it is unlikely to contribute meaningfully to lipid peroxidation during normal aging.

In contrast to iron and copper, zinc is redox inert. However, zinc binds readily to nitrogen and sulfur donors and plays a key role in the function of many different enzymes. In plasma and cerebrospinal fluid the vast majority of zinc is bound to proteins such as albumin. Interestingly, zinc concentrations are several orders of magnitude greater in brain tissue than in plasma or cerebrospinal fluid. The regulation of free zinc levels in tissues is controlled by zinc-binding proteins called metallothioneins and by specific membrane transporters (Ebadi, 1991; McMahon and Cousins, 1998). Three different zinc transporters have been identified: ZnT-1, ZnT-2, and ZnT-3; only ZnT-1 and ZnT-3 are expressed in the brain. ZnT-1 is localized in the plasma membrane and is responsible for the extrusion of zinc from cells, whereas ZnT-3 is localized in secretory vesicles and serves to concentrate zinc therein. Zinc concentrations vary dramatically among neuronal populations. For example, dentate granule neurons concentrate zinc at very high levels in their axon terminals, whereas very little zinc is present in cholinergic neurons of the basal forebrain. Zinc is released from axon terminals along with neurotransmitters (particularly the excitatory amino acid glutamate) and may serve a neuromodulatory function.

Other metals appear to play less widespread roles in brain neurochemistry. Selenium is central to the function of several enzymes involved in free radical metabolism, including glutathione peroxidase (Allan *et al*., 1999). Manganese is an essential trace element required for the proper function of several different enzymes, and imbalances in manganese regulation can result in brain damage (Newland, 1999). However, metals such as aluminum, cadmium, mercury, and cobalt serve few or no important functions in cells, and their presence in tissues is generally considered deleterious to cell function.

The divalent cations of several of these metals, particularly Cd^{2+} and Co^{2+}, can block voltage-dependent Ca^{2+} channels in neurons, thereby compromising cell function.

B. Roles of Iron and Copper in AD

Good and co-workers (1992) reported that levels of iron and aluminum are increased in neurofibrillary tangle-bearing neurons compared to tangle-free neurons in AD brain tissue sections. Levels of copper and iron are also increased in senile plaques in several different brain regions of AD patients (Lovell *et al*., 1998). Histochemical analyses suggest that levels of iron and ferritin are increased in association with senile plaques (Jellinger *et al*., 1990; Connor *et al*., 1992a). Levels of the iron-binding protein transferrin are decreased consistently in brain tissue from AD patients, particulary in white matter (Connor *et al*., 1992b). Immunostaining of brain sections from AD and control patients with antibodies against the iron regulatory proteins IRP-1 and IRP-2 revealed that neurofibrillary tangle-bearing neurons exhibit a marked increase in IRP-2 immunoreactivity, but no increase in IRP-1 immunoreactivity (Smith *et al*., 1998b). In addition, the soluble form of the iron-binding protein p97 is increased significantly in serum from AD patients compared to age-matched control patients (Kennard *et al*., 1996). There is considerable evidence for alterations in the iron transport proteins lactotransferrin and melanotransferrin in the brains of patients with Parkinson's disease (Qian and Wang, 1998). Data suggest that levels of transferrin receptors are decreased in hippocampal and cortical cells in AD, but are unchanged in cerebral blood vessels (Kalaria *et al*., 1992). Moreover, genetic links between altered iron homeostasis are suggested by data demonstrating an association between the C2 transferrin allele and late-onset AD (Namekata *et al*., 1997).

Experimental data further support a role for Fe^{2+} in the pathogenesis of neuronal dysfunction and degeneration in AD. Neurons are highly sensitive to cell injury and death following exposure to Fe^{2+}. For example, cultured primary hippocampal neurons undergo apoptosis following exposure of concentrations of Fe^{2+} in the upper nanomolar to low micromolar range (Kruman *et al*., 1997). Studies of cortical synaptosomes have shown that Fe^{2+} can disrupt the function of membrane glucose and glutamate transporters and can cause mitochondrial dysfunction (Keller *et al*., 1997a). Thus, Fe^{2+} may contribute to the dysfunction and degeneration of synapses that occur prior to neuronal cell death in AD (Mattson *et al*., 1998). Infusion of $FeCl_2$ into the basal forebrain of adult rats causes lipid peroxidation, depletion of choline acetyltransferase, and impairment of visuospatial memory (Bruce-Keller *et al*., 1998). The aggregation of Aβ is accelerated dramatically by Fe^{2+} as the result of metal-catalyzed oxidation (Dyrks *et al*., 1992; Mantyh *et al*., 1993). Studies of the neurotoxic actions of Aβ in primary hippocampal cell cultures have shown that Fe^{2+} enhances the neurotoxicity of the peptide (Goodman and Mattson, 1994). These experimental findings suggest a role for Fe^{2+} in amyloid aggregation and amyloid-induced neuronal degeneration in AD. A reproducible finding in studies of AD is that levels of membrane lipid peroxidation are increased in association with the neurodegenerative process (see Section II). Fe^{2+} is a potent inducer of membrane lipid peroxidation, and exposure of cultured neurons to Fe^{2+} can induce several alterations similar to those observed in studies of AD patients, including impairment of glucose and glutamate transport (Mark *et al*., 1997a; Keller *et al*., 1997a), disruption of cellular calcium homeostasis (Mark *et al*., 1995a,b, 1997b), and changes in the cytoskeleton similar to those seen in neurofibrillary tangles (Mattson *et al*., 1997b). In addition, the antioxidants vitamin E and estrogen (which data suggest may reduce risk for, and slow the course of, AD) suppress membrane lipid peroxidation, thereby counteracting the damaging effects of Fe^{2+} and Aβ (Goodman and Mattson, 1994; Mark *et al*., 1995; Goodman *et al*., 1996; Keller *et al*., 1997b). Lipid peroxidation induced by iron is likely to have a wide range of effects on neuronal function and degeneration (Mattson, 1998).

Copper plays roles in several different neurodegenerative disorders. Inherited disorders of copper metabolism such as Wilson and Menkes diseases manifest profound neurodegenerative phenotypes, and mutations in copper/zinc superoxide dismutase are responsible for some cases of familial amyotrophic lateral sclerosis (Waggoner *et al*., 1999). Moreover, it was reported that a frameshift mutation in the ceruloplasmin gene is responsible for a rare form of familial dementia (Harris *et al*., 1996). Thus, there is ample precedence for perturbations in copper metabolism and copper-containing enzymes resulting in neurodegeneration.

Several different actions of copper suggest that it may contribute to the pathogenesis of AD. Copper greatly increases vulnerabilty of cultured neurons to Aβ toxicity (White *et al*., 1999a). The latter study also showed that depletion of cellular glutathione greatly increases the vulnerability of neurons to copper toxicity. Atwood *et al*. (1998) showed that Cu^{2+} can induce aggregation of Aβ, particularly under conditions of acidic pH. More complex interactions of the amyloid precursor protein and copper have been proposed based on evidence that APP interacts with copper to enhance formation of Cu^{+}, thereby promoting oxyradical production (Hesse *et al*., 1994; White *et al*., 1999b). Collective data therefore suggest that further investigations of the possible involvement of copper in AD are warranted.

C. Role of Aluminum in AD

Largely as the result of data suggesting that levels of aluminum are increased in brain tissue from patients with AD, it was proposed that aluminum plays a critical role in the neurodegenerative process (Savory *et al*., 1996). Laser-activated microprobe mass analysis-based measurements demonstrated increased levels of aluminum in neurofibrillary tangle-bearing neurons (Good *et al*., 1992). In contrast, using a similar approach, Lovell and co-workers (1993) found no clear increase in aluminum in tangle-bearing neurons. It should be noted that the association of aluminum with tangles may simply result from a charge-based interaction as the tau proteins that comprises tangles are hyperphosphorylated (negative charge) and ionic aluminum is a cation. Indeed, the concentrations of several other cations (Ca^{2+} and Zn^{2+}) are increased in tangle-bearing neurons (Good *et al*., 1992; Markesbery and Carney, 1999).

Some experimental data are consistent with a role for aluminum in neuronal degeneration in AD. Exposure of neurons to

aluminum *in vivo* and in cell culture can cause cell damage, although the concentrations of aluminum required are greater than concentrations measured in the brains of AD patients. Aluminum administration in rabbits results in decreases in levels of choline acetyltransferase in entorhinal cortex and hippocampus, and reductions in serotonin and norepinephrine levels in the cerebral cortex that are correlated with severity of neurofibrillary degeneration (Beal *et al*., 1989). Aluminum can also potentiate the toxicities of several insults, including exposure to agents that induce Ca^{2+} influx (Mattson *et al*., 1993b) and Fe^{2+} (Xie *et al*., 1996). Al^{3+} can induce aggregation of Aβ, which is reversed by aluminum silicates (Fasman *et al*., 1995). It has also been reported that aluminum can induce the aggregation of PHF-tau (Shin *et al*., 1994), suggesting a role for aluminum in the formation of neurofibrillary tangles. However, in contrast to calcium, aluminum does not induce increased tau phosphorylation (Mattson *et al*., 1993b).

Despite the kinds of indirect evidence just described, the role of aluminum in AD appears negligible. Thus, epidemiological data reveal no obvious relationship between levels of aluminum exposure and risk for AD (Martyn *et al*., 1997). Indeed, there is not even a direct relationship between dietary intake of aluminum and blood levels of this metal because the vast majority of aluminum obtained in the diet never enters the bloodstream. Aluminum was found to have no effect on enzymatic processing or expression of amyloid precursor protein in cultured neural cells (Neill *et al*., 1996). Moreover, cultured neurons can withstand exposure to very high levels of aluminum of up to 1 m*M* (Mattson *et al*., 1993b).

D. Roles of Zinc, Mercury, and Other Metals in AD

Conflicting reports have appeared concerning levels of zinc in blood and brain tissue of AD patients compared to controls. Some data suggest that zinc levels might be decreased in brain tissues of AD patients (Corrigan *et al*., 1993). Measurements of zinc levels using proton-induced X-ray emission spectroscopic methods have shown that zinc levels are increased in hippocampus and amygdala of AD patients (Danscher *et al*., 1997). Lovell *et al*. (1998) reported that levels of zinc are increased in senile plaques. Gonzalez *et al*. (1999) reported a significant association between higher serum zinc, copper, and insulin concentrations and the presence of an epsilon 4 allele of apolipoprotein E, but only greater serum zinc concentration appeared to be an independent risk factor associated with the development of AD. Experimental data are consistent with a possible role for alterations in zinc metabolism in the pathophysiology of AD, although its specific role remains unclear. Several studies have reported that zinc can be directly neurotoxic toward cultured hippocampal and cortical neurons (Choi *et al*., 1988; Freund and Reddig, 1994). Oxidative stress can induce the release of zinc from metallothionein (Fliss and Menard, 1992), suggesting the possibility of increased free zinc resulting from the increased oxidative stress associated with aging and AD.

One action of zinc of clear relevance to AD is its ability to promote aggregation of Aβ (Mantyh *et al*., 1993; Bush *et al*., 1993, 1994a; Huang *et al*., 1997). Bush and co-workers (1994b) reported that zinc promotes aggregation of Aβ and inhibits α-secretase cleavage of APP. Both of these effects of zinc would be expected to promote neuronal degeneration (Mattson, 1997). However, it has been reported that lower concentrations of zinc can protect cultured hippocampal neurons against Aβ toxicity (Lovell *et al*., 1999). Another mechanism whereby increased zinc levels might promote neuronal degeneration in AD is by impairing the biological activity of NGF (Ross *et al*., 1997), as studies have shown that NGF protects neurons against insults relevant to the pathogenesis of AD (Mattson and Lindvall, 1997).

Measurements of heavy metals in the cerebrospinal fluid of AD and control patients have demonstrated increased levels of cadmium and mercury in AD patients (Basun *et al*., 1991). However, a subsequent study revealed no difference in cadmium levels in samples from AD and control patients (Basun *et al*., 1994). Mercury is highly toxic to neurons, as is evident in cases of mercury poisoning and in rodents administered mercury (Atchison and Hare, 1994). Based on data correlating the number of dental carries with mercury levels in the body (Mackert and Berglund, 1997), the known neurotoxicity of mercury, and studies suggesting that mercury levels are increased in blood and brain tissues of AD patients (Ehmann *et al*., 1986; Hock *et al*., 1998), it was suggested that mercury may contribute to the pathogenesis of AD. Even in the absence of any other data, this led to many dentists replacing carries filled with amalgum containing mercury with mercury-free amalgum. Subsequent studies have not supported a relationship between mercury-containing dental amalgam and AD (Saxe *et al*., 1999). Epidemiological studies failed to demonstrate correlations between between mercury or lead exposure and AD (Gun *et al*., 1997). Nevertheless, mercury and lead are certainly neurotoxic and can cause mental retardation in children (Prince, 1998). It is therefore possible that these metals contribute to the neurodegenerative process in some cases of AD.

E. Therapeutic Implications

As heavy metals are obtained almost exclusively in the diet, there are direct applications of research findings on the roles of metals in AD to the prevention and treatment of AD. Reducing the intake of mercury, cadmium, and aluminum makes sense because these metals are neurotoxic and serve no clear biological functions. Based on the evidence that aluminum levels may be increased in AD patients, clinical trials of chelation therapy using deferoxiamine were undertaken, with beneficial effects of the therapy being reported (Kruck *et al*., 1990). However, iron, copper, and zinc serve important roles in the nervous system and chronic chelation of these ions is therefore likely to have severe side effects. Recommendations as to the dietary intake of iron, copper, and zinc from the perspective of prevention of AD cannot yet be made. However, it is becoming clear that the high intake of iron increases the risk for cardiovascular disease (de Valk and Marx, 1999), and because iron may also contribute to the pathogenesis of AD and Parkinson's disease, it would seem prudent not to take iron supplements. Data described previously and in Section II above strongly suggest that the major roles of iron, copper, and zinc in AD are to promote cellular oxidative stress. Antioxidant supplementation (vitamins E and C; Morris *et al*., 1998) and dietary restriction (Bruce-Keller *et al*., 1999; Zhu *et al*., 1999) are therefore the

approaches currently available that are most likely to reduce risk for AD without side effects.

References

Abraham, C. R., Selkoe, D. J., and Potter, H. (1988). Immunohistochemical identification of the serine protease inhibitor α1-antichymotrypsin in the brain amyloid deposits of Alzheimer's disease. *Cell (Cambridge, Mass.)* **52**, 487–501.

Afagh, A., Cummings, B. J., Cribbs, D. H., Cotman, C. W., and Tenner, A. J. (1996). Localization and cell association of C1q in Alzheimer's disease brain. *Exp. Neurol*. **138**, 22–32.

Akiyama, H., Kawamata, T., Dedhar, S., and McGeer, P. L. (1991). Immunohistochemical localization of vitronectin, its receptor and beta-3 integrin in Alzheimer brain tissue. *J. Neuroimmunol*. **32**, 19–28.

Allan, C. B., Lacourciere, G. M., and Stadtman, T. C. (1999). Responsiveness of selenoproteins to dietary selenium. *Annu. Rev. Nutr.* **19**, 1–16.

Ancolio, K., Marambaud, P., Dauch, P., and Checler, F. (1997). Alpha-secretase-derived product of beta-amyloid precursor protein is decreased by presenilin 1 mutations linked to familial Alzheimer's disease. *J. Neurochem.* **69**, 2494–2499.

Araki, N., Higashi, T., Mori, T., Shibayama, R., Kawabe, Y., Kodama, T., Takahashi, K., Shichiri, M., and Horiouchi, S. (1995). Macrophage scavenger receptor mediates the endocytic uptake and degradation of advanced glycation end products of the Maillard reaction. *Eur. J. Biochem*. **230**, 408–415.

Aschner, M., Cherian, M. G., Klaassen, C. D., Palmiter, R. D., Erickson, J. C., and Bush, A. I. (1997). Metallothioneins in brain—the role in physiology and pathology. *Toxicol. Appl. Pharmacol.* **142**, 229–242.

Atchison, W. D., and Hare, M. F. (1994). Mechanisms of methylmercury-induced neurotoxicity. *FASEB J*. **8**, 622–629.

Atwood, C. S., Moir, R. D., Huang, X., Scarpa, R. C., Bacarra, N. M., Romano, D. M., Hartshorn, M. A., Tanzi, R. E., and Bush, A. I. (1998). Dramatic aggregation of Alzheimer abeta by Cu(II) is induced by conditions representing physiological acidosis. *J. Biol. Chem*. **273**, 12817–12826.

Barger, S. W., Horster, D., Furukawa, K., Goodman, Y., Krieglstein, J., and Mattson, M. P. (1995). Tumor necrosis factors α and β protect neurons against amyloid β-peptide toxicity: evidence for involvement of a κB-binding factor and attenuation of peroxide and Ca^{2+} accumulation. *Proc. Natl. Acad. Sci. U.S.A*. **92**, 9328–9332.

Bauer, J., Strauss, S., Schreiter-Gasser, U., Ganter, U., Schlegel, U., Witt, I., Volk, B., and Berger, M. (1991). Interleukin-6 and α2-macroglobulin indicate an acute-phase state in Alzheimer's disease cortices. *FEBS Lett.* **285**, 111–114.

Bauer, M. K. A., Lieb, K., Schulze-Osthoff, K., Berger, M., Gebicke-Haerter, P. J., Bauer, J., and Fiebich, B. L. (1997). Expression and regulation of cyclooxygenase-2 in rat microglia. *Eur. J. Biochem*. **243**, 726–731.

Basun, H., Forssell, L. G., Wetterberg, L., and Winblad, B. (1991). Metals and trace elements in plasma and cerebrospinal fluid in normal aging and Alzheimer's disease. *J. Neural Transm. [Park. Dis. Dementia Sect.]* **3**, 231–258.

Basun, H., Lind, B., Nordberg, M., Nordstrom, M., Bjorksten, K. S., and Winblad, B. (1994). Cadmium in blood in Alzheimer's disease and non-demented subjects: Results from a population-based study. *Biometals* **7**, 130–134.

Beal, M. F., Mazurek, M. F., Ellison, D. W., Kowall, N. W., Solomon, P. R., and Pendlebury, W. W. (1989). Neurochemical characteristics of aluminum-induced neurofibrillary degeneration in rabbits. *Neuroscience* **29**, 339–346.

Begley, J. G., Duan, W., Chan, S., Duff, K., and Mattson, M. P. (1999). Altered calcium homeostasis and mitochondrial dysfunction in cortical synaptic compartments of presenilin-1 mutant mice. *J. Neurochem.* **72**, 1030–1039

Bilan, P. J., and Klip, A. (1990). Glycation of the human erythrocyte glucose transporter in vitro and its functional consequences. *Biochem. J.* **268**, 661–667.

Blanc, E. M., Kelly, J. F., Mark, R. J., and Mattson, M. P. (1997). 4-hydroxynonenal, an aldehydic product of lipid peroxidation, impairs signal transduction associated with muscarinic acetylcholine and metabotropic glutamate receptors: Possible action on $G\alpha_q$/11. *J. Neurochem*. **69**, 570–580.

Blanc, E. M., Keller, J. N., Fernandez, S., and Mattson, M. P. (1998). 4-hydroxynonenal, a lipid peroxidation product, inhibits glutamate transport in astrocytes. *Glia* **22**, 149–160.

Boldt, D. H. (1999). New perspectives on iron: An introduction. *Am. J. Med. Sci*. **318**, 207–212.

Bradbury, M. W. (1997). Transport of iron in the blood-brain-cerebrospinal fluid system. *J. Neurochem.* **69**, 443–454.

Breitner, J. C. (1996). The role of anti-inflammatory drugs in the prevention and treatment of Alzheimer's disease. *Annu. Rev. Med.* **47**, 401–411.

Brownlee, M. (1995). Advanced protein glycosylation in diabetes and aging. *Annu. Rev. Med.* **46**, 223–234.

Bruce, A. J., Boling, W., Kindy, M. S., Peschon, J., Kraemer, P. J., Carpenter, M. K., Holtsberg, F. W., and Mattson, M. P. (1996). Altered neuronal and microglial responses to brain injury in mice lacking TNF receptors. *Nat. Med*. **2**, 788–794.

Bruce, A. J., Bose, S., Fu, W., Butt, C. M., Mirault, M.-E., Taniguchi, N., and Mattson, M. P. (1997). Amyloid β-peptide alters the profile of antioxidant enzymes in hippocampal cultures in a manner similar to that observed in Alzheimer's disease. *Pathogenesis* **1**, 15–30.

Bruce-Keller, A. J., Li, Y. J., Lovell, M. A., Kraemer, P. J., Gary, D. S., Brown, R. R., Markesbery, W. R., and Mattson, M. P. (1998). 4-Hydroxynonenal, a product of lipid peroxidation, damages cholinergic neurons and impairs visuospatial memory in rats. *J. Neuropathol. Exp. Neurol*. **57**, 257–267.

Bruce-Keller, A. J., Umberger, G., McFall, R., and Mattson, M. P. (1999). Food restriction reduces brain damage and improves behavioral outcome following excitotoxic and metabolic insults. *Ann. Neurol*. **45**, 8–15.

Bush, A. I., Multhaup, G., Moir, R. D., Williamson, T. G., Small, D. H., Rumble, B., Pollwein, B., Beyreuther, K., and Masters, C. L. (1993). A novel zinc(II) binding site modulates the function of the βA4 amyloid protein precursor of Alzheimer's disease. *J. Biol. Chem*. **268**, 16109–161122.

Bush, A. I., Pettingel, H., Multhaup, G., Paradis, M. D., Consattel, J. P., Gusella, J. F., Beyreuther, K., Masters, C. L., and Tanzi, R. E. (1994a). Rapid induction of Alzheimer Aβ amyloid formation by zinc. *Science* **265**, 1464–1467.

Bush, A. I., Pettingell, W. H., Paradis, M. D., and Tanzi, R. E. (1994b). Modulation of A beta adhesiveness and secretase site cleavage by zinc. *J. Biol. Chem.* **269**, 12152–12158.

Butterfield, D. A., Hensley, K., Harris, M., and Mattson, M. P. (1994). β-amyloid peptide free radical fragments initiate synaptosomal lipoperoxidation in a sequence-specific fashion: Implications to Alzheimer's disease. *Biochem. Biophys. Res. Commun*. **200**, 710–715.

Ceriello, A. (1999). Hyperglycaemia: The bridge between non-enzymatic glycation and oxidative stress in the pathogenesis of diabetic complications. *Diabetes Nutr. Metab.* **12**, 42–46.

Chan S. L., and Mattson, M. P. (1999). Caspase and calpain substrates: Roles in synaptic plasticity and cell death. *J. Neurosci. Res*. **58**, 167–190.

Chan, S. L., Griffin, W. S. T., and Mattson, M. P. (1999a). Evidence for caspase-mediated cleavage of AMPA receptor subunits in neuronal apoptosis and in Alzheimer's disease. *J. Neurosci. Res*. **57**, 315–323.

Chan, S. L., Tammariello, S. P., Estus, S., and Mattson, M. P. (1999b). Par-4 mediates trophic factor withdrawal-induced apoptosis of hippocampal neurons: Actions prior to mitochondrial dysfunction and caspase activation. *J. Neurochem.* **73**, 502–512.

Chang, J. W., Coleman, P. D., and O'Banion, M. K. (1996). Prostaglandin G/H synthase-2 (cyclooxygenase-2) mRNA expression is decreased in Alzheimer's disease. *Neurobiol. Aging* **17**, 801–808.

Chao, C. C., Hu, S., Kravitz, F. H., Tsang, M., Anderson, W. R., and Peterson, P. K. (1994). Transforming growth factor-β protects human neurons against beta-amyloid-induced injury. *Mol. Chem. Neuropathol*. **23**, 159–178.

Chen, K. S., Masliah, E., Grajeda, H., Guido, T., Huang, J., Khan, K., Motter, R., Soriano, F., and Games, D. (1998). Neurodegenerative Alzheimer-like pathology in PDAPP 717V $\rightarrow$ F transgenic mice. *Prog. Brain Res*. **117**, 327–334.

Cheng, B., and Mattson, M. P. (1991). NGF and bFGF protect rat and human central neurons against hypoglycemic damage by stabilizing calcium homeostasis. *Neuron* **7**, 1031–1041.

Cheng, B., and Mattson, M. P. (1992). Glucose deprivation elicits neurofibrillary tangle-like antigenic changes in hippocampal neurons: Prevention by NGF and bFGF. *Exp. Neurol*. **117**, 114–123.

Cheng, B., and Mattson, M. P. (1994). NT-3 and BDNF protect hippocampal, septal, and cortical neurons against metabolic compromise. *Brain Res*. **640**, 56–67.

Cheng, B., McMahon, D., and Mattson, M. P. (1993). Modulation of calcium current, intracellular calcium levels and cell survival by hypoglycemia, NGF and bFGF in cultured hippocampal neurons. *Brain Res*. **607**, 275–285.

Cheng, B., Christakos, S., and Mattson, M. P. (1994). Tumor necrosis factors protect neurons against excitotoxic/metabolic insults and promote maintenance of calcium homeostasis. *Neuron* **12**, 139–153.

Choi, D. W., Yokoyama, M., and Koh, J. (1988). Zinc neurotoxicity in cortical cell culture. *Neuroscience* **24**, 67–79.

Connor, J. R., Menzies, S. L., St. Martin, S. M., and Mufson, E. J. (1992a). A histochemical study of iron, transferrin, and ferritin in Alzheimer's diseased brains. *J. Neurosci. Res*. **31**, 75–83.

Connor, J. R., Snyder, B. S., Beard, J. L., Fine, R. E., and Mufson, E. J. (1992b). Regional distribution of iron and iron-regulatory proteins in the brain in aging and Alzheimer's disease. *J. Neurosci. Res*. **31**, 327–335.

Cornett, C. R., and Markesbery, W. R. (1998). Imbalances of trace elements related to oxidative damage in Alzheimer's disease brain. *Neurotoxicology* **19**, 339–346.

Corrigan, F. M., Reynolds, G. P., and Ward, N. I. (1993). Hippocampal tin, aluminum and zinc in Alzheimer's disease. *Biometals* **6**, 149–154.

Cummings, B. J., Su, J. H., and Cotman, C. W. (1993). Neuritic involvement within bFGF immunopositive plaques of Alzheimer's disease. *Exp. Neurol*. **124**, 315–325.

Danscher, G., Jensen, K. B., Frederickson, C. J., Kemp, K., Andreasen, A., Juhl, S., Stoltenberg, M., and Ravid, R. (1997). Increased amount of zinc in the hippocampus and amygdala of Alzheimer's diseased brains: A proton-induced X-ray emission spectroscopic analysis of cryostat sections from autopsy material. *J. Neurosci. Methods* **76**, 53–59.

DeKosky, S. T., Scheff, S. W., and Styren, S. D. (1996). Structural correlates of cognition in dementia: Quantification and assessment of synapse change. *Neurodegeneration* **5**, 417–421.

de Valk, B., and Marx, J. J. (1999). Iron, atherosclerosis, and ischemic heart disease. *Arch. Intern. Med*. **159**, 1542–1548.

Dickson, D. W., Lee, S. C., Mattiace, L. A., Yen, S. H., and Brosnan, C. (1993). Microglia and cytokines in neurological disease, with special reference to AIDS and Alzheimer's disease. *Glia* **7**, 75–83.

Doble, A. (1999). The role of excitotoxicity in neurodegenerative disease: Implications for therapy. *Pharmacol. Ther*. **81**, 163–221.

Duan, W., and Mattson, M. P. (1999). Dietary restriction and 2-deoxyglucose administration improve behavioral outcome and reduce degeneration of dopaminergic neurons in models of Parkinson's disease. *J. Neurosci. Res*. **57**, 195–206.

Duan, W., Rangnekar, V. M., and Mattson, M. P. (1999). Prostate apoptosis response-4 production in synaptic compartments following apoptotic and excitotoxic insults: Evidence for a pivotal role in mitochondrial dysfunction and neuronal degeneration. *J. Neurochem*. **72**, 2312–2322.

Dyrks, T., Dyrks, E., Hartmann, T., Masters, C., and Beyreuther, K. E. (1992). Amyloidogenicity of beta A4 and beta A4-bearing amyloid protein precursor fragments by metal-catalyzed oxidation. *J. Biol. Chem*. **267**, 18210–18217.

Ebadi, M. (1991). Metallothioneins and other zinc-binding proteins in brain. *In* "Methods in Enzymology" (J. F. Riordari and B. L. Vallee, eds.), Vol. 205, pp. 363–387. Academic Press, San Diego, CA.

Eckert, A., Forstl, H., Zerfass, R., Hartmann, H., and Muller, W. E. (1996). Lymphocytes and neutrophils as peripheral models to study the effect of β-amyloid on cellular calcium signalling in Alzheimer's disease. *Life Sci*. **59**, 499–510.

Ehmann, W. D., Markesbery, W. R., Alauddin, M., Hossain, T. I. M., and Brubaker, E. H. (1986). Brain trace elements in Alzheimer's disease. *Neurotoxicology* **7**, 197–206.

Eikelenboom, P., and Stam, F. C. (1982). Immunoglobulins and complement factors in senile plaques. *Acta Neuropathol.* **57**, 239–242.

Eikelenboom, P., Hack, C. E., Rozemuller, J. M., and Stam, F. C. (1989). Complement activation in amyloid plaques in Alzhiemer's dementia. *Virchows Arch*. **56**, 259–262.

Eikelenboom, P., Zhan, S. S., Kamphorst, W., van der Valk, P., and Rozemuller, J. M. (1994). Cellular and substrate adhesion molecules (integrins) and their ligands in cerebral amyloid plaques in Alzheimer's disease. *Virchows Arch*. **424**, 421–427.

Elenkov, I. J., Webster, E. L., Torpy, D. J., and Chrousos, G. P. (1999). Stress, corticotropin-releasing hormone, glucocorticoids, and the immune/inflammatory response: Acute and chronic effects. *Ann. N. Y. Acad. Sci*. **876**, 1–11.

Fasman, G. D., Perczel, A., and Moore, C. D. (1995). Solubilization of β-amyloid-(1-42)-peptide: Reversing the beta-sheet conformation induced by aluminum with silicates. *Proc. Natl. Acad. Sci. U.S.A*. **92**, 369–371.

Fillit, H., Ding, W. H., Buee, L., Kaltman, J., Altstiel, L., Lawlor, B., and Wolf-Klein, G. (1991). Elevated circulating tumor necrosis factor levels in Alzheimer's disease. *Neurosci. Lett*. **129**, 318–320.

Finch, C. E., and Morgan, T. E. (1997). Food restriction and brain aging. *Adv. Cell Aging Gerontol*. **2**, 279–297.

Finch, C. E., Laping, N. J., Morgan, T. E., Nichols, N. R., and Pasinetti, G. M. (1993). TGF-β1 is an organizer of responses to neurodegeneration. *J. Cell. Biochem*. **53**, 314–322.

Flanders, K. C., Lippa, C. F., Smith, T. W., Pollen, D. A., and Sporn, M. B. (1995). Altered expression of transforming growth factor-beta in Alzheimer's disease. *Neurology* **45**, 1561–1569.

Fliss, W. P., and Menard, M. (1992). Oxidant-induced mobilization of zinc from metallothionein. *Arch. Biochem. Biophys*. **293**, 195–199.

Freund, W. D., and Reddig, S. (1994). AMPA/Zn^{2+}-induced neurotoxicity in rat primary cortical cultures: Involvement of L-type calcium channels. *Brain Res.* **654**, 257–264.

Fu, W., Luo, H., Parthasarathy, S., and Mattson, M. P. (1998). Catecholamines potentiate amyloid β-peptide neurotoxicity: Involvement of oxidative stress, mitochondrial dysfunction, and perturbed calcium homeostasis. *Neurobiol. Dis.* **5**, 229–243.

Furukawa, K., Sopher, B., Rydel, R. E., Begley, J. G., Martin, G. M., and Mattson, M. P. (1996). Increased activity-regulating and neuroprotective efficacy of α-secretase-derived secreted APP is conferred by a C-terminal heparin-binding domain. *J. Neurochem*. **67**, 1882–1896.

Giometto, B., Argentiero, V., Sanson, F., Ongaro, G., and Tavolato, B. (1988). Acute phase proteins in Alzheimer's disease. *Eur. Neurol*. **28**, 30–33.

Gonzalez, C., Martin, T., Cacho, J., Brenas, M. T., Arroyo, T., Garcia-Berrocal, B., Navajo, J. A., and Gonzalez-Buitrago, J. M. (1999). Serum zinc, copper, insulin and lipids in Alzheimer's disease epsilon 4 apolipoprotein E allele carriers. *Eur. J. Clin. Invest*. **29**, 637–642.

Good, P. F., Perl, D. P., Bierer, L. M., and Schmeidler, J. (1992). Selective accumulation of aluminum and iron in the neurofibrillary tangles of

Alzheimer's disease: A laser microprobe (LAMMA) study. *Ann. Neurol*. **31**, 286–292.

Good, P. F., Werner, P., Hsu, A., Olanow, C. W., and Perl, D. P. (1996). Evidence of neuronal oxidative damage in Alzheimer's disease. *Am. J. Pathol*. **149**, 21–28.

Goodman, Y., and Mattson, M. P. (1994). Secreted forms of β-amyloid precursor protein protect hippocampal neurons against amyloid β-peptide-induced oxidative injury. *Exp. Neurol*. **128**, 1–12.

Goodman, Y., Steiner, M. R., Steiner, S. M., and Mattson, M. P. (1994). Nordihydroguaiaretic acid protects hippocampal neurons against amyloid β-peptide toxicity, and attenuates free radical and calcium accumulation. *Brain Res*. **654**, 171–176.

Goodman, Y., Bruce, A. J., Cheng, B., and Mattson, M. P. (1996). Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury, and amyloid β-peptide toxicity in hippocampal neurons. *J. Neurochem*. **66**, 1836–1844.

Griffin, W. S., Sheng, J. G., Roberts, G. W., and Mrak, R. E. (1995). Interleukin-1 expression in different plaque types in Alzheimer's disease: Significance in plaque evolution. *J. Neuropathol. Exp. Neurol*. **54**, 276–281.

Griffin, W. S., Sheng, J. G., Royston, M. C., Gentleman, S. M., McKenzie, J. E., Graham, D. I., Roberts, G. W., and Mrak, R. E. (1998). Glial-neuronal interactions in Alzheimer's disease: The potential role of a 'cytokine cycle' in disease progression. *Brain Pathol*. **8**, 65–72.

Gun, R. T., Korten, A. E., Jorm, A. F., Henderson, A. S., Broe, G. A., Creasey, H., McCusker, E., and Mylvaganam, A. (1997). Occupational risk factors for Alzheimer disease: A case-control study. *Alzheimer Dis. Assoc. Disord*. **11**, 21–27.

Gutteridge, J. M. (1994). Hydroxyl radicals, iron, oxidative stress, and neurodegeneration. *Ann. N. Y. Acad. Sci*. **738**, 201–213.

Guo, Q., Furukawa, K., Sopher, B. L., Pham, D. G., Robinson, N., Martin, G. M., and Mattson, M. P. (1996). Alzheimer's PS-1 mutation perturbs calcium homeostasis and sensitizes PC12 cells to death induced by amyloid β-peptide. *NeuroReport* **8**, 379–383.

Guo, Q., Sopher, B. L., Pham, D. G., Furukawa, K., Robinson, N., Martin, G. M., and Mattson, M. P. (1997). Alzheimer's presenilin mutation sensitizes neural cells to apoptosis induced by trophic factor withdrawal and amyloid β-peptide: Involvement of calcium and oxyradicals. *J. Neurosci*. **17**, 4212–4222.

Guo, Q., Fu, W., Xie, J., Luo, H., Sells, S. F., Geddes, J. W., Bondada, V., Rangnekar, V. M., and Mattson, M. P. (1998a). Par-4 is a mediator of neuronal degeneration associated with the pathogenesis of Alzheimer's disease. *Nat. Med*. **4**, 957–962.

Guo, Q., Robinson, N., and Mattson, M. P. (1998b). Secreted APPα counteracts the pro-apoptotic action of mutant presenilin-1 by activation of NF-κB and stabilization of calcium homeostasis *J. Biol. Chem*. **273**, 12341–12351.

Guo, Q., Christakos, S., Robinson, N., and Mattson, M. P. (1998c). Calbindin blocks the pro-apoptotic actions of mutant presenilin-1: Reduced oxidative stress and preserved mitochondrial function. *Proc. Natl. Acad. Sci. U.S.A*. **95**, 3227–3232.

Guo, Q., Fu, W., Sopher, B. L., Miller, M. W., Ware, C. B., Martin, G. M., and Mattson, M. P. (1999a). Increased vulnerability of hippocampal neurons to excitotoxic necrosis in presenilin-1 mutant knockin mice. *Nat. Med*. **5**, 101–107.

Guo, Q., Sebastian, L., Sopher, B. L., Miller, M. W., Ware, C. B., Martin, G. M., and Mattson, M. P. (1999b). Increased vulnerability of hippocampal neurons from presenilin-1 mutant knock-in mice to amyloid β-peptide toxicity: Central roles of superoxide production and caspase activation. *J. Neurochem*. **72**, 1019–1029.

Guo, Q., Sebastian, L., Sopher, B. L., Miller, M. W., Glazner, G. W., Ware, C. B., Martin, G. M., and Mattson, M. P. (1999c). Neurotrophic factors [activity-dependent neurotrophic factor (ADNF) and basic fibroblast growth factor (bFGF)] interrupt excitotoxic neurodegenerative cascades promoted by a presenilin-1 mutation. *Proc. Natl. Acad. Sci. U.S.A*. **96**, 4125–4130.

Harkany, T., De Jong, G. I., Soos, K., Penke, B., Luiten, P. G. M., and Gulya, K. (1995). β-amyloid1-42 affects cholinergic but not parvalbumin-containing neurones in the septal complex of the rat. *Brain Res*. **698**, 270–274.

Harris, Z. L., Migas, M. C., Hughes, A. E., Logan, J. I., and Gitlin, J. D. (1996). Familial dementia due to a frameshift mutation in ceruloplasmin gene. *Q. J. Med*. **89**, 355–359.

Heese, K., Fiebich, B. L., Bauer, J., and Otten, U. (1998). NF-κB modulates lipopolysaccharide-induced microglial nerve growth factor expression. *Glia* **22**, 401–407.

Hensley, K., Carney, J. M., Mattson, M. P., Aksenova, M., Harris, M., Wu, J. F., Floyd, R., and Butterfield, D. A. (1994). A model for β-amyloid aggregation and neurotoxicity based on free radical generation by the peptide: Relevance to Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A*. **91**, 3270–3274.

Hesse, L., Beher, D., Masters, C. L., and Multhaup, G. (1994). The βA4 amyloid precursor protein binding to copper. *FEBS Lett*. **349**, 109–116.

Ho, L., Pieroni, C., Winger, D., Purohit, D. P., Aisen, P. S., and Pasinetti, G. M. (1999). Regional distribution of cyclooxygenase-2 in the hippocampal formation in Alzheimer's disease. *J. Neurosci. Res*. **57**, 295–303.

Hock, C., Drasch, G., Golombowski, S., Muller-Spahn, F., Willershausen Zonnchen, B., Schwarz, P., Hock, U., Growdon, J. H., and Nitsch, R. M. (1998). Increased blood mercury levels in patients with Alzheimer's disease. *J. Neural Transm*. **105**, 59–68.

Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F., and Cole, G. (1996). Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. *Science* **274**, 99–102.

Huang, X., Atwood, C. S., Moir, R. D., Hartshorn, M. A., Vonsattel, J. P., Tanzi, R. E., and Bush, A. I. (1997). Zinc-induced Alzheimer's Aβ1–40 aggregation is mediated by conformational factors. *J. Biol. Chem*. **272**, 26464–26470.

Itagaki, S., McGeer, P. L., and Akiyama, H. (1988). Presence of T-cytotoxic suppressor and leucocyte common antigen positive cells in Alzheimer's disease brain tissue. *Neurosci. Lett*. **91**, 259–264.

Jellinger, K., Paulus, W., Grundke-Iqbal, I., Riederer, P., and Youdim, M. B. (1990). Brain iron and ferritin in Parkinson's and Alzheimer's diseases. *J. Neural Transm. [Park. Dis. Dementia Sect.]* **2**, 327–340.

Jellinger, K. A. (1999). The role of iron in neurodegeneration: Prospects for pharmacotherapy of Parkinson's disease. *Drugs Aging* **14**, 115–140.

Jiang, H., Burdick, D., Glabe, C. G., Cotman, C. W., and Tenner, A. J. (1994). β-amyloid activates complement by binding to a specific region of the collagen-like domain of C1q A chain. *J. Immunol*. **152**, 5050–5059.

Kalaria, R. N., Sromek, S. M., Grhaovac, I., and Harik, S. I. (1992). Transferrin receptors of rat and human brain and cerebral microvessels and their status in Alzheimer's disease. *Brain Res* **585**, 87–93.

Kaltschmidt, B., Uherek, M., Volk, B., Baeuerle, P. A., and Kaltschmidt, C. (1997). Transcription factor NF-βB is activated in primary neurons by amyloid β peptides and in neurons surrounding early plaques from patients with Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A*. **94**, 2642–2647.

Katori, T., Bannai, C., Hayashi, Y., and Yamashita, K. (1999). Decreased Na,K-ATPase activity by glycation at the catalytic center. *Horm. Metab. Res*. **31**, 5–7.

Keller, J. N., and Mattson, M. P. (1997). 17β-estradiol attenuates oxidative impairment of synaptic Na^+/K^+-ATPase activity, glucose transport and glutamate transport induced by amyloid β-peptide and iron. *J. Neurosci. Res*. **50**, 522–530.

Keller, J. N., Pang, Z., Geddes, J. W., Begley, J. G., Germeyer, A., Waeg, G., and Mattson, M. P. (1997a). Impairment of glucose and glutamate transport and induction of mitochondrial oxidative stress and dysfunction in synaptosomes by amyloid β-peptide: Role of the lipid peroxidation product 4-hydroxynonenal. *J. Neurochem*. **69**, 273–284.

Keller, J. N., Germeyer, A., Begley, J. G., and Mattson, M. P. (1997b). 17β-estradiol attenuates oxidative impairment of synaptic Na^+/K^+-ATPase activity, glucose transport, and glutamate transport induced by amyloid β-peptide and iron. *J. Neurosci. Res*. **50**, 522–530.

Keller, J. N., Kindy, M. S., Holtsberg, F. W., St Clair, D. K., Yen, H.-C., Germeyer, A., Steiner, S. M., Bruce-Keller, A. J., Hutchins, J. B., and Mattson, M. P. (1998a). Mitochondrial MnSOD prevents neural apoptosis and reduces ischemic brain injury: Suppression of peroxynitrite production, lipid peroxidation and mitochondrial dysfunction. *J. Neurosci*. **18**, 687–697.

Keller, J. N., Guo, Q., Holtsberg, F. W., Bruce-Keller, A. J., and Mattson, M. P. (1998b). Increased sensitivity to mitochondrial toxin-induced apoptosis in neural cells expressing mutant presenilin-1 is linked to perturbed calcium homeostasis and enhanced oxyradical production. *J. Neurosci*. **18**, 4439–4450.

Kelly, J., Furukawa, K., Barger, S. W., Mark, R. J., Rengen, M. R., Blanc, E. M., Roth, G. S., and Mattson, M. P. (1996). Amyloid β-peptide disrupts carbachol-induced muscarinic cholinergic signal transduction in cortical neurons. *Proc. Natl. Acad. Sci. U.S.A*. **93**, 6753–6758.

Kennard, M. L., Feldman, H., Yamada, T., and Jefferies, W. A. (1996). Serum levels of the iron-binding protein p97 are elevated in Alzheimer's disease. *Nat. Med.* **2**, 1230–1235.

Kimura, T., Takamatsu, J., and Araki, K. (1995). Are advanced glycation end products associated with amyloidosis in Alzheimer's disease? *NeuroReport* **6**, 866–868.

Kimura, T., Takamatzsu, J., Ikeda, K., Kondo, A., Miyadawa, T., and Horinichi, S. (1996). Accumulation of advanced glycation end products of the Malliard reaction with age in human hippocampal neurons. *Neurosci. Lett*. **208**, 53–56.

Ko, L. W., Ko, E. C., Nacharaju, P., Liu, W. K., Chang, E., Kenessey, A., and Yen, S. H. (1999). An immunochemical study on tau glycation in paired helical filaments. *Brain Res*. **830**, 301–313.

Koeppen, A. H. (1995). The history of iron in the brain. *J. Neurol. Sci*. **134**(Suppl.); 1–9.

Kruck, T. P., Fisher, E. A., and McLachlan, D. R. (1990). Suppression of deferoxamine mesylate treatment-induced side effects by coadministration of isoniazid in a patient with Alzheimer's disease subject to aluminum removal by ion-specific chelation. *Clin. Pharmacol. Ther*. **48**, 439–446.

Kruman, I., Bruce-Keller, A. J., Bredesen, D. W., Waeg, G., and Mattson, M. P. (1997). Evidence that 4-hydroxynonenal mediates oxidative stress-induced neuronal apoptosis. *J. Neurosci*. **17**, 5089–5100.

Ledesma, M. D., Bonay, P., and Avila, J. (1995). Tau protein from Alzheimer's disease patients is glycated at the tubulin-binding domain. *J. Neurochem*. **65**, 1658–1664.

Lee, J., Bruce-Keller, A. J., Kruman, U., Chan, S., and Mattson, M. P. (1999). 2-deoxy-D-glucose protects hippocampal neurons against excitotoxic and oxidative injury: Involvement of stress proteins. *J. Neurosci. Res*. **57**, 48–61.

Li, J. J., Sruini, M., Caticas, S., Kawashima, E., and Bouras, C. (1995). Age-dependent accumulation of advanced glycosylation end products in human neurons. *Neurobiol. Aging* **16**, 69–76.

Linder, M. C., and Hazegh-Azam, M. (1996). Copper biochemistry and molecular biology. *Am. J. Clin. Nutr*. **63**, 797S–811S.

Logroscino, G., Marder, K., Coté, L., Tang, M. X., Shea, S., and Mayeux, R. (1996). Dietary lipids and antioxidants in Parkinson's disease: A population-based, case-control study. *Ann. Neurol*. **39**, 89–94

Loo, D., Copani, A., Pike, C., Whittemore, E., Walencewicz, A., and Cotman, C. W. (1993). Apoptosis is induced by β-amyloid in cultured central nervous system neurons. *Proc. Natl. Acad. Sci. U.S.A*. **90**, 7951–7955.

Lovell, M. A., Ehmann, W. D., and Markesbery, W. R. (1993). Laser microprobe analysis of brain aluminum in Alzheimer's disease. *Ann. Neurol*. **33**, 36–42.

Lovell, M. A., Ehmann, W. D., Mattson, M. P., and Markesbery, W. R. (1997). Elevated 4-hydroxynonenal levels in ventricular fluid in Alzheimer's disease. *Neurobiol. Aging* **18**, 457–461.

Lovell, M. A., Robertson, J. D., Teesdale, W. J., Campbell, J. L., and Markesbery, W. R. (1998). Copper, iron and zinc in Alzheimer's disease senile plaques. *J. Neurol. Sci*. **158**, 47–52.

Lovell, M. A., Xie, C., and Markesbery, W. R. (1999). Protection against amyloid beta peptide toxicity by zinc. *Brain Res*. **823**, 88–95.

Mackert, J. R., and Berglund, A. (1997). Mercury exposure from dental amalgam fillings: Absorbed dose and the potential for adverse health effects. *Crit. Rev. Oral Biol. Med*. **8**, 410–436

Malmstrom, B. G., and Leckner, J. (1998). The chemical biology of copper. *Curr. Opin. Chem. Biol*. **2**, 286–292.

Mantyh, P. W., Ghilardi, J. R., Rogers, S., DeMaster, E., Allen, C. J., Stimson, E. R., and Maggio, J. E. (1993). Aluminum, iron and zinc ions promote aggregation of physiological concentrations of β-amyloid peptide. *J. Neurochem*. **61**, 1171–1174.

Mark, R. J., Ashford, J. W., and Mattson, M. P. (1995a). Anticonvulsants attenuate amyloid β-peptide neurotoxicity and promote maintenance of calcium homeostasis. *Neurobiol. Aging* **16**, 187–198.

Mark, R. J., Hensley, K., Butterfield, D. A., and Mattson, M. P. (1995b). Amyloid β-peptide impairs ion-motive ATPase activities: Evidence for a role in loss of neuronal Ca^{2+} homeostasis and cell death. *J. Neurosci*. **15**, 6239–6249.

Mark, R. J., Lovell, M. A., Markesbery, W. R., Uchida, K., and Mattson, M. P. (1997a). A role for 4-hydroxynonenal in disruption of ion homeostasis and neuronal death induced by amyloid β-peptide. *J. Neurochem*. **68**, 255–264.

Mark, R. J., Pang, Z., Geddes, J. W., Uchida, K., and Mattson, M. P. (1997b). Amyloid β-peptide impairs glucose uptake in hippocampal and cortical neurons: Involvement of membrane lipid peroxidation. *J. Neurosci*. **17**, 1046–1054.

Markesbery, W. R. (1997). Oxidative stress hypothesis in Alzheimer's disease. *Free Radical Biol. Med*. **23**, 134–147.

Markesbery, W. R., and Carney, J. M. (1999). Oxidative alterations in Alzheimer's disease. *Brain Pathol*. **9**, 133–146.

Martyn, C. N., Coggon, D. N., Inskip, H., Lacey, R. F., and Young, W. F. (1997). Aluminum concentrations in drinking water and risk of Alzheimer's disease. *Epidemiology* **8**, 281–286.

Masliah, E., Mallory, M., Alford, M., Tanaka, S., and Hansen, L. A. (1998). Caspase dependent DNA fragmentation might be associated with excitotoxicity in Alzheimer disease. *J. Neuropathol. Exp. Neurol*. **57**, 1041–1052.

Mattson, M. P. (1988). Neurotransmitters in the regulation of neuronal cytoarchitecture. *Brain Res. Rev*. **13**, 179–212.

Mattson, M. P. (1990). Antigenic changes similar to those seen in neurofibrillary tangles are elicited by glutamate and calcium influx in cultured hippocampal neurons. *Neuron* **4**, 105–117.

Mattson, M. P. (1997). Cellular actions of β-amyloid precursor protein, and its soluble and fibrillogenic peptide derivatives. *Physiol. Rev*. **77**, 1081–1132.

Mattson, M. P. (1998). Modification of ion homeostasis by lipid peroxidation: Roles in neuronal degeneration and adaptive plasticity. *Trends Neurosci*. **21**, 53–57.

Mattson, M. P., and Begley, J. G. (1998). Amyloid β-peptide induces apoptosis-related events in synapses and dendrites. *Brain Res*. **807**, 167–176.

Mattson, M. P., and Duan, W. (1999). "Apoptotic" biochemical cascades in synaptic compartments: Roles in adaptive plasticity and neurodegenerative disorders. *J. Neurosci. Res*. **58**, 152–166.

Mattson, M. P., and Furukawa, K. (1996). Programmed cell life: Anti-apoptotic signaling and therapeutic strategies for neurodegenerative disorders. *Restorative Neurol. Neurosci*. **9**, 191–205.

Mattson, M. P., and Kater, S. B. (1989). Excitatory and inhibitory neurotransmitters in the generation and degeneration of hippocampal neuroarchitecture. *Brain Res*. **478**, 337–348.

Mattson, M. P., and Lindvall, O. (1997). Neurotrophic factors and the aging brain. *Adv. Cell Aging Gerontol*. **2**, 299–345.

Mattson, M. P., and Pedersen, W. A. (1998). Effects of amyloid precursor protein derivatives and oxidative stress on basal forebrain choliner-

gic systems in Alzheimer's disease. *Int. J. Dev. Neurosci*. **16**, 737–753.

Mattson, M. P., Cheng, B., Davis, D., Bryant, K., Lieberburg, E., and Rydel, R. E. (1992). β-amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity. *J. Neurosci*. **12**, 376–389.

Mattson, M. P., Barger, S. W., Cheng, B., Lieberburg, I., Smith-Swintosky, V. L., and Rydel, R. E. (1993a). β-Amyloid precursor protein metabolites and loss of neuronal Ca^{2+} homeostasis in Alzheimer's disease. *Trends Neurosci*. **16**, 409–414.

Mattson, M. P., Lovell, M., Ehmann, W. D., and Markesbery, W. R. (1993b). Comparison of the effects of elevated intracellular aluminum and calcium levels on neuronal survival and tau immunoreactivity. *Brain Res*. **602**, 21–31.

Mattson, M. P., Tomaselli, K., and Rydel, R. E. (1993c). Calcium-destabilizing and neurodegenerative effects of aggregated β-amyloid peptide are attenuated by basic FGF. *Brain Res*. **621**, 35–49.

Mattson, M. P., Cheng, B., Culwell, A., Esch, F., Lieberburg, I., and Rydel, R. E. (1993d). Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of β-amyloid precursor protein. *Neuron* **10**, 243–254.

Mattson, M. P., Lovell, M. A., Furukawa, K., and Markesbery, W. R. (1995). Neurotrophic factors attenuate glutamate-induced accumulation of peroxides, elevation of $[Ca^{2+}]_i$ and neurotoxicity, and increase antioxidant enzyme activities in hippocampal neurons. *J. Neurochem*. **65**, 1740–1751.

Mattson, M. P., Barger, S. W., Furukawa, K., Bruce, A. J., Wyss-Coray, T., Mark, R. J., and Mucke, L. (1996). Cellular signaling roles of TGFβ, TNFα and βAPP in brain injury responses and Alzheimer's disease. *Brain Res. Rev*. **23**, 47–61.

Mattson, M. P., Goodman, Y., Luo, H., Fu, W., and Furukawa, K. (1997a). Activation of NF-κB protects hippocampal neurons against oxidative stress-induced apoptosis: Evidence for induction of Mn-SOD and suppression of peroxynitrite production and protein tyrosine nitration. *J. Neurosci. Res*. **49**, 681–697.

Mattson, M. P., Fu, W., Waeg, G., and Uchida, K. (1997b). 4-hydroxy-nonenal, a product of lipid peroxidation, inhibits dephosphorylation of the microtubule-associated protein tau. *NeuroReport* **8**, 2275–2281.

Mattson, M. P., Guo, Q., Furukawa, K., and Pedersen, W. A. (1998a). Presenilins, the endoplasmic reticulum, and neuronal apoptosis in Alzheimer's disease. *J. Neurochem*. **70**, 1–14.

Mattson, M. P., Partin, J., and Begley, J. G. (1998b). Amyloid β-peptide induces apoptosis-related events in synapses and dendrites. *Brain Res*. **807**, 167–176.

Mattson, M. P., Zhu, H., Yu, J., and Kindy, M. S. (2000a). Presenilin-1 mutation increases neuronal vulnerability to focal ischemia in vivo, and to hypoxia and glucose deprivation in cell culture: Involvement of perturbed calcium homeostasis. *J. Neurosci*. **20**, 1358–1364.

Mattson, M. P., Culmsee, C., Yu, Z., and Camandola, S. (2000b). Roles of NF-κB in neuronal survival and plasticity. *J. Neurochem*. **74**, 443–456.

Mayeux, R., Costa, R., Bell, K., Merchant, C., Tung, M. X., and Jacobs, D. (1999). Reduced risk of Alzheimer's disease among individuals with low calorie intake. *Neurology* **59**, S296–S297.

McMahon, R. J., and Cousins, R. J. (1998). Mammalian zinc transporters. *J. Nutr*. **128**, 667–670.

Meda, L., Cassatella, M. A., Szendrei, G. I., Otvos, L., Baron, P., Villalba, M., Ferrari, D., and Rossi, F. (1995). Activation of microglial cells by β-amyloid protein and interferon-γ. *Nature (London)* **374**, 647–650.

Moccoci, P., MacGarvey, M. S., and Beal, M. F. (1994). Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. *Ann. Neurol*. **36**, 747–751.

Montine, K. S., Olson, S. J., Amarnath, V., Whetsell, W. O., Graham, D. G., and Montine, T. J. (1997). Immunohistochemical detection of 4-hydroxy-2-nonenal adducts in Alzheimer's disease is associated with inheritance of ApoE4. *Am. J. Pathol*. **150**, 437–443.

Morris, C. M., Kerwin, J. M., and Edwardson, J. A. (1994). Non-haem iron histochemistry of the normal and Alzheimer's disease hippocampus. *Neurodegeneration* **3**, 267–275.

Morris, M. C., Beckett, L. A., Scherr, P. A., Hebert, L. E., Bennett, D. A., Field, T. S., and Evans, D. A. (1998). Vitamin E and vitamin C supplement use and risk of incident Alzheimer disease. *Alzheimer Dis. Assoc. Disord*. **12**, 121–126.

Mufson, E. J., and Kordower, J. H. (1997). Nerve growth factor and its receptors in the primate forebrain: Alterations in Alzheimer's disease and potential use in experimental therapeutics. *In* "Neuroprotective Signal Transduction" (M. P. Mattson, ed.), pp. 23–59. Humana Press, Totowa, NJ.

Mullarkey, C. J., Edelstein, D., and Brownlee, M. (1990). Free radical generation by early glycation products: A mechanism for accelerated atheogenesis in diabetes. *Biochem. Biophys. Res. Commun*. **173**, 932–939.

Munch, G., Mayer, S., Michaelis, J., Hipkiss, A. R., Riederer, P., Muller, R., Neumann, A., Schinzel, R., and Cunningham, A. M. (1997). Influence of advanced glycation end-products and AGE-inhibitors on nucleation-dependent polymerization of β-amyloid peptide. *Biochim. Biophys. Acta* **1360**, 17–29.

Namekata, K., Imagawa, M., Terashi, A., Ohta, S., Oyama, F., and Ihara, Y. (1997). Association of transferrin C2 allele with late-onset Alzheimer's disease. *Hum. Genet*. **101**, 126–129.

Neill, D., Leake, A., Hughes, D., Keith, A. B., Taylor, G. A., Allsop, D., Rima, B. K., Morris, C., Candy, J. M., and Edwardson, J. A. (1996). Effect of aluminium on expression and processing of amyloid precursor protein. *J. Neurosci. Res*. **46**, 395–403.

Netland, E. E., Newton, J. L., Majocha, R. E., and Tate, B. A. (1998). Indomethacin reverses the microglial response to amyloid beta protein. *Neurobiol. Aging* **19**, 201–204.

Newland, M. C. (1999). Animal models of manganese's neurotoxicity. *Neurotoxicology* **20**, 415–432.

Nicholson, D. W., and Thornberry, N. A. (1997). Caspases: Killer proteases. *Trends Biochem. Sci*. **22**, 299–306.

Palmert, M. R., Usiak, M., Mayeux, B., Raskind, M., Tourtellotte, W. W., and Younkin, S. G. (1990). Soluble derivatives of the β-amyloid protein precursor in cerebrospinal fluid: Alterations in normal aging and in Alzheimer's disease. *Neurology* **40**, 1028–1034.

Pedersen, W. A., Culmsee, C., Ziegler, D., Herman, J. P., and Mattson, M. P. (1999). Aberrant stress response associated with severe hypoglycemia in a transgenic mouse model of Alzheimer's disease. *J. Mol. Neurosci*. **13**, 159–165.

Pedersen, W. A., Chan, S. L., and Mattson, M. P. (2000). A mechanism for the neuroprotective effect of apolipoprotein E: Isoform-specific modification by the lipid peroxidation product 4-hydroxynonenal. *J. Neurochem*. **74**, 1426–1433.

Phillips, H. S., Hains, J. M., Armanini, M., Laramee, G., Johnson, S. A., and Winslow, J. W. (1991). BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disese. *Neuron* **7**, 695–702.

Prehn, J. H. M., Backhaub, C., and Krieglstein, J. (1993). Transforming growth factor-β1 prevents glutamate neurotoxicity in rat neocortical cultures and protects mouse from ischemic injury in vivo. *J. Cereb. Blood Flow Metab*. **13**, 521–525.

Prince, M. (1998). Is chronic low-level lead exposure in early life an etiologic factor in Alzheimer's disease? *Epidemiology* **9**, 618–621.

Prohaska, J. R. (1987). Functions of trace elements in brain metabolism. *Physiol. Rev*. **67**, 858–901.

Qian, Z. M., and Wang, Q. (1998). Expression of iron transport proteins and excessive iron accumulation in the brain in neurodegenerative disorders. *Brain Res. Rev*. **27**, 257–267.

Rahbar, S., Kumar Yernini, K., Scott, S., Gonzales, N., and Lalezari, I. (1999). Novel inhibitors of advanced glycation endproducts. *Biochem. Biophys. Res. Commun*. **262**, 651–656.

Raskind, M. A., Peskind, E., Rivard, M. F., Veith, R., and Barnes, R. (1982). The dexamethasone suppression test and cortisol circadian

rythm in primary degenerative dementia. *Am. J. Psychiatry* **139**, 1468–1471.

Rogers, J., Cooper, N. R., Webster, S., Schultz, J., McGeer, P. L., Styren, S. D., Civin, W. H., Brachova, L., Bradt, B., and Lieberburg, I. (1992). Complement activation by β-amyloid in Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 10016–11120.

Rogers, J., Kirby, L. C., Hempleman, S. R., Berry, D. L., McGeer, P. L., Kaszniak, A. W., Zalinski, J., Cofield, M., Mansukhani, L., and Wilson, P. (1993). Clinical trial of indomethacin in Alzheimer's disease. *Neurology* **43**, 1609–1611.

Ross, G. M., Shamovsky, I. L., Lawrance, G., Solc, M., Dostaler, S. M., Jimmo, S. L., Weaver, D. F., and Riopelle, R. J. (1997). Zinc alters conformation and inhibits biological activities of nerve growth factor and related neurotrophins. *Nat. Med.* **3**, 872–878.

Rozemuller, J. M., Eikelenboom, P., Pals, S. T., and Stam, F. C. (1989). Microglial cells around amyloid plaques in Alzheimer's disease express leucocyte adhesion molecules of the LFA-1 family. *Neurosci. Lett.* **101**, 288–292.

Rozemuller, J. M., Stam, F. C., and Eikelenboom, P. (1990). Acute phase proteins are present in amorphous plaques in the cerebral but not in the cerebellar cortex in patients with Alzheimer's disease. *Neurosci. Lett.* **119**, 75–78.

Sano, H., Nagai, R., Matsumoto, K., and Horiuchi, S. (1999). Receptors for proteins modified by advanced glycation endproducts (AGE)—their functional role in atherosclerosis. *Mech. Ageing Dev.* **107**, 333–346.

Sano, M., Ernesto, C., Thomas, R. G., Klauber, M. R., Schafer, K., Grundman, M., Woodbury, P., Growdon, J., Cotman, C. W., Pfeiffer, E., Schneider, L. S., and Thal, L. J. (1997). A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's Disease Cooperative Study. *N. Engl. J. Med.* **336**, 1216–1222.

Savory, J., Exley, C., Forbes, W. F., Huang, Y., Joshi, J. G., Kruck, T., McLachlan, D. R., and Wakayama, I. (1996). Can the controversy of the role of aluminum in Alzheimer's disease be resolved? What are the suggested approaches to this controversy and methodological issues to be considered? *J. Toxicol. Environ. Health* **48**, 615–635.

Saxe, S. R., Wekstein, M. W., Kryscio, R. J., Henry, R. G., Cornett, C. R., Snowdon, D. A., Grant, F. T., Schmitt, F. A., Donegan, S. J., Wekstein, D. R., Ehmann, W. D., and Markesbery, W. R. (1999). Alzheimer's disease, dental amalgam and mercury. *J. Am. Dent. Assoc.* **130**, 191–199.

Schmidt, A. M., Hori, O., Brett, J., Yan, S. D., Wautier, J. L., and Stern, D. (1994). Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. *Arterioscler. Thromb.* **14**, 1521–1528.

Scott, S. A., Mufson, E. J., Weingartner, J. A., Skau, K. A., and Crutcher, K. A. (1995). Nerve growth factor in Alzheimer's disease: Increased levels throughout the brain coupled with declines in nucleus basalis. *J. Neurosci.* **15**, 6213–6221.

Shin, R. W., Lee, V. M., and Trojanowski, J. Q. (1994). Aluminum modifies the properties of Alzheimer's disease PHF tau proteins in vivo and in vitro. *J. Neurosci.* **14**, 7221–7233.

Smale, G., Nichols, N. R., and Brady, D. R. (1995). Evidence for apoptotic cell death in Alzheimer's disease. *Exp. Neurol.* **133**, 225–230.

Smith, C. D., Carney, J. M., Starke-Reed, P. E., Oliver, C. N., Stadtman, E. R., Floyd, R. A., and Markesbery, W. R. (1991). Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 10540–10543.

Smith, M. A., Taneda, S., Richey, P. L., Miyata, S., Yan, S. D., Stern, D., Sayre, L. M., Monnier, V. M., and Perry, G. (1994). Advance maillard reaction end products are associated with Alzheimer's disease pathogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 5710–5714.

Smith, M. A., Harris, P. L. R., Sayre, L. M., Beckman, J. S., and Perry, G. (1997a). Widespread peroxynitrite-mediated damage in Alzheimer's disease. *J. Neurosci.* **17**, 2653–2657.

Smith, M. A., Harris, P. L. R., Sayre, L. M., and Perry, G. (1997b). Iron accumulation in Alzheimer's disease is a source of redox-generated free radicals. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 9866–9868.

Smith, M. A., Hirai, K., Hsiao, K., Pappolla, M. A., Harris, P. L., Siedlak, S. L., Tabaton, M., and Perry, G. (1998a). Amyloid-beta deposition in Alzheimer transgenic mice is associated with oxidative stress. *J. Neurochem.* **70**, 2212–2215.

Smith, M. A., Wehr, K., Harris, P. L. R., Siedlak, S. L., Connor, J. R., and Perry, G. (1998b). Abnormal localization of iron regulatory protein in Alzheimer's disease. *Brain Res.* **788**, 232–236.

Smith-Swintosky, V. L., Pettigrew, L. C., Craddock, S. D., Culwell, A. R., Rydel, R. E., and Mattson, M. P. (1994). Secreted forms of β-amyloid precursor protein protect against ischemic brain injury. *J. Neurochem.* **63**, 781–784.

Sohal, R. S., and Weindruch, R. (1996). Oxidative stress, caloric restriction, and aging. *Science* **273**, 59–63.

Stein-Behrens, B., Mattson, M. P., Chang, I., Yeh, M., and Sapolsky, R. M. (1994). Stress excacerbates neuron loss and cytoskeletal pathology in the hippocampus. *J. Neurosci.* **14**, 5373–5380.

Strittmatter, W. J., and Roses, A. D. (1996). Apolipoprotein E and Alzheimer's disease. *Annu. Rev. Neurosci.* **19**, 53–77.

Su, J. H., Anderson, A. J., Cummings, B., and Cotman, C. W. (1994). Immunocytochemical evidence for apoptosis in Alzheimer's disease. *NeuroReport* **5**, 2529–2533.

Tang, M. X., Jacobs, D., Stern, Y., Marder, K., Schofield, P., Gurland, B., Andrews, H., and Mayeux, R. (1996). Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease. *Lancet* **348**, 429–432.

Terry, R. D. (1994). Neuropathological changes in Alzheimer disease. *Prog. Brain Res.* **101**, 383–390.

Torp, R., Su, J. H., Deng, G., and Cotman, C. W. (1998). GADD45 is induced in Alzheimer's disease, and protects against apoptosis in vitro. *Neurobiol. Dis.* **5**, 245–252.

Tortosa, A., Lopez, E., and Ferrer, I. (1998). Bcl-2 and Bax protein expression in Alzheimer's disease. *Acta Neuropathol.* **95**, 407–412.

van der Wal, E. A., Gomez-Pinilla, F., and Cotman, C. W. (1993). Transforming growth factor-β1 is in plaques in Alzheimer and Down pathologies. *NeuroReport* **4**, 69–72.

Veerhuis, R., van der Valk, P., Janssen, I., Zhan, S. S., Van Nostrand, W. E., and Eikelenboom, P. (1995). Complement activation in amyloid plaques in Alzheimer's disease does not proceed further than C3. *Virchows Arch.* **426**, 603–610.

Vitek, M. P., Bhattacharya, K., Glendening, J. M., Stopa, E., lasara, H., Bucala, R., Manogue, K., and Cerami, A. (1994). Advance glycation end products contribute to amyloidosis in Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 4766–4770.

Vlassara, H., Brownlee, M., and Cerami, A. (1986). Novel macrophage receptor for glucose-modified proteins is distinct from previously described scavenger receptors. *J. Exp. Med.* **164**, 1301–1309.

Vlassara, H., Brownlee, M., Manogue, K., Dinarello, C., and Pasagian, A. (1988). Cachectin/TNF and IL-1 induced by glucose-modified proteins: Role in normal tissue remodeling. *Science* **240**, 1546–1548.

Waggoner, D. J., Bartnikas, T. B., and Gitlin, J. D. (1999). The role of copper in neurodegenerative disease. *Neurobiol. Dis.* **6**, 221–230.

Webster, S., O'Barr, S., and Rogers, J. (1994). Enhanced aggregation and β structure of the amyloid β peptide after coincubation with C1q. *J. Neurosci. Res.* **39**, 448–456.

White, A. R., Bush, A. I., Beyreuther, K., Masters, C. L., and Cappai, R. (1999a). Exacerbation of copper toxicity in primary neuronal cultures depleted of cellular glutathione. *J. Neurochem.* **72**, 2092–2098.

White, A. R., Multhaup, G., Maher, F., Bellingham, S., Camakaris, J., Zheng, H., Bush, A. I., Beyreuther, K., Masters, C. L., and Cappai, R. (1999b). The Alzheimer's disease amyloid precursor protein modulates copper-induced toxicity and oxidative stress in primary neuronal cultures. *J. Neurosci.* **19**, 9170–9179.

Wyss-Coray, T., Feng, L., Masliah, E., Ruppe, M. D., Lee, H. S., Toggas, S. M., Rockenstein, E. M., and Mucke, L. (1995). Increased central nervous system production of extracellular matrix components and development of hydrocephalus in transgenic mice overexpressing transforming growth factor-β1. *Am. J. Pathol*. **147**, 53–67.

Xie, C. X., Mattson, M. P., Lovell, M. A., and Yokel, R. A. (1996). Intraneuronal aluminum potentiates iron-induced oxidative stress in cultured rat hippocampal neurons. *Brain Res*. **743**, 271–277.

Yan, S. D., Yan, S. F., Chen, X., Fu, J., Chen, M., Kupusamy, P., Smith, M. A., Perry, G., Godman, G. C., Nawroth, P., and Stern, D. (1995). Non-enzymatically glycated tau in Alzheimer's disease induces neuronal oxidant stress resulting in cytokine gene expresion and release of amyloid β-peptide. *Nat. Med*. **1**, 693–699.

Yan, S. D., Chen, X., Fu, J., Chen, M., Zhu, H., Roher, A., Slattery, T., Zhao, L., Nagashima, M., Morser, J., Migheli, A., Nawroth, P., Stern, D., and Schmidt, A. M. (1996). RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. *Nature (London)* **382**, 685–691.

Yu, Z. F., and Mattson, M. P. (1999). Dietary restriction and 2-deoxyglucose administration reduce focal ischemic brain damage and improve behavioral outcome: Evidence for a preconditioning mechanism. *J. Neurosci. Res*. **57**, 830–839.

Yu, Z. F., Zhou, D., Bruce-Keller, A. J., Kindy, M. S., and Mattson, M. P. (1999). Lack of the p50 subunit of NF-κB increases the vulnerability of hippocampal neurons to excitotoxic injury. *J. Neurosci*. **19**, 8856–8865.

Zhu, H., Guo, Q., and Mattson, M. P. (1999). Dietary restriction protects hippocampal neurons against the death-promoting action of a presenilin-1 mutation. *Brain Res*. **842**, 224–229.

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Donald K. Ingram

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Rodent Models of Age-Related Memory Impairment

I. Introduction

In characterizing the effects of aging on memory processes in humans, any review must deal with the issue of diversity. To be sure, age-related loss in memory function occurs, but the degree of functional decline is highly dependent upon what component of memory processing is being assessed (see reviews in Keefover, 1998; Grady and Craik, 2000). Particular components are generally spared, e.g., sensory memory, while others are greatly impaired, e.g., secondary or working memory. In addition, aged individuals differ greatly in the degree of memory impairment observed in any particular component of memory processing. At advanced ages, groups of individuals may perform equivalent to younger cohorts in a working memory task, while others are markedly impaired. In addition, it has become increasingly apparent that memory impairments observed in Alzheimer's disease differ from those observed in normal aging, a fact that adds to the diversity issue (Albert, 1997).

In reviewing the literature on rodent models of age-related memory impairments, the same issue of diversity emerges quite distinctly. The degree of age-related decline observed in a memory task is greatly dependent upon the task and specific parameters of the task. Marked individual differences in performance can also be observed among aged rodents tested in the same memory task. Moreover, differences in the age-related pattern of performance decline in memory tasks can be observed between different rodent strains.

While this diversity may on first glance appear to complicate the analysis of aging on memory function, on further consideration the diversity offers insight and direction into the most fruitful areas of investigation. What components of memory function are most affected in human aging? Are there appropriate rodent models, strains, and paradigms that can be used? Given evidence in humans and rodents that a particular component of memory processing is impaired in aging, does this evidence offer hypotheses about which neural systems might underlie the impairment?

The literature surveyed herein has been addressed in previous reviews that have attempted synthesis of results along various outlines (Sarter, 1987; Gallagher and Pelleymounter, 1988; Barnes, 1990, 1998; Ingram *et al*., 1994; Gallagher and Rapp, 1997). The current review follows the conventional approach of outlining results among categories of behavioral tasks. This approach is offered to provide the reader a description of the various behavioral paradigms that have been used to assess age-related impairments in rodent models. It is important to understand how rodent studies are conducted in order to consider the relevance of the results to other paradigms as well as their relevance to the human literature. In applying this categorical approach, the attempt is also made to synthesize and summarize the findings which demonstrate what aspects of memory processing are most affected in aging. The review will not cover recent studies attempting to assess whether mouse models of Alzheimer's disease exhibit memory impairments. The focus will be on behavior; thus, no discussion will be offered regarding linkage of the behavioral performance to underlying neurobiological mechanisms.

When reviewing this literature, the reader should be cognizant that memory processes are hypothetical constructs that are deduced from examining the ''performance'' of rodents in a particular paradigm. This is not a trivial matter when considering the assessment of age differences in memory function in rodents (Ingram, 1985). Before concluding that a performance difference in a particular test is due to impaired memory function, there must be awareness that other noncognitive performance factors, such as sensory, motor, and motivational differences, can impact upon the performance of aged subject. Aged rodents can have degenerated retina, which can impact upon their visual abilities in a maze; they can be too slow in their reaction time in an operant task requiring speed of response; they may be less responsive to food restriction in an appetitive task or have a higher threshold for pain in an aversive task. Behavioral paradigms can often permit the investigator to assess the involvement of such noncognitive performance factors, yet unfortunately many investigators do

not construct their paradigms in this manner. In addition, just as in human studies, particular diseases, such as anemia, may impact upon performance in a memory task (Spangler and Ingram, 1996). While some investigators implement health screens in attempting to eliminate possible moribund rats, unfortunately many others do not. The lack of attention to extraneous variation caused by noncognitive performance factors can complicate general conclusions regarding the age-related memory impairment.

Another complication for producing rodent models for examining age-related memory impairments is that the tasks do not translate well into theoretical models of human memory processes. Memory processing can be viewed within the basic structure of sensory memory, short-term memory, and long-term memory (Craik and Jennings, 1992). Sensory memory is the limited capacity, very short-term memory for sensory events. Short-term memory includes primary memory (e.g., digit span) as well as working memory, in which events to be remembered are held longer and require active transformation or must be held while further incoming information is processed. Long-term memory includes episodic memory (e.g., free recall), semantic memory (e.g., vocabulary), and procedural memory (skill learning). No rodent equivalent exists for some of these elements, such as, semantic memory. Fortunately age-related impairments in humans have been recorded primarily in tasks involving working and episodic memory, where some rodent tasks are have been useful for investigating as models.

When one examines the large number of studies in this research area, several different rat and mouse strains have been used, but two are predominant, the Fischer-344 (F344) rat and the C57BL/6J mouse.

II. Classical Conditioning

Classical conditioning, also known as Pavlovian conditioning, is considered reflex learning and represents one of the most evolutionarily primitive forms of associative processes. In a conventional classical conditioning paradigm, an initially neutral stimulus, such as a tone, is paired with a noxious stimulus, such as foot shock. The foot shock elicits a reflexive unconditioned response (UR), for example, a decrease in heart rate. After repeated pairing of the tone (or conditioned stimulus, CS) with the foot shock (or unconditioned stimulus, US) the CS itself will elicit the UR, which is then referred to as the conditioned response (CR). The CS–US pairing can be contiguous in time (simultaneous conditioning) or the US can be delayed after offset of the CS (delayed conditioning) or while the CS is still active (trace conditioning). The delay can be manipulated parametrically from a few milliseconds (e.g., 300 msec) to a few seconds (e.g., 3 sec). The primary dependent variables are the number of trails to a criterion measured typically as conditioned responses.

A. Eyeblink and Heart Rate Conditioning

In human studies, the most widely used paradigm is eyeblink conditioning which involves a tone (CS) paired with a puff of air to the eye (US) to elicit an eyeblink (CR) in a trace conditioning paradigm. Clear age-related deficits in this paradigm have been reported but are dependent on the CS–US interval (Solomon *et al*., 1991; Woodruff-Pak *et al*., 1999). Specifically, at shorter trace intervals (400 msec) older persons showed a slower rate of conditioning; however, the age differences disappear when longer intervals are imposed (1000–1500 msec). Persons diagnosed with early Alzheimer's disease also show slow acquisition compared to normal aged controls in this conditioning paradigm (Woodruff-Pak *et al*., 1990).

The value of the classical conditioning paradigm is the potential for direct comparisons between human studies and studies of animal models. Eyeblink conditioning studies have been used successfully in rabbits (Buchanan and Powell, 1988; Woodruff-Pak *et al*., 1990); however, rats and mice are less tolerant to the restraint procedures required in these protocols. Thus, there have been relatively few aging studies in rats and none in mice that have assessed the effect of aging on classical conditioning using eyblink or heart rate conditioning.

Heart rate deceleration has been studied as the CR in F344 rats with a tone as the CS and shock as the US (Buchanan and Ginn, 1988). An age-related decline in acquisition and magnitude of the CR was reported. Leg flexion as the CR has also been analyzed in F344 rats with deficits in conditioning observed in male but not females with a tone CS and shock as the US (Prescott *et al*., 1989).

To overcome problems with the use of restraint in rat conditioning studies, Weiss and Thompson (1992) assessed eyeblink conditioning in freely moving F344 rats with noise as the CS and a periorbital shock at the US in a trace paradigm. An age-related reduction in the rate of conditioning was noted. In a follow-up study, the same authors assessed both F344 rats and the F344 × Brown Norway hybrid (Weiss and Thompson, 1992). Conditioning was superior in the hybrid strain, but age-related decline in the rate of conditioning was still evident in both strains. This paradigm has been further developed for use in rats by introducing an air puff to the eye as the US (Weiss *et al*., 1999) and demonstrating hippocampal involvement in conditioning (Weiss *et al*., 1999), again in freely moving rats.

Besides the advantages of direct human–rodent comparisons, the classical conditioning paradigm also offers the advantage of providing control over noncognitive factors in assessing the effects of aging on performance. Specifically, age differences in response to the US and the CS and be assessed independently of the rate of conditioning either by using pseudoconditioning groups or by parametric manipulations of the variables. In humans and rats there appear to be conditions, such as simultaneous conditioning or under longer delays or traces where there no significant age effects are emerged. Age differences are observed under specific trace conditions, an observation that indicates that the defect is likely specific to cognitive variables.

B. Conditioned Taste Aversion

Conditioned taste aversion is another form or classical conditioning in which the CR can be learned after as few as one pairing of the CS with the US. Typically the CS is a novel tasting liquid (e.g., a saccharin flavored solution) paired with lithium chloride (the US), which will induce a malaise or gastrointestinal upset (UR). The subsequent CR is evidenced by the avoidance of the saccharin solution when the animal is given the opportunity to consume it during later trials, usually occurring 1–2 days after the conditioning. The dependent vari-

able is typically the absolute or percentage consumption of the CS.

There are several methodological advantages to the conditioned taste adversion paradigm. First, the conditioning is one-trial and robust with evidence of the learning remaining over intervals of several weeks. Second, the methodological control over noncognitive factors described above for other classical conditioning paradigms can be maintained. Third, no specialized equipment is required. The major disadvantage of the conditioned taste adversion paradigm is that direct comparisons to human experiments are virtually impossible.

No significant age differences in acquisition and retention of conditioned taste adversion have been noted in a variety of rodent strains including Wistar rats (Guanowsky *et al*., 1983; Martinez and Rigter, 1983), F344 rats (Ingram and Peacock, 1980), and C57BL/6J mice (Springer and Fraley, 1981). Typically the delay between CS and US is on the order of 10–60 min in these paradigms with the strength of resulting conditioned taste adversion decreasing as the interval is increased. However, when the delay between CS and US was increased to 3 hr, clear age differences emerged in Wistar rats (Hinderliter and Misanin, 1993). This difference is not due to a possibly greater US intensity because of a heavier body weight of older rats (Misanin and Hinderliter, 1994) nor to age differences in contextual cues (Misanin and Hinderliter, 1995).

Age differences emerge in during extinction testing in the conditioned taste adversion paradigm. In the classical conditioning paradigm, extinction is defined as the gradual loss of ability of the UC to elicit the CR when the UC is no longer present. An age-related increase in resistance to extinction of conditioned taste adversion has been reported in male F344 rats (Ingram and Peacock, 1980). However, another study using SD rats and multiple CS–US pairings found more rapid extinction in aged rats (Cooper *et al*., 1980). Differences in strain and methodology probably account for the discrepancies in conditioned taste adversion extinction.

C. Fear Conditioning

Conditioned responses, such as freezing, can be observed in relationship to environmental cues that were previously paired with an aversive stimulus, such as a brief foot shock. Such paradigms are referred to as *fear conditioning*. The main manipulation is the interval between the aversive conditioning and the observation of the CR. Environmental cues can also be manipulated to examine the impact of context on the response. The main dependent variable is the time spent freezing. Several procedural advantages can identified for this paradigm. As in conditioned taste adversion conditioning, typically only one pairing of the CS (the environment) and the US (the shock) is conducted. The behavioral variable (freezing) is easily observable and quantifiable. The equipment needed is minimal. The disadvantage is the relevance to human applications as noted for the conditioned taste adversion paradigm.

Recently this paradigm has come into extensive general use in mouse studies, but its application to gerontological studies is still rather limited. Oler and Markus (1998) and Houston *et al*. (1999) reported an age-related decrease in the retention of fear conditioning as a function of the delay interval between conditioning and test in F344 rats. Typically freezing behavior strengthens with time presumably because of the ''memory incubation.'' This phenomenon was observed in young rats, whereas, after 20 days reduced freezing behavior was reported in the aged rats (Houston *et al*., 1999).

III. Operant Conditioning

Operant conditioning differs from classical conditioning because the animal must actively operate in the process as opposed to the passive nature of classical conditioning. The animal must make the association between its behavioral response to experimentally controlled presentation of stimuli and reinforcement.

Operant tasks are typically conducted in small, enclosed chambers allowing for maximal control over cue presentation, behavioral responses, and reinforcement delivery. Cues are often lights, tones, or levers, the behavioral response is often lever pressing, and the reinforcement is generally food or water, which can be delivered on different types of schedules (fixed interval, fixed ratio, variable intervals, variable ratio, or combinations thereof). In the case of positive reinforcement, animals are typically placed on a deprivation schedule for food or water, while foot shock is used as negative reinforcement. Although many dependent variables can be examined, some measure of the number or percentage of correct responses represent the dependent variables investigated most frequently.

Studies of the effects of age on operant conditioning in rodents have produced conflicting results (Sarter, 1987). Summarizing many early studies of simple operant paradigms, Arenberg and Robertson-Tchabo (1977) reported both increases and decreases with age in lever pressing behavior. While operant paradigms can permit control of experimental conditions, many reports of age-related differences in bar pressing may reflect differences in general activity levels, motivation, and sensorimotor abilities of the animals rather than deficits in associative learning per se. For example, an initial impairment of aged male Wistar rats to learn to press one of two levers in a reversal task for a water reward was attributed to an age-related decrease in the probability of initiating a novel response (Stephens *et al*., 1985). Other evidence suggests that the reaction time of male Long–Evans rats becomes longer with age (Burwell and Gallagher, 1993). Thus, many variables must be considered to properly interpret an operant analyses of aging.

In male F344 rats no age difference was observed in the acquisition of a fixed interval reinforcement schedule for food (Campbell and Haroutunian, 1981), and only an initial impairment was observed in aged male SD rats trained in a differential reinforcement schedule (Hamm *et al*., 1983). Soffie and Lejeune (1991) noted that aged rats were impaired in a differential reinforcement ''low'' which indicated the animals ability to respond to changes in the reinforcement schedule.

For the clearest interpretation of age differences in operant performance, paradigms in which parametric manipulation is made of the experimental variables are most useful. Such paradigms would include delayed matching to sample (DMS) or delayed nonmatching to sample (DNMS) tasks. These tasks involve the presentation of a sample stimulus (a lever, light, or tone) followed by the presentation of the previous stimulus and a novel stimulus concurrently with a delay between the presentations typically ranging from 0 to 60 sec. In the DMS

task the animal must respond to the previous stimulus to receive the reinforcement, while in the DNMS task, to the novel stimulus. Performance in all age groups declines as a function of the delay between presentation of the first and second stimulus presentations. Dunnett *et al*., (1988) introduced these paradigms to gerontological research. At the 0-delay interval, aged rats were unimpaired in either the DMS or DNMS versions of the task. This observation was important for establishing experimental control over the paradigm. Aged rats were equally capable and motivated for performing the task. As the delay was increased from 0 to 24 sec, a clear age-related decline in performance was observed in both versions of the task. In a later replication of the these findings, Dunnett (1991) demonstrated that the delay-dependent deficits in the performance of aged rats were independent of any increased susceptibility to proactive interference, that is, the ability to remember the stimuli within the context of the demands that multiple similar trials place on memory processing. The observation of a delay-dependent deficit in operant responding with a DMS or DNMS task has been replicated in other rat studies (Roux *et al*., 1994; Soffie *et al*., 1999).

Winocur (1986, 1992) used a related version of the task in which LE rats were required to lever press when the brightness of two lights were the same and withhold a response when they were different. Age-related deficits in correct responses were evident at the longer delays (15 sec) between the two stimuli presentations. Similarly Pontecorvo *et al*. (1988) used a paradigm in which a stimulus pair (two consecutive stimuli, light or tone) was presented. The SD rats were reinforced for responding on one lever to a matching pair of stimuli and on another lever for a nonmatching pair. Again at short delays the performance of young and aged subjects was equivalent but the aged rats' performance at longer delays was impaired. Pontecorvo *et al*. (1996) proposed a distinction between delayed response tasks (those tasks for which all information necessary to determine the correct response is available prior to the retention interval) and delayed comparison tasks (those in which the subject must compare stimuli presented prior to and after the retention interval in order to determine the correct response). Delayed comparison procedures were considered to provide potentially purer assessment of the subject's memory capacities, but are also more difficult for rodents to acquire.

These DMS and DNMS tasks are the most useful for assessing the effects of aging on primary memory performance across short intervals within an high interference context. If aged animals can perform the task at short intervals equivalent to the performance of young animals, then control over noncognitive performance factors can be achieved. Another advantage of these types of operant paradigms is that similar designs can be used in assessment of cognitive aging in nonhuman primates (Buccafusco and Jackson, 1991) as well as in humans (Oscar-Berman and Bonner, 1985). What remains unclear, however, is the influence of attentional variables on operant performance of aged animals. Using operant paradigms that assess vigilance and sustained attention, it is clear that aged rats are impaired in these cognitive abilities (McGaughy and Sarter, 1995a,b), which could impact their performance in tasks requiring memory for highly similar stimuli across various delay intervals.

IV. Instrumental Conditioning

Instrumental conditioning again implies that the animal is actively involved in making the association between stimulus and response. Thus, the response typically precedes the stimulus; whereas, the opposite is the true for classical conditioning tasks. A ''reinforcement'' is also involved similar to operant conditioning. The reinforcement can be positive, such as food or water, or negative, such as foot shock. Instrumental conditioning is conducted in a variety of apparatuses.

A. Active Avoidance

The active avoidance paradigm requires the animal to make a locomotor response to avoid a negative reinforcement (e.g., foot shock). In its most common form the rat must move from one side of a two-sided chamber in response to a light or tone cue in order to escape or avoid the negative reinforcement. This is referred to as one-way avoidance. When the animal must return to the previous side of the chamber, such paradigms are referred to as two-way, or shuttle, avoidance. Failure to avoid the negative reinforcement is interpreted as a failure to associate the cue with the necessary response. Other variations of the one-way avoidance paradigm exist such as the following: (1) step-up to a platform, (2) jump onto a pole, (3) run to a particular side of a T- or Y-maze. The primary dependent variable is the number or percentage successful avoidances of the negative reinforcement.

In early studies examining the effect of age on active avoidance performance, there was a lack of consistent results (see reviews in Arenberg and Robertson-Tchabo, 1977; Goodrick, 1980; Sarter, 1987; Barnes, 1990). However, there have been reports of significant age-related decline in acquisition and retention in these tasks. Rat studies supporting this claim include two-way avoidance paradigms (Nakamura and Ishihara, 1989; Valerio *et al*., 1989; Petkov *et al*., 1990; Ghirardi *et al*., 1992), although one study in F344 rats reported superior performance among the aged group (Vasquez *et al*., 1983). An age-related decline in retention of the avoidance response was reported for F344 rats in a earlier study (Gold *et al*., 1981). Using a one-way active avoidance task requiring light–dark discriminations in SD rats, Thompson and Fitzsimons (1976) noted age-related decline in acquisition and retention of the avoidance response. Arendash *et al*. (1995) used a pole jump active avoidance task to document an age-related impairment in performance in SD rats.

Mouse studies have employed a variety of different paradigms other than shuttle type tasks. Using a step-up one-way active avoidance task, Stavnes and Sprott (1975) noted an age-related decline in the performance of C57BL/6J mice but no age differences in DBA/2J which exhibited generally poor performance at all ages. The findings in C57BL/6J mice were replicated by Forster *et al*. (1988) who also noted an age-related decline in retention of this avoidance response over a 48-hr period. In the same study, strains with various degrees of autoimmunity (NZB/BINJ, MRL/MpJ-1pr, BXSB/MpJ, MRL/Mpj-+, and NZB-WF1/J) also showed the age-related decline in performance that was related to their degree of autoimmunity.

Investigators have many reasons for examining active avoidance performance. The protocols, particularly for two-way paradigms, can be automated and the ability for analyzing acquisition and retention is available. Problems with such paradigms for gerontological research that have not been addressed in the parametric fashion reported for operant tasks include possible age differences in aversive threshold of shock, reaction to shock, sensory detection of the stimuli, exploratory activity and emotionality. Aged animals would appear to be handicapped in the main performance requirements in such tasks, specifically quick response to the sensory stimulus and rapid movement to a safe area. In addition, translation to human studies is impossible, and the memory processes being assessed are difficult to discern. Moreover, because of the stressful reinforcement being used, many of the paradigms, particularly the two-way avoidance tasks, generate approach-avoidance conflicts for the animal. Specifically the animal must return to a place where it had previously been punished. All these factors combine to complicate the application of such paradigms for aging research.

B. Passive Avoidance

Passive avoidance tasks offer many advantages over active avoidance tasks in the control of noncognitive performance factors. In passive avoidance tasks, rodents are trained to inhibit a natural response. In a typical paradigm, the animal is placed in a two-chamber apparatus that is designed to promote movement from one chamber to an adjacent chamber. For example, driven by their general photophobia, rats and mice naturally move from a brightly lighted compartment to a darkened one. Upon entry to this chamber, a foot shock is delivered (eg., 1–3 sec in duration) and the animal is quickly removed. After a delay ranging from a few seconds to several days, the animal is returned to the bright compartment. The dependent variable is the time spent in the lighted chamber during this test trial, typically referred to as ''latency.'' Movement into the dark chamber is interpreted as a failure to recall the previously delivered foot shock. Increasing the delay between the original trial and the second ''memory'' trial parametrically increases the memory load of this task. Other versions of this task can involve a ''step-down'' avoidance in which the animal must inhibit its tendency to move from a narrow shelf onto a more open floor where it had received footshock previously.

A potential major confound in the paradigm would be if aged animals displayed differences in their initial entrance into the darkened compartment or onto the floor where they had previously received foot shock. In addition, if they showed impairments shortly after the conditioning experience, this would confound the interpretation of a memory defect. For example, if aged animals showed reduced latency a few minutes after the conditioning trial, then the impairment would have to be considered as a defect in conditioning, or acquisition, rather than memory, or retention. Importantly, in nearly all studies using the passive avoidance paradigm, it would appear that neither potential confound represents a major problem. In general, aged rats show no differences in their initial entry into the darkened chamber, nor are they impaired relative to young rats at short delays. As examples, latency was equivalent among aged and young F344 rats at short (1- to 2-hr) delays but impaired at longer acquisition–retest intervals of 4 hr up to 6 weeks (Lippa *et al*., 1980; Gold *et al*., 1981; Collier *et al*., 1988; Komiskey *et al*., 1988). Similar results have been reported for a variety of rat strains, males and females, when the delay interval is at least 24 hr (Martinez and Rigter, 1983; Jaenicke *et al*., 1991; Miettinen *et al*., 1993; Riekkinen *et al*., 1996; Silva *et al*., 1996; Vannucchi *et al*., 1997). In reviews of the literature, Bartus *et al*. (1982a,b) and Martinez *et al*. (1988) concluded that old rats forget newly acquired information more rapidly than do young rats.

Similar findings have emerged from mouse studies involving a variety of strains and paradigms (Bartus *et al*., 1980; Dean *et al*., 1981; Kubanis *et al*., 1982; Gower and Lamberty, 1993; Scheuer *et al*., 1995; Carrie *et al*., 1999; Reddy and Kulkarni, 1999). However, results are not always consistent. For example, Puglisi-Allegra *et al*. (1986) reported an age-related decline in passive avoidance retention in BALB/c mice but not in C57BL/6J mice. Several previous studies had documented that the latter strain is deficient in this performance (e.g., Dean *et al*., 1981; Kubanis *et al*., 1982). One strain that has shown consistent age-related decline in passive avoidance retention in numerous studies is the P8 line of senescence accelerated mice (SAM/P8) (see review in Flood and Morley, 1998).

One of the strengths of the task is also its weakness. Although one-trial paradigms, such as passive avoidance, are quickly learned, they lack a performance curve. This makes it difficult to separate age-related increases in forgetting from age-related decreases in acquisition. In fact, when the procedures are altered so that it takes the rat more than one trial to learn the task (multiple-trial passive avoidance), aged F344 rats did not show accelerated forgetting compared to young rats (Bartus *et al*., 1982a,b). There are also problems with possible motivational factors to consider. The passive avoidance task is touted as a measure of forgetting, but it is forgetting within an ''approach-avoidance'' conflict. The animal must choose between one naturally aversive spot (light chamber) and another that is preferred (dark chamber) but has been associated one time previously with negative consequences. Another problem is that parallels between the passive avoidance task and human memory tasks are difficult to draw.

C. Maze Learning

Various maze learning tasks have provided the most used instrumental conditioning paradigms in gerontological research in rodents. Maze learning requires locomotion beginning from a specified start area and proceeding through an artificial environment in which the animal must acquire and integrate position discriminations (go left: go right) or spatial locations aided by specific visual cues provided in the environment. Rodents are quite adept in such behavior. In positive reinforcement paradigms, the animals are either food- or water-deprived and provided with the respective reinforcement in a ''goal'' area. In negative reinforcement paradigms, escape or avoidance of footshock, water, or bright light can be used. Simple mazes offer a limited number of discriminations (one

to four choices), whereas the memory demands can be manipulated by introducing delays after informational trials or by introducing other learning events as interference events. Complex mazes usually have a great number of choices to be made or require the use of a myriad of stimuli in order to locate the goal.

1. Simple Maze Learning

Spontaneous alternation refers to the observation that when given repeated exploratory trials in a simple T-maze or Y-maze, rats and mice tend to naturally alternate between choosing the right or the left arm of the maze in the absence of any specifically supplied reinforcement. It is hypothesized that these rodents prefer to explore the more novel part of the maze as part of an evolutionary adapted foraging behavior. Failure to alternate is generally interpreted as failure of the animal to recall the arm entered on the previous trial. Performance is measured as the percentage alternation across several trials, typically more than 10. The intertrial interval can be increased from a few seconds to hours to increase the demand on memory.

Results have been generally mixed with regard to age differences observed in spontaneous alternation. With short intertrial intervals (<3 min), no age differences were observed in rats from several different strains including F344, Brown Norway, and SD (Barnes, 1979, 1990; Zornetzer *et al*., 1982; Bhatnagar *et al*., 1997; Stemmelin *et al*., 1999). However, with the introduction of longer delays, aged rats exhibit less alternation than young rats (Winocur and Hasher, 1999; Willig *et al*., 1987; Zornetzer *et al*., 1982); however, the results are not always consistent and may depend on strain and delay interval (Barnes, 1990). Age-related decline in spontaneous alternation has been observed in mouse studies as well (Lamberty and Gower, 1992; Miller *et al*., 1999), including the SAMP/8 strain (Maurice *et al*., 1996).

The appeal of this task is the simplicity of apparatus and procedure. Age-related decline in memory performance is most convincing when the delay interval is manipulated parametrically (e.g., Zornetzer *et al*., 1982) to demonstrate that the age difference in performance is not present at short intervals but emerges at longer intervals. The weakness of the task is that the behavior is not contingent upon overt reinforcement. It is presumably ''spontaneous''; thus, there is no penalty to the animal for a mistake. This situation should stimulate consideration of motivational factors; maybe aged animals perform worse at longer intervals for factors other than memory.

When the same type of simple apparatus used in spontaneous alternation is used with a reinforcement, the task becomes a learning task requiring a two-choice discrimination. The discrimination can be for position (left-right) or between visual stimuli. The reinforcement can be a positive one to obtain food or water provided in the maze or to escape or avoid a negative reinforcement such as foot shock or water.

Thus, in this simple two-choice positively reinforced situation when the intertrial interval is generally short (<60 sec), rats and mice show no age-related learning impairment (Barnes *et al*., 1980; Dean *et al*., 1981; Sarter and Markowitsch, 1983; Wincour, 1984; Lowy *et al*., 1985; Stephens *et al*., 1985). Spangler and Ingram (1986) noted no significant age differences in the performance of C57BL/6J mice in simple visual discrimination task requiring escape from water. Thus, analysis of discrimination learning requiring few choices and short intertrial intervals reveals little evidence of age differences. Age differences in performance do emerge when a reversal of a simple discrimination is imposed; that is, the correct response must be made to the previously negative stimulus. Such findings have been reported for mice (Dean *et al*., 1981) and rats (Stephens *et al*., 1985) and are interpreted to represent an age-related increase in perseverative behavior (Arenberg and Robertson-Tchabo, 1977). Similarly, if some event designed to interfere with learning, such as training with similar stimuli, is imposed during the intertrial intervals, then age differences are observed (Winocur, 1984, 1988).

The difficulty of the two-choice discrimination task can be increased in several additional ways. For example, if the discrimination task is modified so that the rat must alternate left and right choices on successive trials with a 5-sec delay, aged F344 rats were impaired relative to young rats (Greene and Naranjo, 1987). Similarly, Ritzmann *et al*. (1993) noted age-related decline in the performance of Swiss–Webster mice with a delay in alternation of 30 sec but not 10 sec.

As another example, in a forced-choice, delayed nonmatch-to-sample (DNMS) task, one arm of the T-maze is blocked in the *sample* trial, forcing the rat to enter the arm containing the reward (i.e., food or water). Memory for the arm just visited is assessed in the choice trial where the rat is free to enter either arm. The reward is located in the arm not visited in the sample trial. Thus, entry into the arm visited in the sample trial is considered a memory error. Including a delay between the sample and the choice trial can parametrically increase the memory demand of the task. Aged rats are only mildly impaired in this task when short (< 5 sec) delays are imposed, but show increasing impairments at longer delays (Ordy *et al*., 1988; Aggleton *et al*., 1989). A water escape paradigm can also be used to in a similar fashion to show age-related decline in the performance of rats (Means and Kennard, 1991; Markowska *et al*., 1996). Willig *et al*. (1987) suggested that such impairments can be reduced or eliminated by habituating old rats to the test apparatus.

Another version of this task is a split-stem T-maze that can permit simultaneous examination of two different types of discriminations. The stem of the T-maze is divided; one side allows access to the arms, and the other side is a dead end. The correct path is constant for every trial; thus, entries into the incorrect side are interpreted as errors in reference memory. Reference memory refers to maintenance of information that remains invariant across trials, e.g., always go to the left here. In the arms of the maze, an alternation response is rewarded. Thus, entries into the arm most recently visited are interpreted as errors in working memory. Working memory requires use of temporary and changing information, (e.g., go left now because I went right a few minutes ago). While Ordy *et al*. (1988) observed an age-related impairment only in the working memory component of this task with Long–Evans rats, Lowy *et al*. (1985) noted age-related impairments in both components of American Cancer Institute rats.

Age differences in the performance of simple discriminations in a T-maze can be expanded to examine retention perfor-

mance as well. Flood and Roberts (1988) and Flood and Morley (1990) employed a one-way active avoidance task for mice that measured acquisition and retention of a position discrimination (left–right) in a T-maze. A weak criterion to acquisition was used (one avoidance response within the first 25 trials). Aged C57BL/6 mice exhibited impaired acquisition measured as the number of trials to criterion, and they also exhibited impaired retention which was evaluated by trials to criterion 1 week later. This paradigm has also been used extensively to assess the age-related impairment observed in the SAM/P8. Performance deficits were noted to occur much earlier in this line than in the R1 line as well as C57BL/6J mice (see review in Flood and Morley, 1998).

2. Complex Maze Learning

Complex mazes require multiple position discriminations for learning the correct pathway from a start area to a goal area. Two of the oldest types of complex mazes used in behavioral research are the Hebb–Williams maze series and the Stone 14-unit T-maze.

The Hebb–Williams maze consists of 12 different configurations of barriers within an enclosed arena. The animal is usually given repeated trials in each of several or all versions, and they have access to intra- and extramaze visual cues. Errors are counted as turns made that do not represent the most direct pathway to the goal. This scoring is typically conducted by the experimenter. Although this maze has had extensive use to examine the effects of specific brain lesions on learning, it has had little use in gerontological studies. Using two of mazes in the Hebb–Williams series, Winocur and Moscovitch (1990) reported that aged rats exhibited impaired learning for food rewards.

Compared to the Hebb–Williams maze, the Stone 14-unit T-maze has had much more extensive use in gerontological studies. This maze is enclosed and requires the rodent to make a series of left–right position discriminations to correctly locate the reward at the end of the labyrinth. Salient visual cues are not provided, so it is deduced that the rat must rely on an internally driven response algorithm to solve the task (Ingram, 1988). It learns a route rather than a spatial location. Errors are counted as movement in the wrong direction, whether into cul-de-sacs or retracing a pathway previously negotiated during the trial. Using a food motivated version of the maze in Wistar rats, Goodrick (1968, 1972, 1973, 1975) demonstrated the significant age-related decline in learning performance in this maze. Other laboratories employing different training schedules, motivational conditions, and rat strains have also observed the age-related learning impairment (Jordan and Sokoloff, 1959; Berman *et al*., 1988; Bratt *et al*., 1994a,b). In a series of studies involving a variety of rats and mice, Ingram (1988, 1996), has also demonstrated the robust age effects observed in learning this complex maze task (Ingram *et al*., 1994). In these studies footshock avoidance is used as the aversive motivation. In this version scoring of errors is done automatically by a microprocessor.

Another form of a complex enclosed maze is the *tunnel maze*, which utilizes no specific reinforcement but rather relies on the natural tendency of rats to explore their environment in an efficient manner, similar to that seen in spontaneous alternation tasks. The apparatus consists of six arms radiating from a center area, and each arm contains a small blind alley that branches off from a fixed location. No external visual cues are available to the rat. Entries into arms previously visited on that trial are considered less efficient exploration interpreted as errors of working memory. Entries into blind alleys are considered reference memory errors. In Wistar and SD rats age-related increases in working but not reference memory errors were observed (Jucker *et al*., 1988; Welzl *et al*., 1988).

Several issues should be addressed regarding interpretation of the age effects observed in the mazes described above. First, in those tasks in which food motivation is used, the question can arise whether the age differences in performance are due to age differences in the motivation for food. Typically rats are food deprived for a few weeks to arrive at a target body weight, e.g., 80% of baseline, prior to maze training. Since younger rats are generally lighter and leaner, this level of deprivation could be greater for them compared to aged rats and thus produce a greater level of deprivation. Goodrick (1972) addressed this possible confound regarding age differences in Stone maze performance by reducing the body weight of age rats to 70% compared to 80% for young rats. Even with this procedural control, there was no attempt to demonstrate equivalent motivation for food. In the shock motivated version of this maze, Ingram (1988) employed a pretraining procedure in which the rats are required to show a high level of competence in shock avoidance in a runway to offset arguments about decreased motor and motivational factors contributing the age-related impairment in maze learning.

3. Complex Spatial Maze Learning

The most widely used maze learning paradigms in gerontological research are those that assess the rodent's ability to locate a goal using spatial cues. These tasks require integration of information from various sensory sources—vision, proprioceptive, vestibular—and thus heavily tax memory processes. Abilities demonstrated in these tasks are considered highly ecologically relevant to rodents because they must remember the spatial distribution of multiple goals in an environment (i.e., food and water sources in relation to a home burrow) to promote survival in their niche. Investigators have capitalized on the natural ability of rodents to remember spatial locations in the design of several spatial memory tasks.

The most widely used paradigm to assess spatial memory in rodents in the Morris water maze. This is somewhat of a misnomer because the apparatus is not a maze in the strictest sense. In its most basic form, a large circular tank is filled with opaque water and equipped with an escape platform (the goal) located slightly below the surface of the water, which is maintained at room temperature. On each trial the animal is placed in the tank at different start points and allowed to explore until finding the goal or until a predetermined time has elapsed. During the first few trials, the animal swims randomly in the tank until it finds the hidden platform by chance. After repeated trials, rodents learn to swim quickly and more directly to the goal. Because the platform is hidden below the water, the animal must rely on the distal visual cues in the room to learn the location of the platform within the tank.

As a separate measure of spatial memory, a ''probe'' trial in which the goal is removed is often run at the end of training (typically at least 16–20 trials) or interspersed with the learning trials, for example, every 4–6 trials. The tendency of the animal to search for the goal in its previous location is interpreted as a demonstration of a spatial map of the environment. Again, because the animal must use the spatial configuration of the distal cues to locate the goal, this task is presumed to assess spatial memory.

During acquisition, the typical dependent variables measured include swim time or distance to the platform on each trial. For the probe trial, dependent measures can include the percentage time or distance spent in the region near the location previously occupied by the platform. A more exact measure tallies the number of times the animal crosses over the exact platform position. In its simplest application, data are collected in the water maze task by recording time of the parameters of interest. However, most laboratories are now equipped with automated video tracking systems that record distances as well as time.

An age-related decline in acquisition in this task, measured as time or distance to the hidden platform, has been demonstrated in rats of various strains in numerous studies (Gage *et al*., 1984; Biegon *et al*., 1986; Rapp *et al*., 1987; Markowska *et al*., 1989; Barnes *et al*., 1992; Frick *et al*., 1995) and in C57BL/6J mice (Fordyce and Wehner, 1993; Forster *et al*., 1996; Magnusson, 1997; Frick *et al*., 2000). Probe trial performance also exhibited an age-related decline.

Although the overall age effect appears robust in this task, many investigators report their results as a bimodal distribution (Baxter and Gallagher, 1996). Among groups of aged rats tested, many exhibit performance that is equivalent to that of young rats, either during acquisition or during the probe trial, and are those referred to as ''age unimpaired,'' whereas those aged rats that show clear differences from the young group are referred to as ''age impaired.'' This dichotomous performance groupings can then be used for correlative analyses focusing on various neurobiological parameters as well as identifying appropriate groups for interventive studies. While this approach has been useful for many investigators, others have not observed this type of dichotomous performance in certain rat strains (Lindner, 1997).

The basic features of the task can also manipulated to create other versions of this maze. For example, the location of the platform can be moved on every other trial. The first trial is treated as an information trial, and then the interval between the first and second trials can be manipulated from a few seconds to several minutes to produce a delayed response task. Using such paradigms, age-related declines in performance have been noted in different rat strains (Lindner *et al*., 1992; Shukitt-Hale *et al*., 1998).

While the numerous results from studies of swim maze performance indicate a very robust phenomenon, several caveats must be considered. Many of the studies assessing the ability of aged rodents in the water maze used swim time only as the dependent measure. This procedure is problematic because assessment of cognitive performance might be confounded by age differences in motor performance and motivation. For example, if aged animals swim directly to the goal, but slower than young animals, the dependent measure of swim time would incorrectly lead to conclusion that aged animals had impaired memory for the goal location. Obviously including swim distance as a dependent measure as well avoids this issue. Nonetheless, other possible performance confounds can affect the interpretation of age differences in this task. For example, Rick *et al*. (1996) have suggested that performance impairments observed in aged F344 rats in the swim maze could be due in part to increased fatigue and thermal stress relative to that experienced by young rats.

Another procedural problem can be observed during the first few trials when the animal has not yet had the necessary experience to form a memory for the platform location. Because the animals are randomly searching for the platform at the start of testing, the early performance of animals that will be identified as poor performers at the end of testing should be the same as those animals that will be identified as good performers. Statistically significant differences between young and aged animals in the first few trials suggest that noncognitive factors are responsible, such as impaired swimming ability. Furthermore, if a statistically significant difference in the first few trials is followed by an identical rate of learning between young and aged animals, then again noncognitive factors are suspected.

As with all memory paradigms, the investigator can interpret only performance differences. It should be clear that the water maze is heavily dependent upon the use of visual cues. So not only can there be confounding due to motor impairment in aged rats, sensory confounding is also possible if the aged rat has impaired vision and cannot use the visual cues. For example, Spencer and Raz (1995) identified retinal degeneration in aged SD, the degree of which was correlated with swim maze performance.

Thus, in a visually guided task such as this, it is critically important to show that observed age-related impairments in performance are not due to deficits in sensory, motor, or motivational factors. The standard control procedure for this task requires the subject to swim to a visible platform, thus making the task one of visual discrimination. Although this procedure might be a suitable control to conclude that the age groups examined were equivalent in the motivation and motor requirements, it is not completely appropriate for a similar conclusion regarding visual abilities. After all, demonstrating that a rat can see a platform in the water tank is not equivalent to demonstrating that they can see the distal cues. Gallagher and Pelleymounter (1988), have shown age-related impairment in the water maze even when the cues are made so salient that aged rats could easily discriminate them.

Other studies have underscored the need for correlation between performance in acquisition and probe trials. For example, in a study of male 129SvJ mice, Hengemihle *et al*. (1999) documented a marked age-related decline in performance (distance to platform) during acquisition, but not during the probe trials. This mouse strain had unique performance disabilities, particularly a visual pathology that affected the older mice. Such results argue against the use of this strain as a model of age-related memory impairment.

Another example of such problems in mice is presented in the results of Markowska *et al*. (1998). Marked age-related differences in the performance of SAM/P8 mice during acquisition were recorded for swim time but not distance measures. In

addition, when probe trial performance was recorded as percentage time in the quadrant, no significant age differences were observed; however, when probe performance was measured at the number of platform crossings, there was an age-related decline in performance. This discordance in results in addition to other characteristics of this mouse strain, such as the tendency to float among the older mice, would also render this strain less useful as a model in this behavioral paradigm. Other investigators have noted the difficulties that have been observed in assessing swim maze performance of mice in general compared to the relative ease with which many rat strains can be trained in this task (Whishaw, 1995, Whishaw and Tomie, 1996).

The radial arm maze, or the Olton maze, is another paradigm used to assess spatial memory in rodents that has been applied in aging studies. For this task either a food or water reward is placed at the end of several arms (4–17) radiating from a central platform. The optimal strategy is to visit each of the baited arms without repeating entries into previously entered arms. Although several versions of spatial memory can be assessed with this apparatus, most studies examining the effect of age consistently bait all or a subset of the arms on repeated trials and record arm entries. Entries into never baited arm are interpreted as memory errors for the invariant aspect of the task, or reference memory, while errors into arms previously visited on that trial are interpreted as memory errors for the flexible, working memory store, or memories that need to be reset at the start of each trial.

Age-related impairments in reward retrieval efficiency have been found consistently in many rat strains (Wallace *et al*., 1980; Ingram *et al*., 1981; De Toledo-Morrell and Morrell, 1985; Willig *et al*., 1987; Kobayashi *et al*., 1988; Barnes, 1990; Caprioli *et al*., 1990; Stewart and Reid, 1994; Noda *et al*., 1997; McLay *et al*., 1999; Ward *et al*., 1999). Variations of the radial maze paradigm that attempt to dissect working and reference memory components of the performance show age-related impairment in both components, although some reports indicate greater deficits in the reference memory component (Arendash *et al*., 1995).

Other variations of the paradigm are also possible. Winter (1997) described age differences in the performance of F344 rats in a continuous learning paradigm in which different arms are baited with food, and the rats are not allowed to move to the next version until a criterion is met. Intervals can be imposed between choices. Chrobak *et al*. (1995) described an age-related increase in errors made by SD rats as the delay between choices is lengthened. Environmental cues can be changed to determine how flexible responding differs with age. Tanila *et al*. (1997) noted that aged rats apparently encode only part of the available information to them, but do so in a highly rigid fashion.

When rats were tested in nonspatial versions of the radial arm maze task, no age-related impairments in F344 rats was observed (Barnes *et al*., 1987). This observation is important in interpreting that age deficits observed in spatial versions actually represent defects in spatial memory processing. Moreover, with overtraining, old rats can reach a given level of performance and achieve the same level as young rats (Barnes *et al*., 1980; Gallagher *et al*., 1985), suggesting that the sensory/motor abilities of the aged rats are sufficient to solve the task. When these overtrained rats must learn new environmental cues, the age-related impairments reappear (Gallagher *et al*., 1985). There is also evidence to suggest that aged rats use a more rigid response algorithm to solve the radial arm maze task, perhaps as compensation for impaired spatial memory (Kobayashi *et al*., 1988).

The radial maze paradigm has not seen heavy utilization in mouse studies. Similar to the problems with translation of the Morris maze paradigm for use with mice, similar difficulties have affected the application of the radial maze paradigm to mouse studies. Indeed, a couple of studies of C57BL/6J mice have noted no age differences in performance (Bernstein *et al*., 1985; Ammassari-Teule *et al*., 1994). Even in the SAMP/8 mouse, no age effects in working or reference memory components have been reported (Ikegami *et al*., 1992). However, in the BDF1 hybrid strain, Ikegami (1994) noted significant age-related decline in both working and reference memory components.

The radial arm maze is a highly useful paradigm for aging research because of several features. First, rodents are generally well adapted to such tasks. Second, working memory and reference components of performance can be analyzed separately. Third, task demand on memory can be manipulated by imposing delays between choices. Fourth, the task can be used for long-term within-subject analysis, unlike many other mazes. Despite these strengths, there are also weaknesses in this paradigm for gerontological applications. Similar to other tasks using food motivation, questions arise as how to equate motivational levels across age groups. Second, like the water maze task, efficient performance in the radial arm maze requires use of visual cues, usually located in the extramaze environment. Researchers rarely attend to these possible confounds. In addition, some researchers often neglect to hold the rat in the center arena for a few seconds between choice-trials. Ignoring this procedure will likely result in the rat (young and aged) exhibiting a response strategy, such as visiting arms directly across from the last one entered or visiting only adjacent arms. Design of a good experiment using the radial arm requires attention to all these possible factors.

The circular platform, or Barnes maze, is a spatial memory task that does not require the use of food deprivation or water escape for motivation, but relies on photophobia, or the natural tendency of rats and mice to avoid wide-open, brightly lit environments, favoring instead small dark enclosures. This motivation is arguably less stressful on the aged subject than water escape, shock, or food deprivation.

The animal is placed on an open circular platform that has 18 equally spaced holes distributed around the perimeter. The animal must locate the single hole (in a fixed position) that leads to the darkened goal box by remembering the position of the rewarded location in relation to the distal environmental cues surrounding the platform.

Aged mice (Bach *et al*., 1999) and rats of several strains (Barnes, 1979; Barnes *et al*., 1980; Barnes and McNaughton, 1985; Markowska *et al*., 1989; McLay *et al*., 1999) are impaired at learning and remembering the location of the goal location compared to young cohorts. This finding provides convergent evidence (by use of a different task) supporting the overall conclusion that aged rodents are impaired in spatial memory compared to young cohorts.

One of the same procedural concerns as discussed for the swim maze applies to the use of circular maze. Specifically, the ability of the animal to locate the goal is highly dependent upon its use of extramaze visual cues.

4. Relevance of Mazes

The major problem with virtually all rodent models of learning and memory paradigms is their relevance to tasks used to assess human performance. While many of the aversive tasks will likely never have human counterparts because of ethical considerations, there is increased use of mazes for analysis of human memory. Using computer-generated graphics, virtual Morris mazes (Astur *et al*., 1998) and radial arm mazes (O'Connor and Glassman, 1993; Glassman *et al*., 1994) have been developed as well as other types (Kahana *et al*., 1999). With increased use of these paradigms in human studies, their application in rodent models increases in value. If parallels can be drawn regarding the effects of aging as well as specific brain lesions and pharmacological manipulations on performance of rodents and humans in similar mazes, then greater predictive validity will accrue to use of maze learning as models of age-related memory impairment.

V. Conclusions and Caveats

A variety of tasks have been used to assess age differences in learning and memory processes in rodent models. Similar to the literature on age-related changes in learning and memory in humans, rodents have defects in short-term memory processing in which demands are placed by increasing the number of items to be learned or the interval in which they must be held in memory. Thus, aged rodents exhibit no difficulty in learning simple mazes or operant tasks. However, if the number of decisions is increased (e.g., beyond six choices in a maze) or the interval between stimulus and response is increased, significant age differences emerge. Such performance deficits indicate defects in working memory, in both capacity and duration. Working memory also appears to be highly susceptible to interference effects among aged subjects. Age-related impairment is also observed in reference memory tasks when the situation requires integration of a complex variety of cues, such as in complex mazes, particularly those involving spatial navigation. Despite this emphasis on complexity as a major factor for observing age differences in performance, the results of studies of classical conditioning reveal age differences in simple reflex learning. Again, increasing the interval between stimuli generally increases the age differences observed.

Several caveats can be considered regarding this area of research. One is relevance of the rodent models to human memory performance and the tasks used to assess this. Many of the tasks used in rodents, particularly aversive tasks, have little chance of meaningful generalization to humans. However, several tasks offer this potential. Classical conditioning is a leading candidate in this regard. DNMS tasks can also provide generalization across a number of species, including rodents to human and nonhuman primates. The analysis of maze learning is offering new opportunities for species generalization as virtual mazes are being used increasingly in human studies.

Another caveat is whether investigators exercise sufficient control over noncognitive factors when using various tasks to analyze age differences in learning and memory performance. Control over sensory, motor, and motivational factors is not always apparent. Tasks which conduct parametric analysis in which responses of the aged animal are shown to be equivalent under one condition, e.g., a short retention interval, but impaired under another in which the performance requirements are the same, e.g., a long delay interval, offer the potential for control over noncognitive factors (Olton and Markowska, 1988). Another caveat in interpreting age differences in cognitive performance is the role of attentional factors. Some attempts have been made to assess age differences in attentional factors using an operant paradigm (Moore *et al*., 1992), but more effort is needed in this area, and such assessments should be considered when designing tasks. Also, time of testing has not been given adequate consideration in design of studies. Winocur and Hasher (1999) demonstrated that aged rats were highly affected by the time of day when testing occurred, whereas younger rats were not. A final caveat is the issue of environmental factors. In virtually all applications, rodent models of aging are reared in environments offering minimal stimulation. Several studies have demonstrated that environmental enrichment, such as adding objects to the home cages over a period of many weeks, can improve the performance of aged rats (van Gool *et al*., 1985; Berman *et al*., 1988; Van Wass and Soffie, 1996; Winocur, 1998). Although it should be noted that many of the age differences in performance still exist in these studies even after environmental enrichment, additional consideration of environmental design for rodent studies could be given in future efforts for developing rodent models of age-related memory impairment.

The area of research covered in this review has shown increasing interest generated by the search for rodent models of aging and Alzheimer's disease to investigate neurobiological mechanisms as well as assess possible therapeutic interventions. The area will likely generate even more progress through greater consideration for standardization of procedures to assure more valid assessment of learning and memory processes and generalization to the human condition.

References

Aggleton, J. P., Blindt, H. S., and Candy, J. M. (1989). Working memory in aged rats. *Behav. Neurosci*. **103**, 975–983.

Albert, M. S. (1997). The ageing brain: Normal and abnormal memory. *Philos. Trans. R. Soc. London, Ser. B* **352**, 1703–1709.

Ammassari-Teule, M., Fagioli, S., and Rossi-Arnaud, C. (1994). Radial maze performance and open-field behaviours in aged C57BL/6 mice: Further evidence for preserved cognitive abilities during senescence. *Physiol. Behav.* **55**, 341–345.

Arenberg, D., and Robertson-Tchabo, E. A. (1977). Learning and aging. *In* "Handbook of the Psychology of Aging" (J. E. Birren and S. W. Schaie, eds.), pp. 169–196. Van Nostrand-Reinhold, New York.

Arendash, G. W., Sanberg, P. R., and Sengstock, G. J. (1995). Nicotine enhances the learning and memory of aged rats. *Pharmacol. Biochem. Behav.* **52**, 517–523.

Astur, R. S., Ortiz, M. L., and Sutherland, R. J. (1998). A characterization of performance by men and women in a virtual Morris water task: A large and reliable sex difference. *Behav. Brain. Res*. **93**, 185–190.

Bach, M. E., Barad, M., Son, H., Zhuo, M., Lu, Y. F., Shih, R., Mansuy, I., Hawkins, R. D., and Kandel, E. R. (1999). Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation in vitro and attenuated by drugs that enhance the cAMP signaling pathway. *Proc. Natl. Acad. Sci. U. S. A.* **96**, 5280–5285.

Barnes, C. A. (1979). Memory deficits associated with senescence: A neurophysiological and behavioral study in the rat. *J. Comp. Physiol. Psychol.* **93**, 74–104.

Barnes, C. A. (1987). Neurological and behavioral investigations of memory failure in aging animals. *Int. J. Neurol.* **22**, 130–136.

Barnes, C. A. (1990). Animal models of age-related cognitive decline. *In* ''Handbook of Neuropsychology'' (F. Boller and J. Grafman, eds.), pp. 169–189. Elsevier, Amsterdam.

Barnes C. A. (1998). Memory changes during normal aging: Neurobiological correlates. *Neurobiol. Learn. Mem.*, pp. 247–287.

Barnes, C. A., and McNaughton, B. L. (1985). An age comparison of the rates of acquisition and forgetting of spatial information in relation to long-term enhancement of hippocampal synapses. *Behav. Neurosci.* **99**, 1040–1048.

Barnes, C. A., Nadel, L., and Honig, W. K. (1980). Spatial memory deficit in senescent rats. *Can. J. Psychol.* **34**, 29–39.

Barnes, C. A., Green, E. J., Baldwin, J., and Johnson, W. E. (1987). Behavioural and neurophysiological examples of functional sparing in senescent rat. *Can. J. Psychol.* **41**, 131–140.

Barnes, C. A., Rao, G., Foster, T. C., and McNaughton, B. L. (1992). Region-specific age effects on AMPA sensitivity: Electrophysiological evidence for loss of synaptic contacts in hippocampal field CA1. *Hippocampus* **2**, 457–468.

Bartus, R. T., Dean, R. L., Goas, J. A., and Lippa, A. S. (1980). Age-related changes in passive avoidance retention: Modulation with dietary choline. *Science* **209**, 301–303.

Bartus, R. T., Dean, R. L., and Beer, B. (1982a). Neuropeptide effects on memory in aged monkeys. *Neurobiol. Aging* **3**, 61–68.

Bartus, R. T., Dean, R. L., 3rd, Beer, B., and Lippa, A. S. (1982b). The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217**, 408–414.

Baxter, M. G., and Gallagher, M. (1996). Neurobiological substrates of behavioral decline: Models and data analytic strategies for individual differences in aging. *Neurobiol. Aging* **17**, 491–495.

Berman, R. F., Goldman, H., and Altman, H. J. (1988). Age-related changes in regional cerebral blood flow and behavior in Sprague-Dawley rats. *Neurobiol. Aging* **9**, 691–696.

Bernstein, D., Olton, D. S., Ingram, D. K., Waller, S. B., Reynolds, M. A., and London, E. D. (1985). Radial maze performance in young and aged mice: Neurochemical correlates. *Pharmacol. Biochem. Behav.* **22**, 301–307.

Bhatnagar, M., Cintra, A., Chadi, G., Lindberg, J., Oitzl, M., De Kloet, E. R., Moller, A., Agnati, L. F., and Fuxe, K. (1997). Neurochemical changes in the hippocampus of the Brown Norway rat during aging. *Neurobiol. Aging* **18**, 319–327.

Biegon, A., Greenberger, V., and Segal, M. (1986). Quantitative histochemistry of brain acetylcholinesterase and learning rate in the aged rat. *Neurobiol. Aging* **7**, 215–217.

Bratt, A. M., Kelly, M. E., Domeney, A. M., Naylor, R. J., and Costall, B. (1994a). Ondansetron fails to attenuate a scopolamine-induced deficit in a Stone maze task. *NeuroReport* **5**, 1921–1924.

Bratt, A. M., Kelly, M. E., Domeney, A. M., Naylor, R. J., and Costall, B. (1994b). Aged Stone maze-impaired rats exhibit reduced striatal muscarinic receptor densities. *NeuroReport* **5**, 2678–2682.

Buccafusco, J. J., and Jackson, W. J. (1991). Beneficial effects of nicotine administered prior to a delayed matching-to-sample task in young and aged monkeys. *Neurobiol. Aging* **12**, 233–238.

Buchanan, S. L., and Ginn, S. R. (1988). Classically conditioned cardiac responses in ''old'' and ''young'' Fischer 344 rats. *Psychol. Aging* **3**, 51–58.

Buchanan, S. L., and Powell, D. A. (1988). Age-related changes in associative learning: Studies in rabbits and rats. *Neurobiol. Aging* **9**, 523–534.

Burwell, R. D., and Gallagher, M. (1993). A longitudinal study of reaction time performance in Long-Evans rats. *Neurobiol. Aging* **14**, 57–64.

Campbell, B. A., and Haroutunian, V. (1981). Effects of age on long-term memory: Retention of fixed interval responding. *J. Gerontol.* **36**, 338–341.

Caprioli, A., Ghirardi, O., Ramacci, M. T., and Angelucci, L. (1990). Age-dependent deficits in radial maze performance in the rat: Effect of chronic treatment with acetyl-L-carnitine. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **14**, 359–369.

Carrie, I., Debray, M., Bourre, J. M., and Frances, H. (1999). Age-induced cognitive alterations in OF1 mice. *Physiol. Behav.* **66**, 651–656.

Chrobak, J. J., Hanin, I., Lorens, S. A., and Napier, T. C. (1995). Within-subject decline in delayed-non-match-to-sample radial arm maze performance in aging Sprague-Dawley rats. *Behav. Neurosci.* **109**, 241–245.

Collier, T. J., Gash, D. M., and Sladek, J. R., Jr. (1988). Transplantation of norepinephrine neurons into aged rats improves performance of a learned task. *Brain Res.* **448**, 77–87.

Cooper, R. L., McNamara, M. C., and Thompson, W. G. (1980). Vasopressin and conditioned flavor aversion in aged rats. *Neurobiol. Aging* **1**, 53–57.

Craik, F. I. M., and Jennings, J. M. (1992). Human memory. *Handb. Aging Cogn.*, pp. 51–83.

Dean, R. L., 3rd, Scozzafava, J., Goas, J. A., Regan, B., Beer, B., and Bartus, R. T. (1981). Age-related differences in behavior across the life span of the C57BL/6J mouse. *Exp. Aging Res.* **7**, 427–451.

De Toledo-Morrell, L., and Morrell, F. (1985). Electrophysiological markers of aging and memory loss in rats. *Ann. N. Y. Acad. Sci.* **444**, 296–311.

Dunnett, S. (1991). Cholinergic grafts, memory and ageing. *Trends Neurosci.* **14**, 371–376.

Dunnett, S. B., Badman, F., Rogers, D. C., Evenden, J. L., and Iversen, S. D. (1988). Cholinergic grafts in the neocortex or hippocampus of aged rats: Reduction of delay-dependent deficits in the delayed non-matching to position task. *Exp. Neurol.* **102**, 57–64.

Flood, J. F., and Morley, J. E. (1990). Pharmacological enhancement of long-term memory retention in old mice. *J. Gerontol.* **45**, B101–B104.

Flood, J. F., and Morley, J. E. (1998). Learning and memory in the SAMP8 mouse. *Neurosci. Biobehav. Rev.* **22**, 1–20.

Flood, J. F., and Roberts, E. (1988). Dehydroepiandrosterone sulfate improves memory in aging mice. *Brain Res.* **448**, 178–181.

Fordyce, D. E., and Wehner, J. M. (1993). Effects of aging on spatial learning and hippocampal protein kinase C in mice. *Neurobiol. Aging* **14**, 309–317.

Forster, M. J., Popper, M. D., Retz, K. C., and Lal, H. (1988). Age differences in acquisition and retention of one-way avoidance learning in C57BL/6NNia and autoimmune mice. *Behav. Neural Biol.* **49**, 139–151.

Forster, M. J., Dubey, A., Dawson, K. M., Stutts, W. A., Lal, H., and Sohal, R. S. (1996). Age-related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain. *Proc. Natl. Acad. Sci. U. S. A.* **93**, 4765–4769.

Frick, K. M., Baxter, M. G., Markowska, A. L., Olton, D. S., and Price, D. L. (1995). Age-related spatial reference and working memory deficits assessed in the water maze. *Neurobiol. Aging* **16**, 149–160.

Frick, K. M., Burlingame, L. A., Arters, J. A., and Berger-Sweeney, J. (2000). Reference memory, anxiety and estrous cyclicity in C57BL/6NIA mice are affected by age and sex. *Neuroscience* **95**, 293–307.

Gage, F. H., Dunnett, S. B., and Bjorklund, A. (1984). Spatial learning and motor deficits in aged rats. *Neurobiol. Aging* **5**, 43–48.

Gallagher, M., and Pelleymounter, M. A. (1988). Spatial learning deficits in old rats: A model for memory decline in the aged. *Neurobiol. Aging* **9**, 549–556.

Gallagher, M., and Rapp, P. R. (1997). The use of animal models to study the effects of aging on cognition. *Annu. Rev. Psychol*. **48**, 339–370.

Gallagher, M., Bostock, E., and King, R. (1985). Effects of opiate antagonists on spatial memory in young and aged rats. *Behav. Neural Biol*. **44**, 374–385.

Ghirardi, O., Caprioli, A., Milano, S., Giuliani, A., Ramacci, M. T., and Angelucci, L. (1992). Active avoidance learning in old rats chronically treated with levocarnitine acetyl. *Physiol. Behav.* **52**, 185–187.

Glassman, R. B., Garvey, K. J., Elkins, K. M., Kasal, K. L., and Couillard, N. L. (1994). Spatial working memory score of humans in a large radial maze, similar to published score of rats, implies capacity close to the magical number 7 +/− 22. *Brain Res. Bull*. **34**, 151–159.

Gold, P. E., McGraugh, J. L., and Hankins, L. L., *et al*. (1981). Age-dependent changes in retention in rats. *Exp. Aging Res*. **8**, 53–58.

Goodrick, C. L. (1968). Learning, retention, and extinction of a complex maze habit for mature-young and senescent Wistar albino rats. *J. Gerontol*. **23**, 298–304.

Goodrick, C. L. (1972). Learning by mature-young and aged Wistar albino rats as a function of test complexity. *J. Gerontol*. **27**, 353–357.

Goodrick, C. L. (1973). Maze learning of mature-young and aged rats as a function of distribution of practice. *J. Exp. Psychol*. **98**, 344–349.

Goodrick, C. L. (1975). Behavioral differences in young and aged mice: Strain differences for activity measures, operant learning, sensory discrimination, and alcohol preference. *Exp. Aging Res*. **1**, 191–207.

Goodrick, C. L. (1980). Effects of long-term voluntary wheel exercise on male and female Wistar rats. I. Longevity, body weight, and metabolic rate. *Gerontology* **26**, 22–33.

Gower, A. J., and Lamberty, Y. (1993). The aged mouse as a model of cognitive decline with special emphasis on studies in NMRI mice. *Behav. Brain Res*. **57**, 163–173.

Grady, C. L., and Craik, F. I. (2000). Changes in memory processing with age. *Curr. Opin. Neurobiol*. **10**, 224–231.

Greene, E., and Naranjo, J. N. (1987). Degeneration of hippocampal fibers and spatial memory deficit in the aged rat. *Neurobiol. Aging* **8**, 35–43.

Guan, X., and Dluzen, D. E. (1994). Age related changes of social memory/recognition in male Fischer 344 rats. *Behav. Brain Res*. **61**, 87–90.

Guanowsky, V., Misanin, J. R., and Riccio, D. C. (1983). Retention of conditioned taste aversion in weanling, adult, and old-age rats. *Behav. Neural Biol*. **37**, 173–178.

Hamm, R. J., Knisely, J. S., and Dixon, C. E. (1983). An animal model of age changes in short-term memory: The DRL schedule. *Exp. Aging Res*. **9**, 23–25.

Hengemihle, J. M., Long, J. M., Betkey, J., Jucker, M., and Ingram, D. K. (1999). Age-related psychomotor and spatial learning deficits in 129/SvJ mice. *Neurobiol. Aging* **20**, 9–18.

Hinderliter, C. F., and Misanin, J. R. (1993). Context familiarity and delayed conditioned taste aversion in young-adult and old-age rats. *Percept. Mot. Skills* **77**, 1403–1406.

Houston, F. P., Stevenson, G. D., McNaughton, B. L., and Barnes, C. A. (1999). Effects of age on the generalization and incubation of memory in the F344 rat. *Learn. Mem*. **6**, 111–119.

Ikegami, S. (1994). Behavioral impairment in radial-arm maze learning and acetylcholine content of the hippocampus and cerebral cortex in aged mice. *Behav. Brain Res*. **65**, 103–111.

Ikegami, S., Shumiya, S., and Kawamura, H. (1992). Age-related changes in radial-arm maze learning and basal forebrain cholinergic systems in senescence accelerated mice (SAM). *Behav. Brain Res*. **51**, 15–22.

Ingram, D. K. (1985). Analysis of age-related impairments in learning and memory in rodent models. *Ann. N. Y. Acad. Sci*. **444**, 312–331.

Ingram, D. K. (1988). Complex maze learning in rodents as a model of age-related memory impairment. *Neurobiol. Aging* **9**, 475–485.

Ingram, D. K. (1996). Brain-behavior linkages in aged rodent models: Strategies for examining individual differences. *Neurobiol. Aging* **17**, 497–499.

Ingram, D. K., and Peacock, L. J. (1980). Conditioned taste aversion as a function of age in mature male rats. *Exp. Aging Res*. **6**, 113–123.

Ingram, D. K., London, E. D., and Goodrick, C. L. (1981). Age and neurochemical correlates of radial maze performance in rats. *Neurobiol. Aging* **2**, 41–47.

Ingram, D. K., Spangler, E. L., Iijima, S., Ikari, H., Kuo, H., Greig, N. H., and London, E. D. (1994). Rodent models of memory dysfunction in Alzheimer's disease and normal aging: Moving beyond the cholinergic hypothesis. *Life Sci*. **55**, 2037–2049.

Jaenicke, B., Kim. E. J., Ahn, J. W., and Lee, H. S. (1991). Effect of Panax ginseng extract on passive avoidance retention in old rats. *Arch. Pharm. Res*. **14,** 25–29

Jordon, J., and Sokoloff, B. (1959). Air ionization, age, and maze learning in rats. *J. Gerontol*. **32**, 344–348.

Jucker, M., Oettinger, R., and Battig, K. (1988). Age-related changes in working and reference memory performance and locomotor activity in the Wistar rat. *Behav. Neural Biol*. **50**, 24–36.

Kahana, M. J., Sekuler, R., Caplan, J. B., Kirschen, M., and Madsen, J. R. (1999). Human theta oscillations exhibit task dependence during virtual maze navigation. *Nature (London)* **399**, 781–784.

Keefover, R. W. (1998). Aging and cognition. *Neurol. Clin*. **16**, 635–648.

Kobayashi, S., Kametani, H., Ugawa, Y., and Osanai, M. (1988). Age difference of response strategy in radial maze performance of Fischer-344 rats. *Physiol Behav.* **42**, 277–280.

Komiskey, H. L., Rahman, A., and Mundinger, K. L. (1988). Aging: Changes in a passive-avoidance response with brain levels of temazepam. *Pharmacol. Biochem. Behav.* **31**, 611–615.

Kubanis, P., Zornetzer, S. F., and Freund, G. (1982). Memory and postsynaptic cholinergic receptors in aging mice. *Pharmacol. Biochem. Behav.* **17**, 313–322.

Lamberty, Y., and Gower, A. J. (1992). Age-related changes in spontaneous behavior and learning in NMRI mice from middle to old age. *Physiol. Behav.* **51**, 81–88.

Lindner, M. D. (1997). Reliability, distribution, and validity of age-related cognitive deficits in the Morris water maze. *Neurobiol. Learn. Mem*. **68**, 203–220.

Lindner, M. D., Balch, A. H., and VanderMaelen, C. P. (1992). Short forms of the ''reference-''and ''working-memory'' Morris water maze for assessing age-related deficits. *Behav. Neural Biol*. **58**, 94–102.

Lippa, A. S., Pelham. R. W., Beer, B., Critchett, D. J., Dean, R. L., and Bartus, R. T. (1980). Brain cholinergic dysfunction and memory in aged rats. *Neurobiol. Aging* **1**, 13–19.

Lowy, A. M., Ingram, D. K., Olton, D. S., Waller, S. B., Reynolds, M. A., and London, E. D. (1985). Discrimination learning requiring different memory components in rats: Age and neurochemical comparisons. *Behav. Neurosci*. **99**, 638–651.

Magnusson, K. R. (1997). The effects of age and dietary restriction on metabotropic glutamate receptors in C57B1 mice. *J. Gerontol. A* **52**. B291–B299.

Markowska, A. L., Stone, W. S., Ingram, D. K., Reynolds, J., Gold, P. E., Conti, L. H., Pontecorvo, M. J., Wenk, G. L., and Olton, D. S. (1989). Individual differences in aging: behavioral and neurobiological correlates. *Neurobiol. Aging* **10**, 31–43.

Markowska, A. L., Price, D., and Koliatsos, V. E. (1996). Selective effects of nerve growth factor on spatial recent memory as assessed by a delayed nonmatching-to-position task in the water maze. *J. Neurosci*. **16**, 3541–3548.

Markowska, A. L., Mooney, M., and Sonntag, W. E. (1998). Insulin-like growth factor-1 ameliorates age-related behavioral deficits. *Neuroscience* **87**, 559–569.

Martinez, J. L., Jr., and Rigter, H. (1983). Assessment of retention capacities in old rats. *Behav. Neural Biol*. **39**, 181–191.

Martinez, J. L., Jr., Schulteis, G., Janak, P. H., and Weinberger, S. B. (1988). Behavioral assessment of forgetting in aged rodents and its relationship to peripheral sympathetic function. *Neurobiol. Aging* **9**, 697–708.

Maurice, T., Roman, F. J., Su, T. P., and Privat, A. (1996). Beneficial effects of sigma agonists on the age-related learning impairment in the senescence-accelerated mouse (SAM). *Brain Res*. **733**, 219–230.

McGaughy, J., and Sarter, M. (1995a). Behavioral vigilance in rats: Task validation and effects of age, amphetamine, and benzodiazepine receptor ligands. *Psychopharmacology* **117**, 340–357.

McGaughy, J., and Sarter, M. (1995b). Effects of chlordiazepoxide and scopolamine, but not aging, on the detection and identification of conditional visual stimuli. *J. Gerontol.* **50**, B90–B96.

McLay, R. N., Freeman, S. M., Harlan, R. E., Kastin, A. J., and Zadina, J. E. (1999). Tests used to assess the cognitive abilities of aged rats: Their relation to each other and to hippocampal morphology and neurotrophin expression. *Gerontology* **45**, 143–155.

Means, L. W., and Kennard, K. J. (1991). Working memory and the aged rat: Deficient two-choice win-stay water-escape acquisition and retention. *Physiol. Behav.* **49**, 301–307.

Miettinen, R., Sirvio, J., Riekkinen, P., Sr., Laakso, M. P., Riekkinen, M., and Riekkinen, P., Jr. (1993). Neocortical, hippocampal and septal parvalbumin- and somatostatin-containing neurons in young and aged rats: Correlation with passive avoidance and water maze performance. *Neuroscience* **53**, 367–378.

Miller, M. M., Hyder, S. M., Assayag, R., Panarella, S. R., Tousignant, P., and Franklin, K. B. (1999). Estrogen modulates spontaneous alternation and the cholinergic phenotype in the basal forebrain. *Neurosci.* **91**, 1143–1153.

Misanin, J. R., and Hinderliter, C. F. (1994). Efficacy of lithium chloride in the taste-aversion conditioning of young-adult and old-age rats: *Psychol. Rep*. **75**, 267–271.

Misanin, J. R., and Hinderliter, C. F. (1995). Lack of age differences in context-illness associations in the long-delay taste-aversion conditioning of rats. *Percept. Mot. Skills* **80**, 595–598.

Moore, H., Dudchenko, P., Bruno, J. P., and Sarter, M. (1992). Toward modeling age-related changes of attentional abilities in rats: Simple and choice reaction time tasks and vigilance. *Neurobiol. Aging* **13**, 759–772.

Nakamura, S., and Ishihara, T. (1989). Region selective increase in activities of CNS cholinergic marker enzymes during learning of memory tasks in aged rats. *Pharmacol. Biochem. Behav.* **34**, 805–810.

Noda, Y., Yamada, K., and Nabeshima, T. (1997). Role of nitric oxide in the effect of aging on spatial memory in rats. *Behav. Brain Res*. **83**, 153–158.

O'Connor, R. C., and Glassman, R. B. (1993). Human performance with a seventeen-arm radial maze analog. *Brain Res. Bull*. **30**, 189–191.

Oler, J. A., and Markus, E. J. (1998). Age-related deficits on the radial maze and in fear conditioning: hippocampal processing and consolidation. *Hippocampus* **8**, 402–415; [erratum: *Ibid*. **10**, 131 (2000)].

Olton, D. S., and Markowska, A. L. (1988). Within-subjects, parametric manipulations to investigate aging. *Neurobiol. Aging* **9**, 469–474.

Ordy, J. M., Thomas, G. J., Volpe, B. T., Dunlap, W. P., and Colombo, P. M. (1988). An animal model of human-type memory loss based on aging, lesion, forebrain ischemia, and drug studies with the rat. *Neurobiol. Aging* **9**, 667–683.

Oscar-Berman, M., and Bonner, R. T. (1985). Matching- and delayed matching-to-sample performance as measures of visual processing, selective attention, and memory in aging and alcoholic individuals. *Neuropsychologia* **23**, 639–651.

Petkov, V. D., Belcheva, S., Stoyanova, V., and Petkov, V. V. (1990). Effects of nootropic agents on the performing of active two-way avoidance tasks in young and old rats. *Acta Physiol. Pharmacol. Bulg*. **16**, 35–42.

Pontecorvo, M. J., Clissold, D. B., and Conti, L. H. (1988). Age-related cognitive impairments as assessed with an automated repeated measures memory task: Implications for the possible role of acetylcholine and norepinephrine in memory dysfunction. *Neurobiol. Aging* **9**, 617–625.

Pontecorvo, M. J., Sahgal, A., and Steckler, T. (1996). Further developments in the measurement of working memory in rodents. *Cogn. Brain Res*. **3**, 205–213.

Prescott, L., Buchanan, S. L., and Powell, D. A. (1989). Leg flexion conditioning in the rat: Its advantages and disadvantages as a model system of age-related changes in associative learning. *Neurobiol. Aging* **10**, 59–65.

Puglisi-Allegra, S., Cabib, S., Ebel, A., Kempf, J., and Castellano, C. (1986). Passive avoidance behavior in mice: Interaction between age and genotype. *Exp. Aging Res*. **12**, 107–109.

Rapp, P. R., Rosenberg, R. A., and Gallagher, M. (1987). An evaluation of spatial information processing in aged rats. *Behav. Neurosci*. **101**, 3–12.

Reddy, D. S., and Kulkarni, S. K. (1999). Sex and estrous cycle-dependent changes in neurosteroid and benzodiazepine effects on food consumption and plus-maze learning behaviors in rats. *Pharmacol. Biochem. Behav.* **62**, 53–60.

Rick, J. T., Murphy, M. P., Ivy, G. O., and Milgram. N. W. (1996). Short intertrial intervals impair water maze performance in old Fischer 344 rats. *J. Gerontol.* **51**, B253–B260.

Riekkinen, P., Jr., Schmidt, B., Stefanski, R., Kuitunen, J., and Riekkinen, M. (1996). Metrifonate improves spatial navigation and avoidance behavior in scopolamine-treated, medial septum-lesioned and aged rats. *Eur. J. Pharmacol*. **309**, 121–130.

Ritzmann, R. F., Kling, A., Melchior, C. L., and Glasky, A. J. (1993). Effect of age and strain on working memory in mice as measured by win-shift paradigm. *Pharmacol. Biochem. Behav.* **44**, 805–807.

Roux, S., Hubert, I., Lenegre, A., Milinkevitch, D., and Porsolt, R. D. (1994). Effects of piracetam on indices of cognitive function in a delayed alternation task in young and aged rats. *Pharmacol. Biochem. Behav.* **49**, 683–688.

Sarter, M. (1987). Measurement of cognitive abilities in senescent animals. *Int. J. Neurosci*. **32**, 765–774.

Sarter, M., and Markowitsch, H. J. (1983). Reduced resistance to progressive extinction in senescent rats: A neuroanatomical and behavioral study. *Neurobiol. Aging* **4**, 203–215.

Scheuer, K., Stoll, S., Paschke, U., Weigel, R., and Muller, W. E. (1995). *N*-methyl-D-aspartate receptor density and membrane fluidity as possible determinants of the decline of passive avoidance performance in aging. *Pharmacol. Biochem. Behav.* **50**, 65–70.

Shukitt-Hale, B., Mouzakis, G., and Joseph, J. A. (1998). Psychomotor and spatial memory performance in aging male Fischer 344 rats. *Exp. Gerontol*. **33**, 615–624.

Silva, R. H., Felicio, L. F., Nasello, A. G., Vital, M. A., and Frussa-Filho, R. (1996). Effect of ganglioside (GM1) on memory in senescent rats. *Neurobiol. Aging* **17**, 583–586.

Soffie, M., and Lejeune, H. (1991). Acquisition and long-term retention of a two-lever DRL schedule: Comparison between mature and aged rats. *Neurobiol. Aging* **12**, 25–30.

Soffie, M., Hahn, K., Terao, E., and Eclancher, F. (1999). Behavioural and glial changes in old rats following environmental enrichment. *Behav. Brain Res*. **101**, 37–49.

Solomon, P. R., Blanchard, S., Levine, E., Velazquez, E., and Groccia-Ellison, M. (1991). Attenuation of age-related conditioning deficits in humans by extension of the interstimulus interval. *Psychol. Aging* **6**, 36–42.

Spangler, E. L., and Ingram, D. K. (1986). Effects of inescapable shock on maze performance as a function of age in mice. *Exp. Aging Res*. **12**, 39–42.

Spangler, E. L., and Ingram, D. K. (1996). Utilization of the rat as a model of mammalian aging: impact of pathology on behavior. *Gerontology* **42**, 301–311.

Spencer, W. D., and Raz, N. (1995). Differential effects of aging on memory for content and context: A meta-analysis. *Psychol. Aging* **10**, 527–539.

Springer, A. D., and Fraley, S. M. (1981). Extinction of a conditioned taste aversion in young, mid-aged, and aged C57/BL6 mice. *Behav. Neural Biol*. **32**, 282–294.

Stavnes, K., and Sprott, R. L. (1975). Effects of age and genotype on acquisition of an active avoidance response in mice. *Dev. Psychobiol*. **8**, 437–445.

Stemmelin, J., Cassel, J. C., Will, B., and Kelche, C. (1999). Sensitivity to cholinergic drug treatments of aged rats with variable degrees of spatial memory impairment. *Behav. Brain Res*. **98**, 53–66.

Stephens, D. N., Weidmann, R., Quartermain, D., and Sarter, M. (1985). Reversal learning in senescent rats. *Behav. Brain Res*. **17**, 193–202.

Stewart, C. A., and Reid, I. C. (1994). Ketamine prevents ECS-induced synaptic enhancement in rat hippocampus. *Neurosci. Lett*. **178**, 11–14; erratum: *Ibid*. **210**, 218 (1996).

Tanila, H., Shapiro, M., Gallagher, M., and Eichenbaum, H. (1997). Brain aging: Changes in the nature of information coding by the hippocampus. *J. Neurosci*. **17**, 5155–5166.

Thompson, C. I., and Fitzsimons, T. R. (1976). Age differences in aversively motivated visual discrimination learning and retention in male Sprague-Dawley rats. *J. Gerontol*. **31**, 47–52.

Valerio, C., Clementi, G., Spadaro, F., D'Agata, V., Raffaele, R., Grassi, M., Lauria, N., and Drago, F. (1989). The effects of acetyl-1-carnitine on experimental models of learning and memory deficits in the old rat. *Funct. Neurol*. **4**, 387–390.

van Gool, W. A., Mirmiran, M., and van Haaren, F. (1985). Spatial memory and visual evoked potentials in young and old rats after housing in an enriched environment. *Behav. Neural Biol*. **44**, 454–469.

Vannucchi, M. G., Scali, C., Kopf, S. R., Pepeu, G., and Casamenti, F. (1997). Selective muscarinic antagonists differentially affect in vivo acetylcholine release and memory performances of young and aged rats. *Neuroscience* **79**, 837–846.

Van Waas, M., and Soffie, M. (1996). Differential environmental modulations on locomotor activity, exploration and spatial behaviour in young and old rats. *Physiol. Behav.* **59**, 265–271.

Vasquez, B. J., Martinez, J. L., Jr., Jensen, R. A., Messing, R. B., Rigter, H., and McGaugh, J. L. (1983). Learning and memory in young and aged Fischer 344 rats. *Arch. Gerontol. Geriatr.* **2**, 279–291.

Wallace, J. E., Krauter, E. E., and Campbell, B. A. (1980). Animal models of declining memory in the aged: Short-term and spatial memory in the aged rat. *J. Gerontol*. **35**, 355–363.

Ward, M. T., Stoelzel, C. R., and Markus, E. J. (1999). Hippocampal dysfunction during aging II: Deficits on the radial-arm maze. *Neurobiol. Aging* **20**, 373–380.

Weiss, C., and Thompson, R. F. (1992). Delayed acquisition of eyeblink conditioning in aged F1 hybrid (Fischer-344 × Brown Norway) rats. *Neurobiol. Aging* **13**, 319–323.

Weiss, C., Knuttinen, M. G., Power, J. M., Patel, R. I., O'Connor, M. S., and Disterhoft, J. F. (1999). Trace eyeblink conditioning in the freely moving rat: Optimizing the conditioning parameters. *Behav. Neurosci*. **113**, 1100–1105.

Welzl, H., Alessandri, B., Oettinger, R., and Batig, K. (1988). The effects of long-term nicotine treatment on locomotion, exploration and memory in young and old rats. *Psychopharmacology* **96**, 317–323.

Whishaw, I. Q. (1995). A comparison of rats and mice in a swimming pool place task and matching to place task: Some surprising differences. *Physiol. Behav.* **58**, 687–693.

Whishaw, I. Q., and Tomie, J. A. (1996). Of mice and mazes: Similarities between mice and rats on dry land but not water mazes. *Physiol. Behav.* **60**, 1191–1197.

Willig, F., Palacios, A., Monmaur, P., M'Harzi, M., Laurent, J., and Delacour, J. (1987). Short-term memory, exploration and locomotor activity in aged rats. *Neurobiol. Aging* **8**, 393–402.

Winocur, G. (1984). The effects of retroactive and proactive interference on learning and memory in old and young rats. *Dev. Psychobiol.* **17**, 537–545.

Winocur, G. (1986). Memory decline in aged rats: A neuropsychological interpretation. *J. Gerontol*. **41**, 758–763.

Winocur, G. (1988). Long-term memory loss in senescent rats: Neuropsychological analysis of interference and context effects. *Psychol. Aging* **3**, 273–279.

Winocur, G. (1992). A comparison of normal old rats and young adult rats with lesions to the hippocampus or prefrontal cortex on a test of matching-to-sample. *Neuropsychologia* **30**, 769–781.

Winocur, G. (1998). Environmental influences on cognitive decline in aged rats. *Neurobiol. Aging* **19**, 589–597.

Winocur, G., and Hasher, L. (1999). Aging and time-of-day effects on cognition in rats. *Behav. Neurosci*. **113**, 991–997.

Winocur, G., and Moscovitch, M. (1990). Hippocampal and prefrontal cortex contributions to learning and memory: Analysis of lesion and aging effects on maze learning in rats. *Behav. Neurosci*. **104**, 544–551.

Winter, J. C. (1997). The effects of age on continuous learning in the radial maze. *Physiol. Behav.* **61**, 609–612.

Woodruff-pak, D. S., Finkbiner, R. G., and Sasse, D. K. (1990). Eyeblink conditioning discriminates Alzheimer's patients from non-demented aged. *NeuroReport* **1**, 45–48.

Woodruff-Pak, D. S., Jaeger, M. E., Gorman, C., and Wesnes, K. A. (1999). Relationships among age, conditioned stimulus-unconditioned stimulus interval, and neuropsychological test performance. *Neuropsychology* **13**, 90–102.

Zornetzer, S. F., Thompson, R., and Rogers, J. (1982). Rapid forgetting in aged rats. *Behav. Neural Biol*. **36**, 49–60.

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25

Genetically Engineered Models of Human Age-Related Neurodegenerative Diseases

Aging-related human diseases such as amyotrophic lateral sclerosis and Alzheimer's disease are both progressive and degenerative conditions for which no ''natural'' nonhuman animal models exist. Specific genes have been implicated in the etiology of both conditions, which has led to the development of transgenic rodent models that may mimic important aspects of the human disease. For Alzheimer's disease, mutations in the amyloid precursor protein and presenilin genes have been linked with familial forms of the disease, and apolipoprotein E gene allelic variations have been identified as risk factors. Transgenic studies have helped clarify the role of these genes in the development of Alzheimer's disease-related pathology, particularly with respect to Aβ amyloid plaque formation. Particular transgenic lines that express high levels of the mutated amyloid precursor protein gene and demonstrate aging-related Aβ deposition into plaques may mimic the earliest stages of Alzheimer's disease, representing an important tool for determining novel therapeutic strategies before substantial neurodegeneration has occurred. In amyotrophic lateral sclerosis, mutations in genes for Cu/Zn superoxide dismutase-1 and neurofilament proteins have been associated with a subset of cases, and transgenic models involving these genes have been shown to replicate important features of the human disease, particularly following aging. Thus, transgenic technology is advancing our understanding of the cause of pathology in human neurodegenerative diseases associated with aging and, perhaps more importantly, will provide an important resource for discovering and evaluating new methods to prevent these diseases.

I. Introduction

One of the major goals of biomedical research is to produce animal models that are relevant to human disease. These animal models may serve a multitude of purposes, from understanding the role of particular proteins or cellular processes in a disease mechanism to replicating important aspects of human pathology so that new therapeutic strategies can be developed. With respect to human age-related neurodegenerative diseases, very few ''natural'' animal models exist where nonhuman species develop pathological changes akin to human diseases. This may be partly due to the unmatched evolutionary complexity of the human nervous system as well as our relative longevity.

Advances in molecular biology and genetic engineering are providing new opportunities to alter the genome of animal species so that they may more closely replicate important aspects of human brain pathology. In this respect, the ability to introduce or delete specific genes, or even crossbreed to combine these genetic backgrounds, may lead to a clarification of the role of particular gene products in specific cellular processes and neurodegenerative conditions. This approach may unravel, for example, the role of a gene mutation in neuronal degeneration or how the absence of a particular protein may affect a degenerative process. Obviously, the ultimate objective of the application of this evolving technology is to recreate a human disease in an easily studied animal, such as a mouse. There has been varying degrees of success in achieving this goal, but data derived are useful for clarifying the role of certain genes and proteins in various human age-related neurodegenerative conditions. Certainly there is the danger that genetic engineering may produce pathology in animals of dubious relevance to human disease, that we are ''inventing'' new brain diseases in rodents that are false leads with respect to understanding pathology in aging human brains. It may also be that providing the necessary genetic background is just one factor in the cascade of brain changes that lead to specific diseases. Further experimental manipulation could be required for these new genetically altered animals to demonstrate their worthiness as human disease ''models.''

This chapter examines various genetically engineered animals that are of relevance to human age-related neurodegenerative diseases. These models will be appraised for their ability to either replicate human brain pathology and/or tell us something interesting about the disease process and the role of a particular genetic background or protein. The focus will be particularly on Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) where the insertion or deletion

of particular genes, implicated previously in familial forms of the disease, into rodent genomes has resulted in a spectrum of interesting and useful animal models.

II. Alzheimer's Disease

AD is a major cause of age-related dementia and is associated with specific pathological changes within the brain. These histopathological features correspond principally to extracellular ''plaques'' and intraneuronal ''neurofibrillary'' changes. Plaques are extracellular spherical masses of fine amyloid fibrils composed of a 4 kDa protein known as β/A4, Aβ, or β-amyloid (Glenner and Wong, 1984; Masters *et al*., 1985). Aβ is normally a soluble protein found within the nervous system (e.g., Seubert *et al*., 1992) but appears to undergo age-related conformational alterations to form amyloid fibrils in particular brain regions in humans and some nonhuman species. The other major histopathological feature of AD is an abnormal transformation of the neuronal cytoskeleton, resulting in paired helical filaments (PHFs). These cytoskeletal changes are associated with abnormal or ''dystrophic'' neurites associated with plaques, the development of filamentous ''tangles'' within nerve cells, and the formation of ''neuropil threads'' within the dendrites of tangle-affected neurons. Neurofibrillary pathology involves major modifications in cytoskeletal proteins such as microtubules and neurofilaments (NFs) as well as associated proteins (Vickers *et al*., 2000). In this respect, an abnormal and highly insoluble form of the microtubule-associated protein tau has been demonstrated to be an integral constituent of neurofibrillary tangles (Wischik *et al*., 1988; Lee *et al*., 1991) and is likely to represent the principal protein of the PHFs, which replace the normal cytoskeleton in degenerating neurons (Vickers *et al*., 1992).

Compared to other major human diseases, there are relatively few risk factors associated with AD. The major risk factor for this condition is age, with the incidence of AD rising exponentially with age from the seventh decade onward (Katzman and Kawas, 1994). However, it should be noted that extreme old age (90+ years of age) may be associated with a relatively reduced risk of AD-related dementia, perhaps due to protective genetic factors that are linked with longevity. Neuropsychological and pathological studies indicate that AD may have a relatively long disease course, characterized by a ''preclinical'' phase, where Aβ plaques are gradually formed in the brain, followed by a more aggressive clinical phase associated with neurofibrillary pathology, neuronal degeneration, and overt clinical symptomatology (Vickers, 1997; Vickers *et al*., 2000).

The other major risk factor for AD is a family history of the condition. Approximately 10% of AD cases have a strong familial association and are linked to mutations in specific genes that are inherited in an autosomal dominant fashion. A further 90% of AD cases are considered ''sporadic'' in nature, but a relative risk of the disease may be affected by the inheritance of particular gene variants.

While symptomatic treatments based on supplementing particular neurotransmitters are available, lack of an effective therapeutic agent for preventing, halting, or even slowing AD can be attributed to the absence of appropriate disease models. Other species do not develop the full spectrum of AD pathology. Particular aged primates, dogs, and bears develop Aβ plaques, and some species such as polar bears, goats, and sheep have been reported to develop intraneuronal structures resembling neurofibrillary tangles (Wisniewski *et al*., 1973; Selkoe *et al*., 1987; Walker *et al*., 1987; Braak *et al*., 1994; Tekirian *et al*., 1996; Giannakopoulos *et al*., 1997). Hence, there has been great interest in developing new animal models of AD and related disorders by genetic engineering, with the view that manipulation of the genome of these animals may reproduce crucial aspects of the AD degenerative process. In addition, the development of a rodent model may be advantageous with respect to aging studies due to the relatively short life span of rats and mice relative to other species.

The genetic engineering approach has resulted in variable degrees of success to date, but the pace and intensity of research in this area, coupled with other approaches to understanding and modeling AD, have provided valuable disease models and important new insights into the sequence of pathological events that may lead to dementia.

A. Amyloid Precursor Protein Transgenics

The Aβ peptide that comprises the amyloid fibrils of plaques in AD is derived from the amyloid precursor protein (APP). APP is encoded by a 19 exon single gene on chromosome 21, which can be alternatively spliced to produce three isoforms of 695, 751, and 770 amino acids, corresponding to a 100 to 140 kDa molecular mass (Octave, 1995). Both of the larger isoforms contain an amino acid sequence derived from exon 7, which is homologous to the Kunitz family of serine protease inhibitors. APP770 also contains an exon 8-derived, 19 amino acid sequence homologous to the OX-2 antigen.

APP is likely to be a single membrane-spanning glycoprotein, which can be processed variably to smaller fragments by the action of unidentified enzymes. A putative ''α-secretase'' cleaves through residues 16 and 17 of the Aβ sequence to release a large, soluble N-terminal fragment of APP (Esch *et al*., 1990; Sisodia *et al*., 1990). A soluble form of the Aβ sequence is also produced by the action of a β-secretase and γ-secretase at the N- and C-terminal sides of the peptide, respectively (Haass *et al*., 1992; Seubert *et al*., 1992; Shoji *et al*., 1992).

While APP is expressed by the brain and other tissues, the precise function of the protein has not been determined. In the nervous system, APP may have a role in neuronal development (Masliah *et al*., 1992), cell surface adhesion (Breen *et al*., 1991), and/or neuronal interactions with the extracellular matrix (Narindrasorasak *et al*., 1992). APP has been shown to undergo fast axonal transport to the synaptic terminals (Koo *et al*., 1990) where it may have a functional role (Schubert *et al*., 1991; Ikin *et al*., 1996). APP is also subject to a range of posttranslational modifications such as phosphorylation, sulfation, and glycosylation (Robakis, 1994), which are likely to affect its function and the secretion of various derivatives.

Two regions of the APP gene demonstrate mutations that are clearly linked with early-onset familial AD (FAD). At codon 717 (APP 770 transcript numbering), amino acid substitutions of Val for Ile, Phe, or Gly have been described in different

families (Chartier-Harlin *et al*., 1991; Goate *et al*., 1991; Murrell *et al*., 1991). A double mutation at codons 670 (Lys to Asn) and 671 (Met to Leu) is also present in two families (probably related) in Sweden (Mullan *et al*., 1992). Both of these mutations are likely to affect the processing of APP, with the codon 717 and 670/71 mutations lying close to the N- and C-terminal ends of the Aβ sequence, respectively. Indeed, individuals who inherit the Swedish APP mutation (APP_{SWE}) show an elevation of secreted levels of Aβ from cultured fibroblasts (Citron *et al*., 1994). Another APP mutation at codon 693 (Glu-Gln), at the 22nd residue of the Aβ sequence, is linked with susceptibility to Dutch-type cerebral hemorrhage with amyloidosis, a condition associated with amyloid deposits along cerebral blood vessels (amyloid angiopathy), strokes, and typically, death in the fifth or sixth decade of life (Levy *et al*., 1990; Van Broeckhoven *et al*., 1990).

The linkage of specific gene mutations in APP with FAD indicates that abnormal processing of APP has a pivotal role in the pathogenesis of AD (Selkoe, 1991). In addition, Down syndrome involving trisomy of chromosome 21 and thus, an extra copy of the APP gene is associated with the development of Aβ plaques relatively early in life (third to fourth decade of life) followed, in subsequent years, by neurofibrillary pathology (Wisniewski *et al*., 1985; Mann and Esiri, 1989). Similarly, data indicate that there is a preclinical phase of AD where Aβ plaques accumulate within the brain prior to the neurodegenerative changes that are linked with the emergence of dementia (Morris *et al*., 1996; Vickers *et al*., 2000). Thus, this type of data lends credence to the ''amyloid cascade hypothesis,'' which proposes that abnormal processing of APP and the development of Aβ deposits in the brain precede, and perhaps in some way cause, the pathological changes in neurons (Selkoe, 1991; Hardy and Higgins, 1992).

From the identification of APP gene mutations linked to FAD, and possible overexpression of APP related to Down syndrome and AD, there have been extensive efforts to introduce particular APP gene constructs into rodents in an attempt to recreate important pathological features of AD in these species. One of the first such reported transgenic mice lines was designed to express the human 751 amino acid isoform of APP (e.g., hAPP751) under regulation by the neuron-specific enolase promoter (Quon *et al*., 1991). The chromosomes of these mice incorporated from one to eight copies of the gene and showed a modest increase in total levels of the APP protein, with Aβ immunolabeling deposits of extracellular material in the brain, particularly in animals greater than 4 months of age. These deposits were stained with silver impregnation methods, sometimes labeled with thioflavine S, and not stained at all by Congo red. No overt neuronal pathology was present, and the authors proposed that the Aβ-immunoreactive deposits may represent a precursor to amyloid fibrils. Further analysis of this line indicated that elderly transgenic animals (22 months old) demonstrate larger Aβ deposits in the brain associated with gliosis and abnormal neurites were labeled with an antibody to the abnormal tau characteristic of AD (Higgins *et al*. 1994, 1995). In addition, abnormal tau was also localized immunohistochemically to increasing numbers of neuronal cell bodies in aging homozygous transgenic mice (Higgins *et al*., 1995). This transgenic line showed deficits in spatial memory acquisition (Morris Water Maze) and alternation (Y-maze choice) at 12 months of age (Moran *et al*., 1995), which is particularly interesting as it would be predicted from previous studies that very few of the older animals would contain significant Aβ deposits.

A second early transgenic model, developed by Wirak *et al*. (1991), utilized the APP promoter itself to drive the transcription of a sequence encoding Aβ1-42. This transgene was expressed at lower levels than the mouse APP (mAPP), and clustered punctate deposits in the brain, particularly the hippocampus, were labeled with antibodies to Aβ in 1-year-old transgenic mice. However, Jucker *et al*. (1992) noted that the C57Bl6 mouse strain used for the genetic background of the transgenic mice of Wirak *et al*. (1991) were particularly susceptible to the development of age-related clusters of granules, originating in the processes of astrocytes. These granular deposits could be labeled with a variety of immunohistochemical markers, including antibodies to Aβ. Wirak *et al*. (1992) accepted the possibility that the abnormal structures observed in their animals were more likely due to the genetic background of their mice rather than the presence of the transgene.

Kammesheidt *et al*. (1992) reported on a transgenic mouse line (C57/Bl6 $\times$ SJL) expressing the 104 amino acids of hAPP695 under the regulation of the dystrophin neural promoter. Expression of the transgene was principally localized to the brain, and abnormal punctate deposits of Aβ immunoreactivity were present within neurons or neuronal processes in different lines (Kammesheidt *et al*., 1992). Furthermore, thioflavine S-stained blood vessels were reported in a subset of transgenic mice. A further study demonstrated that these transgenic mice developed significant brain pathology by 18–22 months of age, including degenerating axons and dendrites, necrotic cell bodies, accumulations of secondary lysosomes, and activation of microglia to a higher degree than age-matched, nontransgenic littermates as well as C57/Bl6 or SJL mice (Oster-Granite *et al*., 1996). Another transgenic mouse line that expresses the just-described construct, including the Flag epitope sequence, appeared to show a similar pattern of pathological changes at 12 months of age, although animals of earlier ages were not examined (Oster-Granite *et al*., 1996). Aging homozygote transgenic mice bearing this latter transcript showed more severe neuronal degeneration in the hippocampus and performed worse on learning and memory tests (Morris water maze) than similarly aged heterozygote animals or nontransgenic control mice (Berger-Sweeney *et al*., 1999).

A similar transgenic mouse line [C-terminal 104 amino acids of hAPP regulated by the low molecular weight NF (NF-L) promoter] showed a modest increase (30–50%) in levels of the C-terminal APP fragment, aging-related deposits of Aβ immunoreactivity in the cortex and hippocampus, astrogliosis, and microglial activation as well as neurodegeneration (20% loss of neurons) in the hippocampus (Nalbantoglu *et al*., 1997). In addition, these mice showed deficits in the maintenance of long-term potentiation (LTP) and decreased performance on Morris water maze tasks.

These studies have been interpreted to support the proposal that an approximately 100 amino acid C-terminal fragment of hAPP may be a significant agent of neurotoxicity in AD rather than the Aβ peptide itself and/or plaque formation (Kammesheidt *et al*., 1992; Oster-Granite *et al*., 1996; Berger-Sweeney *et al*., 1999). In contrast, overexpression of the mouse Aβ

TABLE 25.1 Some Transgenic Lines Demonstrating AD-like Histopathology[a]

FAD-related construct	Aβ plaques	NFT	PHF	Dystrophic neurites			Neuronal degeneration	Reference
				NF	APP	Tau		
$hAPP717_{Val\text{-}Phe}$	+	−	−	+	+	−	−	Games *et al*. (1995)
$hAPP_{SWE}$	+	−	−	+	+	−	−	Hsiao *et al*. (1996)
$hAPP_{SWE}$ + $APP717_{Val\text{-}Ile}$	+	−	ND	+	+	+	+	Sturchler-Pierrat *et al*. (1997)
$hAPP_{SWE}$ or $hAPP717_{Val\text{-}Ile}$	+	−	ND	+	+	+	−	Moechars *et al*. (1999)

[a] Aβ plaques are defined as masses of amyloid fibrils immunoreactive for Aβ; NFT, neurofibrillary tangles; PHF, paired helical filaments; NF, neurofilament; APP, amyloid precursor protein; ND, not determined.

sequence (regulated by the NF-L promoter) in FVB/N mice was related to neurodegeneration, apoptosis, and reactive gliosis in the brain, but not plaques or neurofibrillary pathology, indicating a possible *in vivo* toxicity of the Aβ peptide (LaFerla *et al*., 1995). In addition, these animals displayed heightened seizure activity and premature death.

However, while these constructs lead to age-related neurodegeneration, it has yet to be shown whether the abnormal neuronal changes match authentic AD-specific nerve cell pathology. In addition, there is no direct evidence of APP C-terminal fragment or Aβ toxicity from studies of the AD brain. Another important issue is the genetic background of these various transgenic mice, which may have a major role in determining the pathological and behavioral phenotype of the animals. Hsiao *et al*. (1995) also demonstrated that aging transgenic mice overexpressing mouse or human APP695 on a FVB/N background demonstrate neophobia, increased seizures, impaired spatial alternation in a Y maze, diminished glucose utilization, and astrogliosis in the brain, but that these features can also occur in non-transgenic FVB/N mice with aging. Thus, the presence of APP transgenes appears to lead to a potentiation of normal age-related pathology. The wide range of APP transgenics that do not show AD-like histological hallmarks can also do poorly on such tests, suggesting that a disruption of normal brain function may be linked to the novel gene and/or the overexpression of APP or derivatives.

An alternate approach for introducing larger forms of the APP gene involved germline transmission by injecting mouse blastocysts with embryonic stem cells containing the yeast artificial chromosome (YAC) incorporating the entire hAPP gene (Pearson and Choi, 1993; Lamb *et al*., 1993). Abundant expression of hAPP 695 and 751 isoforms was obtained, at levels similar to endogenous APP species (Lamb *et al*., 1993). Mice containing the YAC–APP gene construct with the Swedish FAD double mutation demonstrate high levels of Aβ in the brain and a decrease in α-secretase-derived fragments (Lamb *et al*., 1997). In addition, transgenic mice bearing YAC–APP gene constructs with the FAD-related, Val-Ile substitution at codon 717 showed particularly high levels of Aβ42/43 (Lamb *et al*., 1997). Thus, while authentic AD-like histopathology has not been reported in these YAC transgenics, they confirm the proposal that FAD-related mutations result in higher levels of Aβ, particularly Aβ species that are likely to be more highly amyloidogenic.

A breakthrough in replicating AD-specific pathology in transgenic mice was heralded by Kawabata *et al*. (1991), who reported on the presence of aging-related amyloid plaques and neurofibrillary tangles in mice expressing a transgene containing the Thy-1 promoter and a sequence corresponding to a C-terminal region of APP, including the Aβ sequence. However, serious concerns emerged regarding the remarkable AD-like pathology in these transgenic mice, and upon failure to replicate the results in further transgenic animals, the original paper was withdrawn (Kawabata *et al*., 1992).

Subsequently, there have been four groups that have developed transgenic mouse models that faithfully replicate the congophilic, amyloid fibril plaques of AD (Games *et al*., 1995; Hsiao *et al*., 1996; Sturchler-Pierrat *et al*., 1997; Moechars *et al*., 1999) (Table 25.1). In the first of such models, the construct utilized was a hAPP minigene, containing introns 6 to 8 for alternative splicing of exons 7 and 8, and also bearing the Val to Phe FAD mutation at codon 717 ($APP717_{Val\text{-}Phe}$) (Games *et al*., 1995). Transgene expression was driven by a platelet-derived growth factor (PDGF)-β promoter and the genetic background was a Swiss Webster × (C57BL/6 × DBA/2) hybrid. Approximately 40 copies of the gene were inserted into the transgenic line 109 genome and greater than 10-fold expression of hAPP relative to mAPP (or AD) was detected in the brain. At approximately 6 to 9 months of age, heterozygote transgenic animals of line 109 developed Aβ deposits in the hippocampus, cerebral cortex, and the corpus callosum, and the density of these plaque-like structures increased with further aging (Games *et al*., 1995; Irizarry *et al*., 1997a). Aβ deposition also showed remarkable specificity in its distribution within the cerebral cortex, closely resembling the pattern of distribution of plaques in AD (Irizarry *et al*., 1997a). Biochemical analysis has demonstrated that Aβ levels, particularly Aβ1-42, were increased massively during aging in transgenic mice (up to 500-fold rise in 18-month compared to 4-month-old animals) (Johnson-Wood *et al*., 1997).

Aging-related plaques in line 109 animals were labeled by Congo red and stained with silver impregnation methods, with a substantial proportion also stained by thioflavine S. The initial report noted that neurofibrillary tangles and/or labeling for tau was absent in these animals (Games *et al*., 1995). However, abnormal neurites labeled with antibodies to hAPP, NF, and synaptophysin were associated with Aβ plaques, as indeed were astrocytes (Games *et al*., 1995; Masliah *et al*., 1996). In addition, there was evidence of microglial acti-

vation within the brains of animals demonstrating plaques. No overt neuronal degeneration was detectable, although a loss of synaptophysin and microtubule-associated protein 2 (MAP2) was noted in particular hippocampal regions.

Further investigation of line 109 using electron microscopy techniques confirmed that these transgenic mice do develop intraparenchymal masses of amyloid-like fibrils with aging (Masliah *et al*., 1996). Furthermore, the plaque-associated dystrophic neurites contained accumulations of NFs as well as abnormal features such as numerous multilamellated bodies and dense-core vesicles. Further neurodegenerative changes in some cortical and hippocampal neurons included apoptotic changes, but it was not clear whether these were spatially related to the Aβ deposits. However, despite the progressive increase of β-amyloid deposition in these mice with aging, stereological analysis revealed no overt nerve cell loss in the hippocampus or in the entorhinal and cingulate cortices up to 18 months of age (Irizarry *et al*., 1997a). An identical PDGF APP717$_{\text{Val-Phe}}$ mutation construct was used to generate another line of mice (H6) that develops aging-related Aβ plaques (Hsia *et al*., 1999). Interestingly, these animals demonstrate a decrease in markers such as MAP2 and synaptophysin in the hippocampus in the months prior to the formation of Aβ deposits. In contrast, animals from line 109 show no such early neuronal changes. Electrophysiological examination of the hippocampus of H6 mice at younger ages (1–4 months) prior to β-amyloid deposition demonstrated no impairment in LTP but deficits in transmission between CA3 and CA1 neurons and increased NMDA receptor activity relative to non-NMDA receptors in postsynaptic potentials. Hsia *et al*. (1999) noted that there may be an insidious deleterious activity of APP transgene products prior to the emergence of plaques, which may be relevant to AD or a consequence of overexpression of APP as observed in numerous other transgenic lines.

Transgenic mice with this construct are also useful for determining the role of other factors in AD. For example, crossing such mice with animals expressing the transforming growth factor (TGF)-β1 results in accelerated Aβ deposition in the brain, suggesting a role of increased levels of this factor in AD (Wyss-Coray *et al*., 1997). In addition, mice bearing the APP717$_{\text{Val-Phe}}$ mutation also show increased cell death in the hippocampus, relative to nontransgenic animals, following experimental brain trauma, indicating that a hAPP$_{\text{mutant}}$ product may augment cellular degeneration following such insults (Smith *et al*., 1998). However, these animals do not show augmented β-amyloid deposition in the form of plaques. In contrast, transgenic animals that overexpress the entire APP gene (via a YAC) do not show elevated neuronal degeneration following brain trauma (Murai *et al*., 1998).

In 1996, Hsiao *et al*. reported on a different transgenic mouse line (Tg2756) that contained a construct comprising the hamster prion protein promoter and the APP695 sequence with the Swedish FAD double mutation at codons 670 (Lys to Asn) and 671 (Met to Leu) (APP$_{\text{SWE}}$ mice). Mice were bred on to a C57Bl6 $\times$ SJL background, and heterozygous transgenic mice were found to have brain levels of total APP from 5- to 6-fold higher than mAPP. Furthermore, there was a 5- and 14-fold increase in Aβ1-40 and Aβ1-42/43 levels, respectively, in old (11–13 months of age) relative to younger (2–8 months of age) transgenic mice. Plaque-like deposits, labeled with antibodies to Aβ and stained with Congo red and thioflavine S, were also present in the 11- to 13-month-old transgenic animals. In addition, astrocytes and silver-stained dystrophic neurites were associated with the plaques in older transgenic mice, but there was no evidence of neurofibrillary tangle formation or nerve cell degeneration reported. Synaptophysin and NF immunoreactive enlarged axonal processes have been localized to plaques in this line (Irizarry *et al*., 1997b), and markers of oxidative stress may also be present within these plaque-related dystrophic neurites (Papolla *et al*., 1998). Furthermore, activated microglia show a close association to Aβ deposits in these animals (Frautschy *et al*., 1998). Despite the presence of Aβ plaques in the hippocampus of APP$_{\text{SWE}}$ mice, stereological analysis demonstrated that there was no associated nerve cell or volume loss in the CA1 subregion, nor loss of synaptophysin labeling in the dentate gyrus (Irizarry *et al*., 1997b).

Hsiao *et al*. (1996) also determined that APP$_{\text{SWE}}$ transgenic mice showed deficits in spatial memory (Morris water maze) and alternation (choice in a Y-maze) tasks at older ages (9 to 10 months), whereas younger transgenic mice performed at equal levels to nontransgenic animals. In addition, further studies confirmed aging-related deficits in spatial memory in APP$_{\text{SWE}}$ mice as well as impaired LTP in the CA1 and dentate gyrus of the hippocampus, indicating an important functional impairment in these animals despite limited neuronal pathology (Chapman *et al*., 1999). In another study, younger transgenic mice that express both APP$_{\text{SWE}}$ and APP717$_{\text{Val-Phe}}$ mutations showed higher levels of Aβ protein in the hippocampus than mice with the APP717$_{\text{Val-Phe}}$ mutation alone (Hsia *et al*., 1999). Furthermore, doubly transgenic mice also demonstrated more severe impairment in synaptic transmission in this brain region than APP717$_{\text{Val-Phe}}$ mice prior to the emergence of plaques, supporting the proposal that elevated nonamyloidogenic Aβ may have deleterious effects on nerve cells (Hsia *et al*., 1999).

The third successful effort at producing AD-like pathology in transgenic mice has come from the laboratories of Novartis Pharma (Sturchler-Pierrat *et al*., 1997). Two lines, expressing either the hAPP751 isoform bearing the APP$_{\text{SWE}}$ mutation and APP717$_{\text{Val-Ile}}$ mutations with the human Thy-1 promoter (line 22) or the hAPP751 isoform bearing the Swedish mutation only driven by the murine Thy-1 promoter (line 23), were shown to develop age-related Aβ plaques. A further ''line 14'' containing the hAPP751 construct with the Swedish mutation and the human Thy-1 promoter did not show Aβ plaque formation with aging. Interestingly, both lines 14 and 22 demonstrated a twofold overexpression of hAPP relative to mAPP, and line 23 showed a sevenfold increase in transgene expression. Mice of line 22 were of a very advanced age (18 months) when they began to show Aβ deposits, whereas line 23 mice demonstrated Aβ deposits by 6 months of age. In addition, plaques in line 22 mice showed reduced levels of Congo red staining and glial reaction as compared with similar structures in line 23 mice. Abnormal neurites stained for acetylcholinesterase or labeled with antibodies to NFs, APP, and synaptophysin were associated with plaques in these animals. Interestingly, congophilic plaques in these two lines were also associated with abnormal neurites labeled with various antibodies to tau, the first such report indicating a link between APP transgenic-associated plaques with AD-like modification in

tau. However, while neurofibrillary tangles were not present in these mice, nerve cell loss of the order of 14 to 25% occurs in the CA1 region of the hippocampus and is correlated with Aβ plaque load, with no appreciable neuronal degeneration in the neocortex (Calhoun *et al*., 1998).

The most recent research group to report on AD-like neuropathology described two different transgenic constructs, FVB/N mice bearing hAPP minigenes with either APP_{SWE} or $APP717_{Val\text{-}Ile}$ mutations under regulation by the mouse Thy-1 promoter (Moechars *et al*., 1999). Heterozygote animals with either of these constructs developed Aβ immunoreactive plaques in various regions of the brain at greater than 12 months of age, whereas transgenic animals overexpressing nonmutated mouse and human APP695 did not. Animals bearing the APP_{SWE} mutation produced higher amounts of β-secretase-derived APP derivatives, indicating the possible consequence of the site of the mutation, whereas mice with the APP mutation at codon 717 demonstrated particularly high levels of Aβ1-42/43. The plaque-like structures were stained with thioflavine S and by silver impregnation. Plaques were surrounded by astrocytes and microglia, as well as by dystrophic neurites labeled with antibodies to NFs, APP, ubiquitin, synaptophysin, MAP1, and tau.

All APP transgenics generated by Moechars *et al*. (1999) showed a heightened risk for early death, which may be related to previous studies of the influence of APP transgenes in the FVB/N mouse strain (Hsiao *et al*., 1995). In addition, these transgenic lines demonstrated abnormal behavior such as heightened aggressiveness, neophobia, and agitation, which occurred at ages long before plaque formation. Furthermore, mice derived from these various APP constructs demonstrated impairments in memory tasks. Further analysis of mice with the codon 717 mutation indicated normal synaptic transmission within the hippocampus, but abnormal decay of LTP (Moechars *et al*., 1999).

Thus, multiple studies involving transgenic mice demonstrate that overexpression of the APP gene (or regions thereof) may have deleterious effects on brain function irrespective of plaque pathology, leading to impairment in synaptic phenomena such as LTP as well as cognitive functioning and behavior. In some mice, overt neuronal degeneration and gliosis also occur. Whether the functional and pathological changes are related to developmental abnormalities in transgenic animals or the toxicity of specific APP fragments remains to be firmly established. Until this is determined, and if evidence of APP/soluble Aβ toxicity in the human brain is established, the value of these animals for replicating important aspects of AD remains to proven.

It is also clear that aging, in addition to the overexpression of APP bearing known FAD mutations, is essential for the emergence of a specific feature of AD-like pathology, the Aβ plaque (Games *et al*., 1995; Hsiao *et al*., 1996; Sturchler-Pierrat *et al*., 1997; Moechars *et al*., 1999). Thus, overexpression of these APP forms is likely to lead to increased levels of Aβ peptide being produced and, in particular, abundant amounts of the more fibrillogenic Aβ1-42/43 may be crucial. The Aβ plaques in these mice are not pathologically inert structures as they are associated with abnormal neurites variably labeled for APP, synaptophysin, NFs, and, in two studies (Sturchler-Pierrat *et al*., 1997; Moechars *et al*., 1999), abnormal tau characteristic of the AD brain. However, there is no evidence of AD-specific intraneuronal abnormal filamentous structures (PHFs) within these affected neurites or in cell bodies. In addition, overt neuron loss around plaques is not obvious in most of these animals, nor are neurofibrillary tangles or neurodegeneration in nerve cells known to be selectively vulnerable in AD (Hof *et al*., 1999). A number of interpretations can thus be made from the current information available. It is possible that Aβ deposits are not particularly vital in the sequence of pathological events leading to neurodegeneration in AD. As indicated by numerous other transgenic models and other studies, a more soluble form of Aβ, or perhaps a different fragment of APP, could be the toxic species in AD. Alternatively, as suggested by Terry *et al*. (1996), APP misprocessing may not be the central early event in AD pathology—abnormal changes in the neuronal cytoskeleton might lead to neuronal degeneration and plaque formation.

However, the correct interpretation of these transgenic models may rely on a closer analysis of the local degenerative changes that are associated with plaque formation. As noted earlier, dystrophic neurites localized to plaques in transgenic mice have a common profile in their labeling with antibodies to APP, synaptophysin, and NFs. In AD, dystrophic neurites can be classified into several subtypes based on their immunolabeling for specific markers. Thus, three main subtypes of dystrophic neurites have been shown to be labeled principally for NFs, tau, or APP/synaptophysin (Dickson *et al*., 1999). As noted in ultrastructural studies by Masliah *et al*. (1996), the plaque-related axonal changes in APP transgenic mice, including an accumulation of NFs and abnormal multilamellated and vesicular structures, bear a striking resemblance to the subset of NF-containing dystrophic neurites in AD. This may be particularly crucial with respect to studies on the preclinical stage of AD, which is characterized by the presence of Aβ plaques but not extensive neurofibrillary pathology or neuronal degeneration (Morris *et al*., 1996; Price and Morris, 1999; Vickers *et al*., 2000). However, neuritic plaques do occur in this early stage of the disease, and these dystrophic neurites are characterized by the presence of APP, synaptic markers, or NF proteins, but not abnormal tau species (summarized in Dickson *et al*., 1999).

Thus, the pathology in transgenic mice demonstrating Aβ plaques most closely resembles the preclinical phase of AD. In a similar fashion to humans, these transgenic animals may require several more years before more profound neurofibrillary pathology could "mature," far beyond the normal longevity of mice. Thus, while these current transgenic models may not ever develop the full spectrum of pathology in AD, their replication of the early stages of the disease will make them extremely valuable for ascertaining new therapeutic strategies to either prevent plaque formation or delay the neuronal reaction to plaque formation, which eventually leads to neuronal degeneration and dementia. In this respect, a study has indicated that early immunization with human Aβ peptides in $hAPP717_{Val\text{-}Phe}$ transgenic mice (Games *et al*., 1995) results in protection against Aβ plaque formation with aging (Schenk *et al*., 1999). Furthermore, immunization at later ages may even reduce Aβ plaque load in animals in which the pathology would already have been established (Schenk *et al*., 1999). The precise mode of effect of the immunization strategy is

unknown, although it is possible that the binding of antibodies to the human Aβ sequence may trigger the immune system to eliminate pathological Aβ species before and after deposition into plaques. If supported by other studies, this report may represent an important step in preventing a key pathological hallmark of AD. However, the vaccination strategy in humans should be approached with caution due to the ubiquitous expression of APP throughout the body (unlike the localized expression of hAPP in transgenic mice) and the possibility of stimulating an ultimately harmful autoimmune/inflammatory response.

The issue of how these plaques cause local changes in axons, and how plaques lead to neurofibrillary pathology in nerve cell bodies in AD, also remains to be firmly established. Plaque-localized oxidative stress may be one mechanism (Papolla *et al*., 1998). Alternatively, we have proposed that the development of masses of amyloid fibrils in the brain leads to compression and deformation of axonal processes (King *et al*., 1997; Vickers, 1997; Dickson *et al*., 1999). The reactive changes in axons would explain the morphological, neurochemical, and ultrastructural features of early forms of dystrophic neurites. In this respect, the neuronal pathology associated with plaques in transgenic mice and early stages of AD can be replicated in experimental models of acute physical injury to the central nervous system (King *et al*., 1997). The chronic stimulation of this response may lead to a localized inflammatory reaction, abnormal processing of tau in dystrophic neurites and the cell body of origin of the damaged axon, and, ultimately, degeneration of the entire neuron (Vickers, 1997; Vickers *et al*., 2000). It is notable that studies of transgenic mice have also described a compression of the neuropil associated with Aβ deposits (Games *et al*., 1995), and plaque-related compression of cell bodies may occur in one transgenic cell line (Calhoun *et al*., 1998).

B. Amyloid Precursor Protein Knockouts

Homologous recombinant techniques can be used to modify endogenous gene structure in embryonic stem cells. This genetic background can then be transferred to embryos and, with appropriate cross-breeding of heterozygous animals, lines in which expression of the gene is disrupted (''knocked-out'') and prevented can be created. Mice in which mAPP has been inactivated with such transgenic techniques are both viable and fertile, but demonstrate reduced weight (Zheng *et al*., 1995). Such animals also develop gliosis in the brain as well as neurological signs such as reduced limb strength and locomotion (Zheng *et al*., 1995). APP knockout animals were impaired in learning tasks associated with the Morris water maze (Phinney *et al*., 1999), but did not show neuron or synapse loss in the hippocampus (Phinney *et al*., 1999). Reduction in MAP2 and synaptophysin labeling, as well as dendrite length, in the hippocampus has been noted in APP knockout mice, in addition to impaired LTP and paired-pulse depression of GABA-mediated postsynaptic activity (Seabrook *et al*., 1999). Cultured neurons lacking the APP were reported to display reduced viability and neurite growth (Perez *et al*., 1997). In contrast, other studies have indicated that cultured neurons from APP-null mice demonstrate normal neuritic growth, as well as reactions to toxic Aβ and oxidative stress that are similar to nontransgenic-cultured neurons (Harper *et al*., 1998; White *et al*., 1998).

Transgenic mice engineered to express only an isoform of APP lacking exon 2 at 5% of normal APP expression levels (a possible ''functional'' knockout of mAPP) are also viable and fertile but showed reduced body weight, impaired learning ability on the Morris water maze, reduced exploratory behavior, and frequent corpus callosum agenesis (Tremml *et al*., 1998; Magara *et al*., 1999). Further analysis of this low-expressing APP_{mutant}, as well as APP knockouts, indicated that these transgenic modifications result in reduced brain weight and decreased size of forebrain commissures (Magara *et al*., 1999). However, the precise genetic background of particular mouse strains appears to regulate the frequency of callosal agenesis in APP_{mutant} and APP knockout mice (Magara *et al*., 1999).

These data indicate that the absence of APP expression may affect neuronal structure and activity in particular brain regions. While a precise indication of the cellular role of APP has not emerged from these studies, some results point toward a role for APP in neuronal development, axonal guidance, and cell–cell interactions. It is also important to note that interpretation of the phenotype associated with knockout of the APP gene may be difficult due to a possible compensatory action of amyloid precursor-like proteins (White *et al*., 1998; Seabrook and Rosahl, 1999).

However, it is interesting that disruption of mAPP expression can result in a phenotype that is similar to many of the structural, pathological, and behavioral changes noted in numerous transgenic animals designed to overexpress various APP constructs. It may be that the alterations in these latter animals are closely associated with a disruption of the normal function of mAPP, rather than overexpression of particular novel forms of the protein.

C. Presenilin Transgenics, Knockouts, and Crosses

APP mutation carriers account for only a minor subset of all FAD pedigrees. A much larger proportion of FAD cases have been linked to mutations (or exon deletion) on genes located on chromosome 14 and 1, which encode structurally similar proteins (67% sequence homology), presenilin (PS) 1 and 2, respectively (Hardy, 1997). PS gene mutations result in an autosomal dominant pattern of inheritance of AD with a relatively early onset of disease symptoms. PS are membrane-bound proteins with multiple (probably eight) transmembrane domains with the amino- and carboxyl-terminal ends likely to be internal (cytosolic) to the membranous structure. FAD-linked mutations occur throughout the protein, particularly in regions conserved between PS-1 and PS-2, with mutations more abundant on PS-1 than on PS-2 (Mattson and Guo, 1997; Hardy, 1997).

The precise cellular function of PS in the brain, as well as their role in AD pathogenesis, remains to be firmly established. PS-1 and PS-2 are similar to *C. elegans* proteins, sel-12 and Spe-4, which are involved in cell fate determination through the lin-12/Notch signaling pathway and protein trafficking in the Golgi during spermatogenesis, respectively (Mattson and Guo, 1997).

PS-1 also appears to have an important role in Notch processing in mammalian cells. A PS-1 gene knockout in mice is

lethal prior to birth, with embryos demonstrating abnormal somite segmentation and differentiation, as well as decreased expression of Notch 1 and associated ligands (Wong *et al*., 1997). Interestingly, APP γ-secretase inhibitors also interfere with the processing of Notch (De Strooper *et al*., 1998). In addition, neuronal cultures derived from PS-1 knockout embryos demonstrate normal α- and β-secretase activity in APP processing, but impairment in APP cleavage at the γ-secretase site, resulting in reduced Aβ and increased C-terminal APP fragments, suggesting the possibility that PS may act as a γ-secretase for APP (DeStrooper *et al*., 1998, 1999). Indeed, studies of cell lines transfected with specific PS-1 mutations have provided evidence that this PS may correspond to the elusive γ-secretase that is important for processing APP into the Aβ peptide (Wolfe *et al*., 1999). Alternatively, PS may act to ''traffic'' proteins such as APP and Notch to the appropriate cellular site where the γ-secretase acts (Hardy and Israel, 1999). These studies therefore indicate that PS mutations may have an important role in the abnormal processing of APP that leads to the generation of amyloidogenic species of Aβ, indicating a toxic ''gain of function'' of the abnormal protein. Loss of function of mutated PS is also contraindicated by the ability to ''rescue'' PS-1-null mice by transgenic introduction of PS-1 genes carrying mutations linked with FAD (Davis *et al*., 1998; Qian *et al*., 1998).

Thus, these data may explain why human PS_{mutant} carriers show elevated A1-42/43 in plasma in a similar fashion to APP_{mutant} carriers (Scheuner *et al*., 1996). In addition, cultured fibroblasts from individuals with PS-1 and PS-2 mutations also show increased release of Aβ1-42/43 (Scheuner *et al*., 1996).

Transgenic mice that overexpress FAD-linked mutations in human PS-1 or PS-2 demonstrate increased levels of the Aβ1-42/43 peptide in the brain relative to transgenic mice expressing wild-type hPS-1 and nontransgenic animals (Duff *et al*., 1996; Oyama *et al*., 1998). Furthermore, an aging-related further increase in Aβ1-42/43 in hPS-2 mice has been reported (Oyama *et al*., 1998). Borchelt *et al*. (1996) demonstrated an increase in brain Aβ1-42/43 levels in crossed transgenic mice expressing a FAD-linked hPS-1 mutation and $hAPP_{SWE}$ as compared to APP_{SWE} alone or transgenic mice expressing wild-type hPS-1 and the Swedish mutation. However, Citron *et al*. (1997) have noted a specific increase in the ratio of Aβ1-42/43 to Aβ1-40 in $PS\text{-}1_{mutant}$ transgenics crossed with mice expressing wild-type hAPP695 at higher levels than mAPP.

PS_{mutant} transgenics do not result in amyloid plaque development, although crossing such animals with transgenic mice with the APP_{SWE} mutation leads to earlier Aβ deposition in the brain (Borchelt *et al*., 1997; Holcomb *et al*., 1998). Cumulatively, studies using the double-transgenic animal models support the contention that PS mutants lead to increased levels of Aβ1-42/43 relative to Aβ1-40, which can accelerate Aβ deposition in the brain. However, it may be possible that PS mutations impart harmful effects independent of Aβ deposition as aged (>13 months) transgenic mice expressing a FAD-linked PS-1 mutation demonstrate apoptotic and degenerative neurons in the hippocampus, cortex, and cerebellum (Chui *et al*., 1999). Furthermore, cultured neurons from transgenic rats expressing high levels of hPS-1 show enhanced susceptibility to apoptosis (Czech *et al*., 1998).

D. Apolipoprotein E

The apolipoprotein E (ApoE) gene is located on chromosome 19 and comes in three allelic variations, ϵ2, ϵ3, and ϵ4, encoding proteins known as ApoE2, ApoE3, and ApoE4, respectively. Inheritance of the ϵ4 allele is associated with a gene-dose related, increased risk of AD, whereas ϵ2 may be associated with decreased risk (Corder *et al*., 1993, 1994). Notably, some ethnic groups may show reduced effects of ApoE gene-related susceptibility to AD (Osuntokun *et al*., 1995; Tang *et al*., 1998). In addition, it is important to note that ϵ4 carriers generally display an increased risk to cardiovascular disease, as well as decreased longevity. It is well known that ApoE has a role in the transport of cholesterol and lipids throughout the body (Mahley, 1988), but the mechanism by which ApoE isoforms may influence relative risk to AD remains undetermined. It has been proposed that ApoE may act as a ''pathological chaperone,'' enhancing the fibrillogenesis of Aβ (Wisniewski and Frangione, 1992; Ma *et al*., 1994). In this respect, ϵ4 carriers show higher levels of Aβ plaque load in AD (Rebeck *et al*., 1993; Schmechel *et al*., 1993; Corder *et al*., 1994; Olichney *et al*., 1996). Alternatively, it has been suggested that ApoE has an important role in regulating the neuronal response to injury by the mobilization and/or redistribution of cholesterol and lipids (Poirier, 1994). Thus, inheritance of particular isoforms of the ApoE gene may influence the neurodegenerative changes in the AD brain. Similarly, it is notable that head injury and ApoE genotype may act synergestically to increase risk for AD (Mayeux *et al*., 1995) and that a poor clinical outcome from head trauma is associated with the inheritance of ϵ4 alleles (Teasdale *et al*., 1997; Jordan *et al*., 1997; Friedman *et al*., 1999).

In the preclinical and end stages of AD, ApoE is immunohistochemically localized to a subset of Aβ deposits and, in particular, the neuritic plaque (Dickson *et al*., 1997). ApoE immunolabeling can also be present in neurofibrillary tangles, but only in the end stage, extracellular forms of this hallmark, indicating a secondary association (Dickson *et al*., 1997). Localization of ApoE to neuritic plaques indicates that these may be the most fibrillogenic plaques or that ApoE becomes secondarily bound to the plaque due to its role in regulating the local neuritic response to injury. Examination of ApoE transgenic animals could, thus, provide important clues to the specific involvement of ApoE in AD pathology.

ApoE knockout mice demonstrate high plasma triglyceride and cholesterol levels and, ultimately, develop artherosclerotic lesions (Higgins *et al*., 1997). These mice are viable and have been shown to develop an age-related decrease in immunolabeling for neuronal markers such as synaptophysin, tubulin, and MAP2, but increased expression of glial markers (Masliah *et al*., 1995). Morphological and ultrastructural studies indicated abnormal dendrites and a loss of synapses in these animals. In addition, ApoE knockout mice show increased brain levels of 3-nitrotyrosine, a possible marker of oxidative stress (Matthews and Beal, 1996). ApoE-deficient animals display impaired ability on Morris water maze tasks, decreased acetylcholinesterase activity in the cortex and hippocampus, and the presence of abnormally hyperphosphorylated tau (Gordon *et al*., 1996). However, Mercken and Brion (1995) have not observed ApoE knockout-related changes in tau expression or

modification. Furthermore, Fagan *et al*. (1998) presented data that indicates no loss of acetylcholinesterase activity with aging in ApoE knockout mice, nor loss of other cholinergic markers with aging or following injury of pertinent cholinergic pathways. In addition, other studies on ApoE knockouts have found minimal age-related brain changes, cognitive impairment, or influence on the neuronal response to injury (Anderson *et al*., 1998). In contrast, other investigations have replicated initial reports, demonstrating that aging in ApoE-null mice is associated with an immunodetectable decrease in synaptophysin and MAP2 labeling, as well as a disruption of NF-immunoreactive axons (Buttini *et al*., 1999) and deficits in cognitive functioning (Raber *et al*., 1998). Interestingly, while noting no effects of the lack of ApoE on the cholinergic system following injury, Fagan *et al*. (1998) demonstrated that damage to other axonal pathways (entorhinal-hippocampus connections) leads to an abnormal prolonged degenerative response in ApoE knockouts. ApoE-null mice also show a greater degree of nerve cell degeneration, associated with motor and cognitive deficits, than wild-type mice following experimental closed head injury (Chen *et al*., 1997).

The underlying cause for the discrepancies in these results with ApoE knockouts remains to be determined: they may be due to genetic background of different strains or, if strains are identical, it has been suggested that animal management-related factors throughout the animal's life may contribute (Buttini *et al*., 1999). Perhaps of greater significance, using transgenic technology to express either $\epsilon 3$ or $\epsilon 4$ genes on the ApoE knockout background has revealed isotype-specific effects on the brain response to various insults. For example, expression of human $\epsilon 3$, but not $\epsilon 4$, minigenes in the brain under regulation of the neuron-specific enolase promoter protects the brain from loss of synaptophysin, MAP2, and NF markers in mice following systemic kainic acid toxicity (Buttini *et al*., 1999). Of particular interest is that $\epsilon 4$-expressing mice do not show a greater extent of neurodegeneration than ApoE knockouts (i.e., the ApoE isoform does not appear to be further deleterious) (Buttini *et al*., 1999), but the same line of $\epsilon 4$ carriers, particularly females, show aging-related impairment in acquisition of the Morris water maze task (Raber *et al*., 1998). These data indicate that more subtle brain changes are linked with specific ApoE isoforms, and the possibility of influences of gender-related effects. Expression of the $\epsilon 3$ gene on the ApoE null background appears to ameliorate the synaptic and dendritic alterations usually associated with aging of the knockout, and expression of the $\epsilon 4$ gene selectively prevents loss of synaptic markers in these animals (Veinvergs *et al*., 1999).

Crossing ApoE knockout mice with $\text{hAPP717}_{\text{Val-Phe}}$ mice resulted in reduced numbers of age-related Aβ deposits, relative to $\text{hAPP717}_{\text{Val-Phe}}$ mice crossed to ApoE-bearing mice, but no changes in either APP expression or Aβ peptide levels in brain (Bales *et al*., 1997). These data strongly support the proposal that ApoE serves to facilitate Aβ plaque formation, rather than having a secondary role in the neuritic response to plaque-induced injury. Likewise, ApoE is localized to amyloid deposits found in peripheral tissues in some APP transgenic animals (Igeta *et al*., 1997). However, a possible role for ApoE isoforms in the neural response to trauma cannot be ruled out, as demonstrated by studies on the response of isoform-specific transgenics following focal brain ischemia (Sheng *et al*., 1998) and excitotoxicity (Buttini *et al*., 1999).

E. Cytoskeletal Proteins

Abnormal changes in cytoskeletal proteins such as microtubules, tau, and NFs are key pathological features of AD (Vickers *et al*., 1999). However, mutations in the genes for these cytoskeletal proteins have not been detected in any FAD cases. Mutations in the tau gene have been linked with a different form of brain degeneration known as frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) (Hardy *et al*., 1998). This disease can be accompanied by AD-like alterations in the tau protein, leading to a classification of FTDP-17 as a form of ''tauopathy.'' It will thus be very interesting to determine how the tau mutations lead to neuronal degeneration in transgenic animals once they become available. Expression of various forms of human tau in transgenic mice has resulted in neuron-specific expression of the transgene and biochemical modifications in tau that resemble those observed in the neurofibrillary pathology of AD (Gotz *et al*., 1995; Brion *et al*., 1999). These animals do not develop tangles per se, or abnormal filaments characteristic of advanced neurofibrillary pathology, leading to the interpretation that tau alterations in these transgenic mice represent the ''pretangle'' stage of AD pathology.

Further interesting data pertinent to AD and other neurodegenerative disease have been derived from studies of transgenic mice expressing human NF proteins. A transgenic mouse line has been developed that expresses the human NF-M protein at levels substantially lower than mNF-M (Lee *et al*., 1992). In young animals, the human NF protein incorporates into filaments containing endogenous NF subunits and no obvious brain pathology is evident (Lee *et al*., 1992). With aging, tangle-like structures were observed in the neocortex, but these inclusions were composed of NFs and not PHFs (Vickers *et al*., 1994). The pathology in these mice may be illustrative of how the human NF-M protein may be susceptible to age-related pathological alterations. In addition, the tangle-like structures showed a remarkable predilection for certain neocortical layers and cell types that correspond to the neurons showing selective vulnerability to neurofibrillary pathology in AD (Hof *et al*., 1990; Vickers *et al*., 1992). Similarly, aging and the emergence of neurofilamentous pathology in these mice were associated with learning deficits on Morris water maze tasks (Haroutunian *et al*., 1996). Other forms of pathology were evident in these mice, including Lewy body-like structures in the cerebral cortex and axonal ''torpedos'' in the cerebellum, with the latter resembling the cerebellar degeneration characteristic of numerous human diseases (Vickers *et al*., 1994). Finally, these mice also demonstrated retinal changes in NFs in select neuronal subpopulations of retinal ganglion cells (Vickers *et al*., 1995a) that were very similar to the neuronal alterations in primate models of glaucoma (Vickers *et al*., 1995b).

Similar forms of neurofilamentous and cellular pathology, often leading to cell death, have been reported in transgenic mice expressing other neuronal intermediate filament gene constructs (Ma *et al*., 1995, 1999; Tu *et al*., 1997; Ching *et al*., 1999). These results emphasize how alterations invol-

ving individual filament proteins can lead to a general disruption in the cytoskeleton and that cell-type-specific collapse or disruption of proteins such as NFs can result in a panorama of pathological inclusions, resembling numerous human neurodegenerative diseases.

III. Amyotrophic Lateral Sclerosis

ALS is the major cause of human motor neuron disease. In a similar fashion to AD, ALS is generally an aging-related condition that involves progressive neurodegenerative changes, with specific subsets of neurons showing particularly high susceptibility to damage. Selectively vulnerable neurons include motor neurons in the spinal cord as well as subsets of, mostly motor, neurons in the brain stem and cortex (Vickers *et al*., 1995c). In addition, alterations in the cytoskeleton also appear to play a role in the degeneration of neurons in ALS. In the spinal cord, extensively phosphorylated NFs are abnormally localized to the perikarya of ventral horn neurons, Lewy body-like (or hyaline) inclusions occur within the cell body, and accumulations of NFs in the proximal axon lead to the development of ''spheroids'' (Leigh and Swash, 1991). Cytoskeletal changes can also occur in the motor cortex, including aberrant NF phosphorylation in perikarya and the accumulation of NF in the terminal boutons of basket cells (Troost *et al*., 1992). In addition, many of the pathological changes in degenerating neurons are ubiquitinated (Leigh and Swash, 1991).

As with AD, the cause of the neurodegenerative process in ALS remains unknown. It may be that the disease mechanisms are related as, in rare conditions, a motor neuron disease syndrome occurs in conjunction with AD-like changes and dementia (e.g., in the native population of Guam; see Chapter 15). An extensive range of causative factors for ALS has been proposed, including viral infection, autoimmunity, metal toxicity, excitotoxicity, oxidative stress, and genetic factors (de Belleroche *et al*., 1996; Ince *et al*., 1998). Perhaps the best insight into ALS pathogenesis lies in determining the role of specific genes in disease etiology, and, thus, significant progress has resulted by the replication of important features of the human disease in transgenic models.

A. Cu/Zn Superoxide Dismutase-1

Most ALS cases appear to be sporadic in origin, although approximately 5 to 10% appear to inherit the disease in an autosomal dominant fashion. Onset of familial ALS (FALS) is approximately 50 years of age (an appreciably younger onset than sporadic cases), and the disease shows a relatively rapid degenerative course (de Belleroche *et al*., 1996). A major advance for ALS research was the identification of mutations in the Cu/Zn superoxide dismutase-1 (SOD-1) gene, with numerous different mutations in the gene accounting for approximately 20% of FALS cases (Rosen *et al*., 1993). SOD-1 is an enzyme that helps protect cells from oxygen radicals, being specifically involved in the catalysis of superoxide radicals to hydrogen peroxide and oxygen. While it has been proposed that SOD-1 gene mutations may lead to free radical toxicity due to ineffective SOD-1 activity or that overactivity of this enzyme may lead to a harmful increase in hydrogen peroxidase and damage to membranes, data examining peripheral effects of these FALS mutations have been conflicting (Ince *et al*., 1998). The ability to express these gene mutations in mice has represented an important and evolving tool for understanding the role of FALS mutations and SOD-1 in disease pathogenesis (Table 25.2).

The first of such transgenic mice generated expressed either the most common human SOD-1 mutation of alanine to valine at codon 4 (A4V mice) or the glycine to alanine mutation at codon 93 (G93A) (Gurney *et al*., 1994). Whereas A4V mice developed normally, one line of G93A mice that expressed the highest amount of hSOD-1_{G93A} developed tremors and signs of hind limb weakness by 3 to 4 months of age. These neurological changes were progressive, with animals practically paralyzed by 5 months of age and unable to feed. Pathological changes occurred in the spinal cord, including a loss of cholinergic ventral horn motor neurons, neurofilamentous accumulation in cell bodies, degeneration of ventral motor roots (but sparing of dorsal roots), loss of axons, and compensatory sprouting in muscles. Further analysis of this and other lines with the G93A mutation revealed that nerve cell damage was restricted to the brain stem and spinal cord and included vacuolar degeneration localized to the rough endoplasmic reticulum and possibly also mitochondria (Dal Canto and Gurney, 1994, 1995; Tu *et al*., 1996). Other pathological changes included NF-containing spheroids, Lewy body-like inclusions in nerve cell bodies and axons, astrogliosis, ubiquitination of cell bodies and neurofilamentous inclusions, and Wallerian-like degeneration of peripheral nerves. Time course studies demonstrated that the development of NF-containing inclusions and astrogliosis was closely correlated with the onset of neurological symptoms at 2 months of age. Vacuolarization

TABLE 25.2 Some Transgenic Lines Demonstrating Motor Neuron Pathology[a]

Construct	Age of onset (weeks)	Limb weakness	ALS-like cytoskeletal pathology	Vacuolarization	References
hSOD-1_{G93A}	12–16	+	+	+	Gurney *et al*. (1994)
hSOD-1_{G37R}	16–24	+	+	+	Wong *et al*. (1995)
mSOD-1_{G86R}	16–24	+	+	−	Ripps *et al*. (1995)
hSOD-1_{G85R}	32–40	+	−	−	Bruijn *et al*. (1997b)
mNF-L	0–1	+	+[a]	−	Xu *et al*. (1993)
hNF-H	12–16	+	+[a]	−	Côtë *et al*. (1993)
mNF-L_{L394P}	2–3	+	+	−	Lee *et al*. (1994)

[a]Overt motor neuron degeneration has not been demonstrated.

(particularly in mitochondria) appears to precede frank neuronal degeneration of motor neurons (Kong and Xu, 1998).

These data show that transgenic mice bearing a known FALS mutation can replicate many of the neurological and pathological features of human ALS. However, it is important to note that some pathological features in G93A mice, such as extensive vacuolarization, may occur in some FALS cases (Sasaki *et al*., 1998) but are not a common feature of the human disease. Interestingly, pathological consequences of the transgene appeared to rely on high expression of hSOD-1_{G93A}. In contrast, high expression of hSOD-1 without mutation did not result in such neurodegeneration (Gurney *et al*., 1994), indicating that ALS-like pathology may be due to a toxic gain of function, thus resembling results from many of the APP_{mutant} transgenic mice. In this fashion, elevated indices of lipid peroxidation occur in the spinal cord prior to the onset of ALS-like symptoms in G93A mice, with further increased peroxidation during the active phase of the condition (Hall *et al*., 1998). Increased free radical production selective for the spinal cord has also been reported (Liu *et al*., 1998). Thus, the G93A mutation may lead to altered SOD-1 activity, resulting in increased levels of free radical species and/or increased generation of hydrogen peroxide with consequent peroxidative damage to cell membranes. G93A mice are being used to develop possible new therapeutic strategies for ALS (Klivenyi *et al*., 1999).

A similar spectrum of neurological and pathological features has been described in transgenic mice bearing a FALS-linked glycine to arginine mutation at codon 37 (G37R) (Wong *et al*., 1995). These mice showed a particularly high expression of the transgene in spinal cord relative to peripheral cell types. At approximately 4 to 6 months of age, hSOD-1_{G37R} mice develop tremors and limb weakness as well as appreciable muscle wasting. In a similar fashion to G93A mice, motor function deteriorates, leading to paralysis. Disease onset was associated with vacuolar degeneration in the spinal cord, but unlike G93A mice, the vacuoles in G37R mice were localized to dendrites and the proximal regions of axons and had a particular association with abnormal mitochondria (Wong *et al*., 1995). Focal accumulations of dephosphorylated NF and SOD-1 immunoreactivity surrounded small vacuoles in axons at this stage (Borchelt *et al*., 1998). In addition, a decrease in ventral horn motor neurons, the abnormal localization of phosphorylated NF epitopes to cell bodies, and NF-containing swollen axons were noted in the earlier phase of neurological impairment (Wong *et al*., 1995).

In advanced stages of neurological impairment in G37R mice, there was clear degeneration of large myelinated axons and ventral motor nerve roots (Wong *et al*., 1995). However, it is notable that appreciable degeneration was present in the dorsal nerve roots, the ascending axons of dorsal columns. In addition, brain stem motor nuclei demonstrated degeneration, but so did the olfactory bulb, piriform cortex, pons, and deep nuclei of the cerebellum. Furthermore, vacuolar changes were present in kidney as well as epithelial cells of the choroid plexus. Vacuolar and cellular degeneration may not be spinal cord specific, but is likely to be the key factor associated with motor deterioration in these transgenic animals. In a similar fashion to Gurney *et al*. (1994), overexpression of wild-type hSOD-1 in different transgenic mice did not lead to an overt degenerative phenotype (Wong *et al*., 1995). In addition, 3-nitrotyrosine levels are increased by two- to three-fold in the spinal cord of G37R mice from the earliest pathological changes onward, but no increase in protein-bound nitrotyrosine was detected, nor evidence of increased hydroxyl radicals or lipid peroxidation (Bruijn *et al*., 1997a).

Ripps *et al*. (1995) examined the effects of a FALS-linked mutation in a mouse SOD-1 transgene [glycine to arginine at codon 86 (G86R), equivalent to codon 85 in hSOD-1]. SOD activity in these animals was equivalent to control (mSOD without mutation) animals. Mice showing relatively high expression of the transgene developed hind limb paralysis by 3 to 4 months of age, which progressed rapidly over the following days to a general loss of motor activity. Neuropathology at this end stage included loss of neurons in the ventral horn, with some neurodegeneration in brain stem motor nuclei, superior colliculus, cerebellar nuclei, basal ganglia, thalamus, and motor cortex (Ripps *et al*., 1995; Nimchinsky *et al*., 2000). In the ventral horn, silver staining demonstrated dystrophic neurites, argyophilic perikarya, and swollen processes (Ripps *et al*., 1995). However, the extensive vacuolar degeneration demonstrated in other SOD-1 transgenic animals was not detected in G86R mice (Morrison *et al*., 1998). At disease onset in G86R mice, phosphorylated NF epitopes were localized to cell bodies in the ventral horn, and ultrastructural examination demonstrated misshapen neuronal nuclei, chromatin aggregation, swollen mitochondria, and microvacuoles bordering the nucleus (Morrison *et al*., 1998). Disease onset was also associated with astrogliosis and the rapid loss of nerve cells, including motor neurons and interneurons. Close examination of the phenotype of vulnerable neurons in these mice determined that those interneurons and motor neurons that degenerate are characterized by their selective content of NF triplet immunoreactivity (Morrison *et al*., 1996), which may thus contribute toward their susceptibility to neurofilamentous pathology and neurodegeneration.

Interestingly, transgenic animals that express relatively low levels of hSOD-1 bearing the G85R mutation develop a relatively late-onset (8 to 10 months of age) but fast-progressing (approximately 2 weeks) ALS-like disease with limb weakness and muscle atrophy leading to extensive paralysis (Bruijn *et al*., 1997b). However, pathological examination revealed the presence of Lewy body-like inclusions in astrocytes and not neurons prior to disease onset (at approximately 6 months of age). From the development of neurological signs, there was a rapid loss of motor neurons and other spinal cord nerve cells as well as prominent astrogliosis with associated Lewy body-like inclusions. Unlike transgenic animals that expressed relatively high levels of mutated hSOD-1 (Gurney *et al*., 1994; Wong *et al*., 1995), there was no evidence of vacuole development or abnormal mitochondria in the G85R mice (Bruijn *et al*., 1997b), indicating that overexpression of mutated SOD-1 is responsible for this unusual form of neuronal pathology. Notably, Bruijn *et al*. (1997b) demonstrated a loss of the glial glutamate transported in the G85R mice, which may be similar to that which occurs in ALS (Rothstein *et al*., 1992), contributing toward a putative excitotoxic mechanism of neuronal degeneration (Rothstein *et al*., 1992; Vickers *et al*., 1995c).

Crossing G85R mice with either SOD-1 knockout mice or mice overexpressing hSOD-1 does not exacerbate or protect against the neurological and degenerative changes associated with aging in the G85R animals (Bruijn *et al*., 1998), strongly

indicating that heightened or decreased enzymatic activity may not be responsible for the pathological phenotype.

B. Neurofilaments

Alterations in NFs have a clear role in the pathological changes within upper and lower motor neurons that lead to neurodegeneration in ALS (Vickers *et al*., 1995c). A characteristic phenotype of most SOD-$1_{mutation}$ transgenic mice is the abnormal processing (phosphorylation) of NFs in the cell body as well as accumulations of NFs in the perikarya and axons. Similar changes have been reported in human FALS SOD-$1_{mutation}$ carriers (Rouleau *et al*., 1996). In addition, NF and SOD-1 are colocalized to the same slow phase of axonal transport as well as particular inclusions in G37R transgenic mice (Borchelt *et al*., 1998). Examination of a large number of sporadic ALS cases indicated that specific deletions in the NF-H gene were associated with the disease (Figlewicz *et al*., 1994), although no such polymorphism was detected in a large number of FALS pedigrees (Vechio *et al*., 1996). A further study has confirmed the presence of specific NF-H carboxy-terminal deletions in three sporadic cases as well as a FALS case (Al-Chalabi *et al*., 1999). While these NF-H tail polymorphisms are not common, they further implicate the NF proteins as having a pivotal role in ALS pathology. In this respect, specific transgenic models expressing particular NF gene constructs can also result in an ALS-like phenotype.

Overexpression (4-fold greater than endogenous gene) of the mouse NF-L gene results in a phenotype similar to ALS (Xu *et al*., 1993). Following birth, these animals develop swollen perikarya, NF accumulations in perikarya and proximal axons, distal axon degeneration, and skeletal muscle atrophy. Most animals die within a few weeks of birth. Transgenic animals that express the entire human NF-H gene ($>$2-fold mNF-H) develop fine tremors, abnormal reflexes, and muscle weakness by 3 to 4 months of age, associated with accumulation of NFs in perikarya and axons in the ventral horn (Côté *et al*., 1993). There was also a generalized loss of NFs in more distal segments of axons leading to atrophy of axons in the ventral spinal roots and sciatic nerve. Neurological degeneration appears to be relatively slow, although animals can experience respiratory difficulties at later ages. Notably, motor deterioration is more severe in homozygous transgenics compared to heterozygotes, indicating a gene-dosage effect (Côté *et al*., 1993). Skeletal muscle atrophy is also present in these animals. However, it is important to note that frank motor neuron degeneration has yet to be demonstrated in hNF-H or mNF-L overexpressing animals (Xu *et al*., 1993; Côté *et al*., 1993). Collard *et al*. (1995) demonstrated that these hNF-H transgenic animals show impaired axonal transport. Thus NF accumulation in the cell body and proximal axons may account for the lack of NFs in more distal axonal segments as well as reduced axonal transport and atrophy. Impaired fast and slow axonal transport has also been demonstrated in a transgenic line bearing the G93A hSOD-1 mutation, in parallel with the emergence of NF-containing inclusions (Zhang *et al*., 1997). Indeed, impairment in certain kinds of axonal transport may precede frank neuronal pathology in SOD-1_{mutant}-bearing mice (Warita *et al*., 1999; Williamson and Cleveland, 1999).

Perhaps the best model of ALS pathology has been produced in transgenic mice expressing a mutated (Leu to Pro at codon 394) mouse NF-L (mNF-L_{L394P}) transgene to approximately 50% of endogenous NF-L proteins (Lee *et al*., 1994). Within a few weeks after birth, mNF-L_{L394P} animals develop weakness in upper and lower limbs and abnormal reflexes, with progressive degeneration continuing until death. The pathological correlate of these neurological signs included stereotypical ALS features, such as loss of ventral motor neurons, perikaryal NF phosphorylation, proximal axonal swellings, degeneration of large axons in the ventral root, astrogliosis, and microgliosis, and atrophy of skeletal muscles with an associated denervation. Ultrastructural examination determined that the assembly of NFs was normal but that these filaments were segregated from microtubules and other membranous organelles.

This latter transgenic model confirms that a specific pattern of NF pathology is sufficient to result in an ALS phenotype. Regardless of different environmental and genetic factors, changes in NFs may be a key pathological alteration leading to neurodegeneration in ALS. Ultimately, neuronal degeneration itself may have more to do with the associated deficit in normal axonal transport. However, studies involving cross-breeding of transgenic mice have resulted in data that demonstrate that the pathogenic pathway in ALS may not be that simple. For example, transgenic mice bearing a NF-H construct ligated to the LacZ gene at the tail sequence of the NF gene develop perikaryal accumulations of NF and decreased NFs in axons associated with decreased calibre (Eyer and Peterson, 1994). Crossing NF-HLacZ mice with the G37R mouse (Wong *et al*., 1995) did not affect motor neuron degeneration characteristic of the G37R line (Eyer *et al*., 1998). Thus, NF disruption appeared to have no effect on SOD-1_{mutant}-related degeneration in this study. However, it should be noted that NF-HLacZ mice already result in motor neuron degeneration and axonal damage, indicating that the threshold for producing an ALS-like phenotype may be less in a G37R mouse crossed into this background (i.e., a protective effect may be masked).

A further curious result involved crossing G37R (Wong *et al*., 1995) mice with hNF-H (Côté *et al*., 1993) mice, which resulted in a phenotype where animals showed a markedly increased life span with less motor and sensory axon degeneration (Couillard-Després *et al*., 1998). Couillard-Després *et al*. proposed that the perikaryal accumulation of NF characteristic of the NF-H line (and also NF-HLacZ mice) may act to protect neurons against calcium-mediated toxicity due to the calcium-binding properties of NFs (Lefebvre and Mushynski, 1988).

Finally, crossing G85R mice onto a NF-L knockout background (where the remaining NF triplet proteins do not form filaments) also results in a delayed onset of the ALS phenotype, an increased life span, and a shift in vulnerable cell types to include sensory axons (Williamson *et al*., 1998). Thus, in this example, the absence of NFs ameliorates the G85R phenotype. In addition, increased expression of NF-M and NF-H is present in NF-L knockout mice, and Williamson *et al*. (1998), in a similar fashion to Couillard-Després *et al*. (1998), have suggested that elevated levels of these NF subunits may possibly serve to chelate excess calcium.

Thus, the precise role of SOD-1 and NF gene mutations and protein alterations in ALS remains to be elucidated. It is not perfectly clear whether the expression of hSOD-1 mutations simply leads to problems relating to oxidative stress, or whether NF accumulations are absolutely necessary for the neuronal pathology that leads to functional impairment. As with transgenic models of AD, delineation of the important cellular alterations leading to neuronal degeneration may become apparent through experiments involving the crossing of various transgenic lines. There may be heterogeneous causes of ALS, originating from genetic and/or environmental factors. Certainly, the identification of gene abnormalities in FALS cases lacking SOD-1 or NF gene mutations, or the determination of gene polymorphisms that may be risk factors, may provide important clues to disease causation and targets for transgenic models. There also needs to be a closer examination of how other leading theories of ALS etiology (e.g., excitotoxic mechanisms) relate to data derived from transgenic studies. It could be that the progressive neuronal degeneration that is central to ALS ultimately relates to impairment in the cellular machinery underlying fundamental cellular processes such as axonal transport, an essential feature of neurons with long-projecting axons.

Furthermore, given conflicting results as to whether the presence of SOD-1 mutations leads to heightened oxidative stress in either humans or transgenic mice, perhaps other cellular roles of SOD-1 may need to be explored. Notably, mice in which Cu/Zn SOD-1 expression has been knocked out develop normally and do not demonstrate motor neuron degeneration spontaneously but do show relatively greater vulnerability to motor neuron degeneration following axonal injury (Reaume *et al*., 1996). The axonal pathways of long projecting neurons may be subject to various extraneous physical forces (e.g., stretching and compression), and possession of a mutated SOD-1 protein may thus precipitate an abnormal neuronal response, ultimately leading to degeneration. Studies of the response of SOD-1 mutant transgenic animals to peripheral nerve injury may be instructive with respect to elucidating novel roles of the SOD-1 protein.

IV. Conclusion

Genetic engineering has provided new avenues for developing animal models that replicate key pathological changes in human neurodegenerative diseases. For research in AD, a number of studies have shown that overexpression of APP genes bearing known FAD-linked mutations in mice results in the development of Aβ amyloid plaques, typically in an age-related fashion. Crossing these animals with other transgenic lines carrying genes implicated in the disease process, such as PS and ApoE, has provided new light on the interaction of these factors in AD pathogenesis. Results to date indicate that all of these genes have crucial roles in contributing toward Aβ plaque formation, further implicating plaque pathology as a central event in AD. However, no transgenic animal models have been successful in producing authentic, PHF-related, neurofibrillary pathology such as tangles. Neuronal changes in plaque-bearing mice are indicative of the earliest stages of AD, thus indicating that a longer time frame may be required for substantial neurofibrillary pathology to develop. Irrespective, the current models represent vital new tools for developing methods to prevent or eliminate plaque pathology or the earliest forms of plaque-related neuronal pathology. It will also be important to determine the cause of neuronal pathology and functional synaptic impairment in APP/PS/ApoE transgenic animals that occurs in the absence of overt AD-like histopathology to establish whether these are changes central to understanding AD or a secondary phenomena related to the transgene or perhaps interactions with the genetic background of individual mice strains.

Similar success in replicating human neuropathology has been obtained with transgenic mice bearing FALS-related genes. However, the precise role of SOD-1 in disease etiology remains mysterious and, particularly, how SOD-1 abnormalities may relate to key neuronal alterations in the cytoskeleton that lead to impaired axonal transport. In this respect, crossbreeding of mice with various transgenic backgrounds may help unravel important pathological pathways leading to ALS. It is clear that the currently existing transgenic SOD-1 and NF transgenic lines will be extremely useful for developing new therapeutic strategies.

V. Addendum

As discussed in this chapter, numerous studies have described transgenic mice expressing mutated human genes involved in familial AD, such as APP or presenilins genes. These animals exhibit an increased amyloid peptide level, and amyloid deposits similar to those observed in AD occur, but ball of these models have failed in developing tau intraneuronal aggregates. The first reports of tau transgenic mouse lines focused on models expressing either the longest or the shortest human tau isoform (Götz *et al*., 1995; Brion *et al*., 1999; Ishihara *et al*., 1999; Spittaels *et al*., 1999; Duff *et al*., 2000). A somatodendritic localization of transgenic tau in brain and spinal cord is a constant feature in these models, as well as phosphorylation at sites that are hyperphosphorylated in PHF-tau proteins in AD (Götz *et al*., 1995; Brion *et al*., Ishihara *et al*., 1999; Spittaels *et al*., 1999; Duff *et al*., 2000). The presence of phosphorylated transgenic tau has also been described in glial cells in mice expressing the shortest transgenic human tau isoform (Brion *et al*., 1999). The somatodendritic transgenic tau exhibits a conformational modification revealed by antibody Alz50 that has been considered as a pretangle stage process. Nevertheless, these transgenic models do not exhibit fibrillar structures exactly similar to AD neurofibrillary lesions. In mice with a high level of expression of the shortest human tau isoform (5- to 15-fold the level of endogenous mouse tau), spheroidal intraneuronal inclusions have been observed early in the spinal cord and appear later in the brain where they are smaller (Ishihara *et al*., 1999). These lesions contain hyperphosphorylated tau proteins and NF, and their size and number are transgene dose dependent, as well as age dependent. A fraction of these transgenic proteins are insoluble, and their amount increases with age and worsening of the pathology. Ultrastructural analysis of these lesions has revealed the formation of straight filaments, rather than the characteristic PHF of AD.

Functionally, the overexpression of transgenic human tau isoform in mice induces axonopathy in the brain and spinal cord, as well as motor impairments. These severity of the pathological features is correlated with the level of expression of the transgene and with age (Götz *et al*., 1995; Spittaels *et al*., 1999; Probst *et al*., 2000). More recently, transgenic mice that overexpress a human tau genomic transgene have been described (Duff *et al*., 2000). The six human tau isoforms were generated in their brain, although the normal 1:1 ratio of three to four repeat isoforms seen in human brain was shifted in favor of an increase in three-repeat tau isoforms. A conformational change of transgenic tau similar to those described in the other mouse models was revealed with antibody MC1, but the characteristic somatodendritic showing that the proportion of the different tau isoforms and their compartmentalization are crucial events in the onset of several neurological disorders.

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References

Al-Chalabi, A., Andersen, P. M., Nilsson, P., Chioza, B., Andersson, J. L., Russ, C., Shaw, C. E., Powell, J. F., and Leigh, P. N. (1999). Deletions of heavy neurofilament subunit tail in amyotrophic lateral sclerosis. *Hum. Mol. Genet.* **8**, 157–164.

Anderson, R., Barnes, J. C., Bliss, T. V., Cain, D. P., Cambon, K., Davies, H. A., Errington, M. L., Fellows, L. A., Gray, R. A., Hoh, T., Stewart, M., Large, C. H., and Higgins, G. A. (1998). Behavioural, physiological and morphological analysis of a line of apolipoprotein E knockout mice. *Neuroscience* **85**, 93–110.

Bales, K. R., Verina, T., Dodel, R. C., Yansheng, D., Altstiel, L., Bender, M., Hyslop, P., Johnstone, E. M., Little, S. P., Cummins, D. J., Piccardo, P., Ghetti, B., and Paul, S. M. (1997). Lack of apolipoprotein E dramatically reduces amyloid β-peptide deposition. *Nat. Genet.* **17**, 263–264.

Berger-Sweeney, J., McPhie, D. L., Arters, J. A., Greenan, J., Oster-Granite, M. L., and Neve, R. L. (1999). Impairments in learning and memory accompanied by neurodegeneration in mice transgenic for the carboxyl-terminus of the amyloid precursor protein. *Mol. Brain Res*. **66**, 150–162.

Borchelt, D. R., Thinakaran, G., Eckman, C. B., Lee, M. K., Davenport, F., Ratovitsky, T., Prada, C. M., Kim, G., Seekins, S., Yager, D., Slunt, H. H., Wang, R., Seeger, M., Levey, A. I., Gandy, S. E., Copeland, N. G., Jenkins, N. A., Price, D. L., Younkin, S. G., and Sisodia, S. S. (1996). Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1-42/1-40 ratio in vitro and in vivo. *Neuron* **17**, 1005–1013.

Borchelt, D. R., Ratovitski, T., van Lare, J., Lee, M. K., Gonzales, V., Jenkins, N. A., Copeland, N. G., Price, D. L., and Sisodia, S. S. (1997). Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. *Neuron* **19**, 939–945.

Borchelt, D. R., Wong, P. C., Becher, M. W., Pardo, C. A., Lee, M. K., Xu, Z.-S., Thinakaran, G., Jenkins, N. A., Copeland, N. G., Sisodia, S. S., Cleveland, D. W., Price, D. L., and Hoffman, P. N. (1998). Axonal transport of mutant superoxide dismutase 1 and focal axonal abnormalities in the proximal axons of transgenic mice. *Neurobiol. Dis*. **5**, 27–35.

Braak, H., Braak, E., and Strothjohann, M. (1994). Abnormally phosphorylated tau protein related to the formation of neurofibrillary tangles and neuropil threads in the cerebral cortex of sheep and goat. *Neurosci. Lett*. **171**, 1–4.

Breen, K. C., Bruce, M., and Anderton, B. H. (1991). Beta amyloid precursor protein mediates neuronal cell-cell and cell-surface adhesion. *J. Neurosci. Res*. **28**, 90–100.

Brion, J.-P., Tremp, G., and Octave, J.-N. (1999). Transgenic expression of the shortest human tau affects its compartmentalization and its phosphorylation as in the pretangle stage of Alzheimer's disease. *Am. J. Pathol*. **154**, 255–270.

Bruijn, L. I., Beal, M. F., Becher, M. W., Schulz, J. B., Wong, P. C., Price, D. L., and Cleveland, D. W. (1997a). Elevated free nitrotyrosine levels, but not protein-bound nitrotyrosine or hydroxyl radicals, throughout amyotrophic lateral sclerosis (ALS)-like disease implicate tyrosine nitration as an aberrant in vivo property of one familial ALS-linked superoxide dismutase 1 mutant. *Proc. Natl Acad. Sci. U.S.A*. **94**, 7606–7611.

Bruijn, L. I., Becher, M. W., Lee, M. K., Anderson, K. L., Jenkins, N. A., Copeland, N. G., Sisodia, S. S., Rothstein, J. D., Borchelt, D. R., Price, D. L., and Cleveland, D. W. (1997b). ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. *Neuron* **18**, 327–338.

Bruijn, L. I., Houseweart, M. K., Kato, S., Anderson, K. L., Anderson, S. D., Ohama, E., Réaume, A. G., Scott, R. W., and Cleveland, D. W. (1998). Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. *Science* **281**, 1851–1854.

Buttini, M., Orth, M., Bellosta, S., Akeefe, H., Pitas, R. E., Wyss-Coray, T., Mucke, L., and Mahley, R. W. (1999). Expression of human apolipoprotein E3 or E4 in the brains of ApoE-/- mice: Isoform-specific effects on neurodegeneration. *J. Neurosci*. **19**, 4867–4880.

Calhoun, M. E., Wiederhold, K. H., Abramowski, D., Phinney, A. L., Probst, A., Sturchler-Pierrat, C., Staufenbiel, M., Sommer, B., and Jucker, M. (1998). Neuron loss in APP transgenic mice. *Nature (London)* **395**, 755–756.

Chapman, P. F., White, G. L., Jones, M. W., Cooper-Blacketer, D., Marshall, V. J., Irizarry, M., Younkin, L., Good, M. A., Bliss, T. V., Hyman, B. T., Younkin, S. G., and Hsiao, K. K. (1999). Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. *Nat. Neurosci*. **2**, 271–276.

Chartier-Harlin, M.-C., Crawford, F., Houlden, H., Warren, A., Hughes, D., Fidani, L., Goate, A., Rossor, M., Roques, P., Hardy, J., and Mullan, M. (1991). Early-onset Alzheimer's disease caused by mutations at codon 717 of the β-amyloid precursor protein gene. *Nature (London)* **353**, 844–846.

Chen, Y., Lomnitski, L., Michaelson, D. M., and Shoshami, E. (1997). Motor and cognitive deficits in apolipoprotein E-deficient mice after closed head injury. *Neuroscience* **80**, 1255–1262.

Ching, G. Y., Chien, C.-L., Flores, R., and Liem, R. K. H. (1999). Overexpression of a-internexin causes abnormal neurofilamentous accumulations and motor coordination deficits in transgenic mice. *J. Neurosci*. **19**, 2974–2986.

Chui, D.-H., Tanahash, H., Ozawa, K., Ikeda, S., Checler, F., Ueda, O., Suzuki, H., Araki, W., Inoue, H., Shirotani, K., Takahashi, K., Gallyas, F., and Tabira, T. (1999). Transgenic mice with Alzheimer presenilin 1 mutations show accelerated neurodegeneration without amyloid plaque formation. *Nat. Med*. **5**, 560–564.

Citron, M., Vigo-Pelfrey, C., Teplow, D. B., Miller, C., Schenk, D., Johnston, J., Winblad, B., Venizelos, N., Lannfelt, L., and Selkoe, D. J. (1994). Excessive production of amyloid beta-protein by peripheral cells of symptomatic and presymptomatic patients carrying the Swedish familial Alzheimer disease mutation. *Proc. Natl Acad. Sci. U.S.A*. **91**, 11993–11997.

Citron, M., Westaway, D., Xia, W., Carlson, G., Diehl, T., Levesque, G., Johnson-Wood, K., Lee, M., Seubert, P., Davis, A., Kholodenko, D., Motter, R., Sherrington, R., Perry, B., Yao, H., Strome, R., Lieberburg,

I., Rommens, J., Kim, S., Schenk, D., Fraser, P., St. George Hyslop, P., and Selkoe, D. J. (1997). Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice. *Nat. Med.* **3**, 67–72.

Collard, J.-F., Côté, F., and Julien, J.-P. (1995). Defective axonal transport in a transgenic mouse model of amyotrophic lateral sclerosis. *Nature (London)* **375**, 61–64.

Corder, E. H., Saunders, A. M., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G. W., Roses, A. D., Haines, J. L., and Pericak-Vance, M. A. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261**, 921–923.

Corder, E. H., Saunders, A. M., Risch, N. J., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Jr., Rimmler, J. B., Locke, P. A., Conneally, P. M., Schmader, K. A., Small, G. W., Roses, A. D., Haines, J. L., and Pericak-Vance, M. A. (1994). Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nat. Genet*. **7**, 180–184.

Côté, F., Collard, J.-F., and Julien, J.-P. (1993). Progressive neuronopathy in transgenic mice expressing the human neurofilament heavy gene: A mouse model of amyotrophic lateral sclerosis. *Cell (Cambridge, Mass.)* 7**3**, 35–46.

Couillard-Després, S., Zhu, Q., Wong, P. C., Price, D. L., Cleveland, D. W., and Julien, J.-J. (1998). Protective effect of neurofilament gene overexpression in motor neuron disease induced by mutant superoxide dismutase. *Proc. Natl Acad. Sci. U.S.A*. **95**, 9626–9630.

Czech, C., Lesort, M., Tremp, G., Terro, F., Blanchard, V., Schombert, B., Carpentier, N., Dreisler, S., Bonici, B., Takashima, A., Moussaoui, S., Hugon, J., and Pradier, L. (1998). Characterization of human presenilin 1 transgenic rats: Increased sensitivity to apoptosis in primary neuronal cultures. *Neuroscience* **87**, 325–336.

Dal Canto, M. C., and Gurney, M. E. (1994). Development of central nervous system pathology in a murine transgenic model of human amyotrophic lateral sclerosis. *Am. J. Pathol*. **145**, 1271–1279.

Dal Canto, M. C., and Gurney, M. E. (1995). Neuropathological changes in two lines of mice carrying a transgene for mutant human Cu, Zn SOD, and in mice overexpressing wild type human SOD: A model of familial amyotrophic lateral sclerosis (FALS). *Brain Res*. **676**, 25–40.

Davis, J. A., Naruse, S., Chen, H., Eckman, C., Younkin, S., Price, D. L., Borchelt, D. R., Sisodia, S. S., and Wong, P. C. (1998). An Alzheimer's disease-linked PS1 variant rescues the developmental abnormalities of PS1-deficient embryos. *Neuron* **20**, 603–609.

de Belleroche, J., Orrell, R. W., and Virgo, L. (1996). Amyotrophic lateral sclerosis: Recent advances in understanding disease mechanisms. *J. Neuropathol. Exp. Neurol*. **55**, 747–757.

De Strooper, B., Saftig, P., Craessaerts, K., Vanderstichele, H., Guhde, G., Annaert, W., von Figura, K., and van Leuven, F. (1998). Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature (London)* **391**, 387–390.

De Strooper, B., Annaert, W., Cupers, P., Saftig, P., Craessaerts, K., Mumm, J. S., Schroeter, E. H., Schrijvers, V., Wolfe, M. S., Ray, W. J., Goate, A., and Kopan, R. (1999). A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain. *Nature (London)* **398**, 518–522.

Dickson, T. C., Saunders, H. L., and Vickers, J. C. (1997). Relationship between apolipoprotein E and the amyloid deposits and dystrophic neurites of Alzheimer's disease. *Neuropathol. Appl. Neurobiol.* **23**, 483–494.

Dickson, T. C., King, C. E., McCormack, G. H., and Vickers, J. C. (1999). Neurochemical diversity of dystrophic neurites in the early and late stages of Alzheimer's disease. *Exp. Neurol.* **156**, 100–110.

Duff, K., Eckman, C., Zehr, C., Yu, X., Prada, C. M., Perez-tur, J., Hutton, M., Buée, L., Harigaya, Y., Yager, D., Morgan, D., Gordon, M. N., Holcomb, L., Refolo, L., Zenk, B., Hardy, J., and Younkin, S. (1996). Increased amyloid-beta 42(43) in brains of mice expressing mutant presenilin 1. *Nature (London)* **383**, 710–713.

Duff, K., Knight, H., Refolo, L. M., Sanders, S., Yu, X., Picciano, M., Malester, B., Hutton, M., Adamson, J., Goedert, M., Bürki, K., and Davies, P. (2000). Characterization of pathology in transgenic mice over-expressing human genomic and cDNA tau transgenes. *Neurobiol. Dis*. **7**, 87–98.

Esch, F. S., Keim, P. S., Beattie, E. C., Blacher, R. W., Culwell, A. R., Oltersdorf, T., McClure, D., and Ward, P. J. (1990). Cleavage of amyloid beta peptide during constitutive processing of its precursor. *Science* **248**, 1122–1124.

Eyer, J., and Peterson, A. (1994). Neurofilament-deficient axons and perikaryal aggregates in viable transgenic mice expressing a neurofilament-β-galactosidase fusion protein. *Neuron* **12**, 389–405.

Eyer, J., Cleveland, D. W., Wong, P. C., and Peterson, A. C. (1998). Pathogenesis of two axonopathies does not require axonal neurofilaments. *Nature (London)* **391**, 584–587.

Fagan, A. M., Murphy, B. A., Patel, S. N., Kilbridge, J. F., Mobely, W. C., Bu, G., and Holtzman, D. M. (1998). Evidence of normal aging of the septo-hippocampal cholinergic system in ApoE (-/-) mice but impaired clearance of axonal degeneration products following injury. *Exp. Neurol.* **151**, 314–325.

Figlewicz, D. A., Krizus, A., Martinoli, M. G., Meininger, V., Dib, M., Rouleau, G. A., and Julien, J. P. (1994). Variants of the heavy neurofilament subunit are associated with the development of amyotrophic lateral sclerosis. *Hum. Mol. Genet*. **3**, 1757–1761.

Frautschy, S. A., Yang, F., Irizarry, M., Hyman, B., Saido, T. C., Hsiao, K., and Cole, G. M. (1998). Microglial response to amyloid plaques in APPsw transgenic mice. *Am. J. Pathol*. **152**, 307–317.

Friedman, G., Froom, P., Sazabon, L., Grinblatt, I., Shochina, M., Tsenter, J., Babaey, S., Ben Yehuda, A., and Groswasser, Z. (1999). Apolipoprotein E-ϵ4 genotype predicts a poor outcome in survivors of traumatic brain injury. *Neurology* **52**, 244–248.

Games, D., Adams, D., Alessandrini, R., Barbour, R., Berthelette, P., Blackwell, C., Carr, T., Clemens, J., Donaldson, T., Gillespie, F., Guido, T., Hagoplan, S., Johnson-Wood, K., Khan, K., Lee, M., Leibowitz, P., Lieberburg, I., Little, S., Masliah, E., McConlogue, L., Montoya-Zavala, M., Mucke, L., Paganini, L., Penniman, E., Power, M., Schenk, D., Seubert, P., Snyder, B., Soriano, F., Tan, H., Vitale, J., Wadsworth, S., Wolozin, B., and Zhao, J. (1995). Alzheimer-type neuropathology in transgenic mice overexpressing V717F β-amyloid precursor protein. *Nature (London)* **373**, 523–527.

Giannakopoulos, P., Silhol, S., Jallageas, V., Mallet, J., Bons, N., Bouras, C., and Delaère, P. (1997). Quantitative analysis of tau protein-immunoreactive accumulations and β amyloid protein deposits in the cerebral cortex of the mouse lemur, *Microcebus murinus. Acta Neuropathol*. **94**, 131–139.

Glenner, G. G., and Wong, C. W. (1984). Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun*. **120**, 885–890.

Goate, A., Chartier-Harlin, M. C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., James, L., Mant, R., Newton, P., Rooke, K., Roques, P., Talbot, C., Pericak-Vance, M., Roses, A., Williamson, R., Rossor, M., Owen, M., and Hardy, J. (1991). Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature (London)* **349**, 704–706.

Gordon, I., Genis, I., Grauer, E., Sehayek, E., and Michaelson, D. M. (1996). Biochemical and cognitive studies of apolipoprotein-E-deficient mice. *Mol. Chem. Neuropathol*. **28**, 97–103.

Götz, J., Probst, A., Spillantini, M. G., Schafer, T., Jakes, R., Burki, K., and Goedert, M. (1995). Somatodendritic localization and hyperphosphorylation of tau protein in transgenic mice expressing the longest human brain tau isoform. *EMBO J*. **14**, 1304–1313.

Gurney, M. E., Pu, H., Chiu, A. Y., Dal Canto, M. C., Polchow, C. Y., Alexander, D. D., Caliendo, J., Hentati, A., Kwon, Y. W., Deng, H.-X., Chen, W. C., Zhai, P., Sufit, R. L., and Siddique, T. (1994). Motor neuro degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. *Science* **264**, 1772–1775.

Haass, C., Schlossmacher, M. G., Hung, A. Y., Vigo-Pelfrey, C., Mellon, A., Ostaszewski, B. L., Lieberburg, I., Koo, E. H., Schenk, D., and Teplow, D. B. (1992). Amyloid β-peptide is produced by cultured cells during normal metabolism. *Nature (London)* **359**, 322–325.

Hall, E. D., Andrus, P. K., Ostveen, J. A., Fleck, T. J., and Gurney, M. E. (1998). Relationship of oxygen radical-induced lipid peroxidative damage to disease onset and progression in a transgenic model of familial ALS. *J. Neurosci. Res*. **53**, 66–77.

Hardy, J. (1997). Amyloid, the presenilins and Alzheimer's disease. *Trends Neurosci*. **20**, 154–159.

Hardy, J., and Israël, A. (1999). In search of gamma-secretase. *Nature (London)* **398**, 466–467.

Hardy, J., Duff, K., Hardy, K. G., Perez-Tur, J., and Hutton, M. (1998). Genetic dissection of Alzheimer's disease and related dementias: Amyloid and its relationship to tau. *Nat. Neurosci*. **1**, 355–358.

Hardy, J. A., and Higgins, G. A. (1992). Alzheimer's disease: The amyloid cascade hypothesis. *Science* **256**, 184–185.

Haroutunian, V., Zhou, Y., Elder, G., Li, C., and Lazzarini, R. A. (1996). Age-dependent spatial memory deficits in transgenic mice expressing the human mid-sized neurofilament gene: I. *Mol. Brain Res*. **42**, 62–70.

Harper, S. J., Bilsland, J. G., Shearman, M. S., Zheng, H., Van der Ploeg, L., and Sirinathsinghji, D. J. (1998). Mouse cortical neurones lacking APP show normal neurite outgrowth and survival responses in vitro. *NeuroReport* **14**, 3053–3058.

Higgins, G. A., Large, C. H., Rupniak, H. T., and Barnes, J. C. (1997). Apolipoprotein E and Alzheimer's disease: A review of recent studies. *Pharmacol. Biochem. Behav*. **56**, 675–685.

Higgins, L. S., Holtzman, D. M., Rabin, J., Mobley, W. C., and Cordell, B. (1994). Transgenic mouse brain histopathology resembles early Alzheimer's disease. *Ann. Neurol*. **35**, 598–607.

Higgins, L. S., Rodems, J. M., Catalano, R., Quon, D., and Cordell, B. (1995). Early Alzheimer disease-like histopathology increases in frequency with age in mice transgenic for β-APP751. *Proc. Natl. Acad. Sci. U.S.A*. **92**, 4402–4406.

Hof, P. R., Cox, C., and Morrison, J. H. (1990). Quantitative analysis of a vulnerable subset of pyramidal neurons in Alzheimer's disease: I. Superior frontal and inferior temporal cortex. *J. Comp. Neurol*. **301**, 44–54.

Hof, P. R., Bouras, C., and Morrison, J. H. (1999). Cortical neuropathology in aging and dementing disorders: Neuronal typology, connectivity and selective vulnerability. *In* "Cerebral Cortex" (A. Peters and J. H. Morrison, eds.), Vol. 14, pp. 175–312. Kluwer Academic-Plenum, New York.

Holcomb, L., Gordon, M. N., McGowan, E., Yu, X., Benkovic, S., Jantzen, P., Wright, K., Saad, I., Mueller, R., Morgan, D., Sanders, S., Zehr, C., O'Campo, K., Hardy, J., Prada, C.-M., Eckman, C., Younkin, S., Hsiao, K., and Duff, K. (1998). Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat. Med*. **4**, 97–100.

Hsia, A. Y., Masliah, E., McConlogue, L., Yu, G.-Q., Tatsuno, G., Hu, K., Kholodenko, D., Malenka, R. C., Nicoll, R. A., and Mucke, L. (1999). Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. *Proc. Natl. Acad. Sci. U.S.A*. **96**, 3228–3233.

Hsiao, K. K., Borchelt, D. R., Olson, K., Johannsdottir, R., Kitt, C., Yunis, W., Xu, S., Eckman, C., Younkin, S., Price, D., Iadecola, C., Clark, H. B., and Carlson, G. (1995). Age-related CNS disorder and early death in transgenic FVB/N mice overexpressing Alzheimer amyloid precursor proteins. *Neuron* **15**, 1203–1218.

Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F., and Cole, G. (1996). Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. *Science* **274**, 99–102.

Igeta, Y., Kawarabayashi, T., Sato, M., Yamada, N., Matsubara, E., Ishiguro, K., Kanai, M., Tomidokoro, Y., Osuga, J., Okamato, K., Hirai, S., and Shoji, M. (1997). Apolipoprotein E accumulates with the progression of A beta deposition in transgenic mice. *J. Neuropathol. Exp. Neurol*. **56**, 1228–1235.

Ikin, A. F., Annaert, W. G., Takei, K., De Camilli, P., Jahn, R., Greengard, P., and Buxbaum, J. D. (1996). Alzheimer amyloid protein precursor is localized in nerve terminal preparations to Rab5-containing vesicular organelles distinct from those implicated in the synaptic vesicle pathway. *J. Biol. Chem.* **271**, 31783–31786.

Ince, P. G., Lowe, J., and Shaw, P. J. (1998). Amyotrophic lateral sclerosis: Current issues in classification, pathogenesis and molecular pathology. *Neuropathol. Appl. Neurobiol*. **24**, 104–117.

Irizarry, M. C., Soriano, F., McNamara, M., Page, K. J., Schenk, D., and Hyman, B. T. (1997a). Aβ deposition is associated with neuropil changes but not with overt neuronal loss in the human amyloid precursor protein V717F (PDAPP) transgenic mouse. *Neuroscience* **17**, 7053–7059.

Irizarry, M. C., McNamara, M., Fedorchak, K., Hsiao, K., and Hyman, B. T. (1997b). APPSw transgenic mice develop age-related Aβ deposits and neuropil abnormalities, but no neuronal loss in CA1. *J. Neuropathol. Exp. Neurol*. **56**, 965–973.

Ishihara, T., Hong, M., Zhang, B., Nakagawa, Y., Lee, M. K., Trojanowski, J. Q., and Lee, V. M. (1999). Age-dependent emergence and progression of a tauopathy in transgenic mice overexpressing the shortest human tau isoform. *Neuron* **24**, 751–762.

Johnson-Wood, K., Lee, M., Motter, R., Hu, K., Gordon, G., Barbour, R., Khan, K., Gordon, M., Tan, H., Games, D., Lieberburg, I., Schenk, D., Seubert, P., and McConlogue, L. (1997). Amyloid precursor protein processing and A beta 42 deposition in a transgenic mouse model of Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A*. **94**, 1550–1555.

Jordan, B. D., Relkin, N. R., Ravdin, L. D., Jacobs, A. R., Bennett, A., and Gandy, S. (1997). Apolipoprotein E epsilon4 associated with chronic traumatic brain injury in boxing. *JAMA, J. Am. Med. Assoc*. **278**, 136–140.

Jucker, M., Walker, L. C., Martin, L. J., Kitt, C. A., Kleinman, H. K., Ingram, D. K., and Price, D. L. (1992). Age-associated inclusions in normal and transgenic mouse brain. *Science* **255**, 1443–1445.

Kammesheidt, A., Boyce, F. M., Spanoyannis, A. F., Cummings, B. J., Ortegón, M., Cotman, C., Vaught, J. L., and Neve, R. L. (1992). Deposition of β/A4 immunoreactivity and neuronal pathology in transgenic mice expressing the carboxyl-terminal fragment of the Alzheimer amyloid precursor in the brain. *Proc. Natl. Acad. Sci. U. S. A*. **89**, 10857–10861.

Katzman, R., and Kawas, C. H. (1994). The epidemiology of dementia and Alzheimer's disease. *In* "Alzheimer Disease" (R. D. Terry, R. Katzman, and K. L. Bick, eds.), pp. 105-122. Raven Press, New York.

Kawabata, S., Higgins, G. A., and Gordon, J. W. (1991). Amyloid plaques, neurofibrillary tangles and neuronal loss in brains of transgenic mice overexpressing a C-terminal fragment of human amyloid precursor protein. *Nature (London)* **354**, 476–478.

Kawabata, S., Higgins, G. A., and Gordon, J. W. (1992). Alzheimer's retraction. *Nature (London)* **356**, 23.

King, C. E., Jacobs, I., Dickson, T. C., and Vickers, J. C. (1997). Physical damage to rat cortical axons mimics early Alzheimer's neuronal pathology. *NeuroReport* **8**, 1663–1665.

Klivenyi, P., Ferrante, R. J., Matthews, R. T., Bogdanov, M. B., Klein, A. M., Andreassen, O. A., Mueller, G., Wermer, M., Kaddurah-Daouk, R., and Beal, M. F. (1999). Neuroprotective effects of creatine in a transgenic animal model of amyotrophic lateral sclerosis. *Nat. Med*. **5**, 347–350.

Kong, J., and Xu, Z. (1998). Massive mitochondrial degeneration in motor neurons triggers the onset of amyotrophic lateral sclerosis in mice expressing a mutant SOD1. *J. Neurosci*. **18**, 3241–3250.

Koo, E. H., Sisodia, S. S., Archer, D. R., Martin, L. J., Weidemann, A., Beyreuther, K., Fischer, P., Masters, C. L., and Price, D. L. (1990). Precursor of amyloid protein in Alzheimer disease undergoes fast anterograde axonal transport. *Proc. Natl. Acad. Sci. U.S.A*. **87**, 1561–1565.

LaFerla, F. M., Tinkle, B. T., Bieberich, C. J., Haudenschild, C. C., and Jay, G. (1995). The Alzheimer's Aβ peptide induces neurodegeneration and apoptotic cell death in transgenic mice. *Nat. Genet*. **9**, 21–30.

Lamb, B. T., Sisodia, S. S., Lawler, A. M., Slunt, H. H., Kitt, C. A., Kearns, W. G., Pearson, P. L., Price, D. L., and Gearhart, J. D. (1993). Introduction and expression of the 400 kilobase amyloid precursor protein gene in transgenic mice. *Nat. Genet*. **5**, 22–30.

Lamb, B. T., Call, L. M., Slunt, H. H., Bardel, K. A., Lawler, A. M., Eckman, C. B., Younkin, S. G., Holtz, G., Wagner, S. L., Price, D. L., Sisodia, S. S., and Gearhart, J. D. (1997). Altered metabolism of familial Alzheimer's disease-linked amyloid precursor protein variants in yeast artificial chromosome transgenic mice. *Hum. Mol. Genet*. **6**, 1535–1541.

Lee, K. L., Marszalek, J. R., and Cleveland, D. W. (1994). A mutant neurofilament subunit causes massive, selective motor neuron death: Implication for the pathogenesis of human motor neuron disease. *Neuron* **13**, 975–988.

Lee, V. M., Balin, B. J., Otvos, L., Jr., and Trojanowski, J. Q. (1991). A68: A major subunit of paired helical filaments and derivatized forms of normal Tau. *Science* **251**, 675–678.

Lee, V. M.-Y., Elder, G. A., Chen, L.-C., Liang, Z., Snyder, S. E., Friedrich, V. L., Jr., and Lazzarini, R. A. (1992). Expression of human mid-sized neurofilament subunit in transgenic mice. *Mol. Brain Res*. **15**, 76–84.

Lefebvre, S., and Mushynski, W. E. (1988). Characterization of the cation-binding properties of porcine neurofilaments. *Biochemistry* **27**, 8503–8508.

Leigh, P. N., and Swash, M. (1991). Cytoskeletal pathology in motor neuron diseases. *Adv. Neurol*. **56**, 115–124.

Levy, E., Carman, M. D., Fernandez-Madrid, I. J., Power, M. D., Lieberburg, I., van Duinen, S. G., Bots, G. T., Luyendijk, W., and Frangione, B. (1990). Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. *Science* **248**, 1124–1126.

Liu, R., Althaus, J. S., Ellerbrock, B. R., Becker, D. A., and Gurney, M. E. (1998). Enhanced oxygen radical production in a transgenic mouse model of familial amyotrophic lateral sclerosis. *Ann. Neurol*. **44**, 763–770.

Ma, D., Descarries, L., Julien, J.-P., and Doucet, G. (1995). Abnormal perikaryal accumulation of neurofilament light protein in the brain of mice transgenic for the human protein: Sequence of postnatal development. *Neuroscience* **68**, 135–149.

Ma, D., Descarries, L., Micheva, K. D., Lepage, Y., Julien, J.-P., and Doucet, G. (1999). Severe neuronal losses with age in the parietal cortex and ventrobasal thalamus of mice transgenic for the human NF-L neurofilament protein. *J. Comp. Neurol*. **406**, 433–448.

Ma, J., Yee, A., Brewer, H. B., Jr., Das, S., and Potter, H. (1994). Amyloid-associated proteins alpha 1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer beta-protein into filaments. *Nature (London)* **372**, 92–94.

Magara, F., Muller, U., Li, Z.-W., Lipp, H.-P., Weissmann, C., Stagljar, M., and Wolfer, D. P. (1999). Genetic background changes the pattern of forebrain commisure defects in transgenic mice underexpressing the β-amyloid precursor protein. *Proc. Natl. Acad. Sci. U.S.A*. **96**, 4656–4661.

Mahley, R. W. (1988). Apolipoprotein E: Cholesterol transport protein with expanding role in cell biology. *Science* **240**, 622–630.

Mann, D. M. A., and Esiri, M. M. (1989). The pattern of acquisition of plaques and tangles in the brains of patients under 50 years of age with Down's syndrome. *J. Neurol. Sci*. **89**, 169–179.

Masliah, E., Mallory, M., Ge, N., and Saitoh, T. (1992). Amyloid precursor protein is localized in growing neurites of neonatal rat brain. *Brain Res*. **593**, 323–328.

Masliah, E., Mallory, M., Ge, N., Alford, M., Veinbergs, I., and Roses, A. D. (1995). Neurodegeneration in the central nervous system of ApoE-deficient mice. *Exp. Neurol*. **136**, 107–122.

Masliah, E., Sisk, A., Mallory, M., Mucke, L., Schenk, D., and Games, D. (1996). Comparison of neurodegenerative pathology in transgenic mice overexpressing V717F beta-amyloid precursor protein and Alzheimer's disease. *J. Neurosci*. **16**, 5795–5811.

Masters, C. L., Simms, G., Weinman, N. A., Multhaup, G., McDonald, B. L., and Beyreuther, K. (1985). Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc. Natl. Acad. Sci. U.S.A*. **82**, 4245–4249.

Matthews, R. T., and Beal, M. F. (1996). Increased 3-nitrotyrosine in brains of Apo E-deficient mice. *Brain Res*. **718**, 181–184.

Mattson, M. P., and Guo, Q. (1997). Cell and molecular neurobiology of presenilins: A role for the endoplasmic reticulum in the pathogenesis of Alzheimer's disease? *J. Neurosci. Res*. **50**, 505–513.

Mayeux, R., Ottmna, R., Maestre, G., Ngai, C., Tang, M.-X., Ginsberg, H., Chun, M., Tycko, B., and Shelanski, M. (1995). Synergistic effects of traumatic head injury and apolipoprotein-E4 in patients with Alzheimer's disease. *Neurology* **45**, 555–557.

Mercken, L., and Brion, J. P. (1995). Phosphorylation of tau protein is not affected in mice lacking apolipoprotein E. *NeuroReport* **6**, 2381–2384.

Moechars, D., Dewachter, I., Lorent, K., Reverse, D., Baekelandt, V., Naidu, A., Tesseur, I., Spittaels, K., Haute, C. V., Checler, F., Godaux, E., Cordell, B., and Van Leuven, F. (1999). Early phenotypic changes in transgenic mice that overexpress different mutants of amyloid precursor protein in brain. *J. Biol. Chem*. **274**, 6483–6492.

Moran, P. M., Higgins, L. S., Cordell, B., and Moser, P. C. (1995). Age-related learning deficits in transgenic mice expressing the 751-amino acid isoform of human β-amyloid precursor protein. *Proc. Natl. Acad. Sci. U.S.A*. **92**, 5341–5345.

Morris, J. C., Storandt, M., McKeel, D. W., Rubin, E. H., Price, J. L., Grant, E. A., and Berg, L. (1996). Cerebral amyloid deposition and diffuse plaques in ''normal'' aging: Evidence from presymptomatic and very mild Alzheimer's disease. *Neurology* **46**, 707–719.

Morrison, B. M., Gordon, J. W., Ripps, M. E., and Morrison, J. H. (1996). Quantitative immunocytochemical analysis of the spinal cord in G86R superoxide dismutase transgenic mice: Neurochemical correlates of selective vulnerability. *J. Comp. Neurol*. **373**, 619–631.

Morrison, B. M., Janssen, W. G., Gordon, J. W., and Morrison, J. H. (1998). Time course of neuropathology in the spinal cord of G86R superoxide dismutase transgenic mice. *J. Comp. Neurol*. **391**, 64–77.

Mullan, M., Crawford, F., Axelman, K., Houlden, H., Lilius, L., Winblad, B., and Lannfelt, L. (1992). A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of β-amyloid. *Nat. Genet*. **1**, 345–347.

Murai, H., Pierce, J. E., Raghupathi, R., Smith, D. H., Saatman, K. E., Trojanowski, J. Q., Lee, V. M., Loring, J. F., Eckman, C., Younkin, S., and McIntosh, T. K. (1998). Twofold overexpression of human beta-amyloid precursor proteins in transgenic mice does not affect the neuromotor, cognitive, or neurodegenerative sequelae following experimental brain injury. *J. Comp. Neurol*. **392**, 428–438.

Murrell, J., Farlow, M., Ghetti, B., and Benson, M. D. (1991). A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease. *Science* **254**, 97–99.

Nalbantoglu, J., Tirado-Santiago, G., Lahsaini, A., Poirier, J., Goncalves, O., Verge, G., Momoli, F., Welner, S. A., Massicotte, G., Julien, J. P., and Shapiro, M. L. (1997). Impaired learning and LTP in mice expressing the carboxy terminus of the Alzheimer amyloid precursor protein. *Nature (London)* **387**, 500–505.

Narindrasorasak, S., Lowery, D. E., Altman, R. A., Gonzalez-DeWhitt, P. A., Greenberg, B. D., and Kisilevsky, R. (1992). Characterization of high affinity binding between laminin and Alzheimer's disease amyloid precursor proteins. *Lab. Invest*. **67**, 643–652.

Nimchinsky, E. A., Young, W. G., Yeung, G., Shah, R. A., Gordon, J. W., Bloom, F. E., Morrison, J. H., and Hof, P. R. (2000). Differential vulnerability of oculomotor, facial, and hypoglossal nuclei in G86R superoxide dismutase transgenic mice. *J. Comp. Neurol.* **416**, 112–125.

Octave, J.-N. (1995). The amyloid peptide and its precursor in Alzheimer's disease. *Rev. Neurosci*. **6**, 287–316.

Olichney, J. M., Hansen, L. A., Galasko, D., Saitoh, T., Hofstetter, C. R., Katzman, R., and Thal, L. J. (1996). The apolipoprotein E epsilon 4 allele is associated with increased neuritic plaques and cerebral amyloid

angiopathy in Alzheimer's disease and Lewy body variant. *Neurology* **47**, 190–196.

Oster-Granite, M., McPhie, D. L., Greenan, J., and Neve, R. L. (1996). Age-dependent neuronal and synaptic degeneration in mice transgenic for the C-terminus of the amyloid precursor protein. *J. Neurosci.* **16**, 6732–6741.

Osuntokun, B. O., Sahota, A., Ogunniyi, A. O., Gureje, O., Baiyewu, O., Adeyinka, A., Oluwole, S. O., Komolafe, O., Hall, K. S., Unverzagt, F. W., Hui, S. L., Yang, M., and Hendrie, H. C. (1995). Lack of an association between apolipoprotein E epsilon 4 and Alzheimer's disease in elderly Nigerians. *Ann. Neurol*. **38**, 463–465.

Oyama, F., Sawamura, N., Kobayashi, K., Morishima-Kawashima, M., Kuramochi, T., Ito, M., Tomita, T., Maruyama, K., Saido, T. C., Iwatsubo, T., Capell, A., Walter, J., Grünberg, J., Ueyama, Y., Haass, C., and Ihara, Y. (1998). Mutant presenilin 2 transgenic mouse: Effects of an age-dependent increase of amyloid β-protein 42 in the brain. *J. Neurochem*. **71**, 1313–1322.

Papolla, M. A., Chyan, Y.-J., Omar, R. A., Hsiao, K., Perry, G., Smith, M. A., and Bozner, P. (1998). Evidence of oxidative stress and in vivo neurotoxicity of β-amyloid in a transgenic mouse model of Alzheimer's disease. *Am. J. Pathol*. **152**, 871–877.

Pearson, B. E., and Choi, T. K. (1993). Expression of the human β-amyloid precursor protein gene from a yeast artificial chromosome in transgenic mice. *Proc. Natl. Acad. Sci. U.S.A*. **90**, 10578–10582.

Perez, R. G., Zheng, H., Van der Ploeg, L. H. T., and Koo, E. K. (1997). The β-amyloid precursor protein of Alzheimer's disease enhances neuron viability and modulates neuronal polarity. *J. Neurosci*. **17**, 9407–9414.

Phinney, A. L., Calhoun, M. E., Wolfer, D. P., Lipp, H. P., Zheng, H., and Jucker, M. (1999). No hippocampal neuron or synaptic bouton loss in learning-impaired aged β-amyloid precursor protein-null mice. *Neuroscience* **90**, 1207–1216.

Poirier, J. (1994). Apolipoprotein E in animal models of CNS injury and in Alzheimer's disease. *Trends Neurosci*. **17**, 525–530.

Price, J. L., and Morris, J. C. (1999). Tangles and plaques in nondemented aging and ''preclinical'' Alzheimer's disease. *Ann. Neurol*. **45**, 358–368.

Probst, A., Götz, J., Wiederhold, K. H., Tolnay, M., Mistl, C., Jaton, A. L., Hong, M., Ishihara, T., Lee, V. M. Y., Trojanowski, J. Q., Jakes, R., Crowther, R. A., Spillantini, M. G., Bürki, K., and Goedert, M. (2000). Axonopathy and amyotrophy inmice transgenic for human four-repeat tau protein. *Acta Neuropathol*. **99**, 469–481.

Qian, S., Jiang, P., Guan, X. M., Singh, G., Trumbauer, M. E., Yu, H., Chen, H. Y., Van de Ploeg, L. H., and Zheng, H. (1998). Mutant human presenilin 1 protects presenilin1 null mouse against embryonic lethality and elevates Aβ1-42/43 expression. *Neuron* **20**, 611–617.

Quon, D., Wang, R., Catalano, R., Marian Scardina, J., Murakami, K., and Cordell, B. (1991). Formation of β-amyloid protein deposits in brains of transgenic mice. *Nature (London)* **352**, 239–241.

Raber, J., Wong, D., Buttini, M., Orth, M., Bellosta, S., Pitas, R. E., Mahley, R. W., and Mucke, L. (1998). Isoform-specific effects of human apolipoprotein E on brain function revealed in *ApoE* knockout mice: Increases susceptibility of females. *Proc. Natl. Acad. Sci. U.S.A*. **95**, 10914–10919.

Reaume, A. G., Elliott, J. L., Hoffman, E. K., Kowall, N. W., Ferrante, R. J., Siwek, D. F., Wilcox, H. M., Flood, D. G., Beal, M. F., Brown, R. H., Jr., Scott, R. W., and Snider, W. D. (1996). Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury. *Nat. Genet*. **13**, 43–47.

Rebeck, G. W., Reiter, J. S., Strickland, D. K., and Hyman, B. T. (1993). Apolipoprotein E in sporadic Alzheimer's disease: Allelic variation and receptor interactions. *Neuron* **11**, 575–580.

Ripps, M. E., Huntley, G. W., Hof, P. R., Morrison, J. H., and Gordon, J. W. (1995). Transgenic mice expressing an altered murine superoxide dismutase gene provide and animal model of amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. U.S.A*. **92**, 689–693.

Robakis, N. K. (1994). β-amyloid and amyloid precursor protein. *In* ''Alzheimer Disease'' (R. D. Terry, R. Katzman, and K. L. Bick, eds.), pp. 179–196. Raven Press, New York.

Rosen, D. R., Siddique, T., Patterson, D., Figlewicz, D. A., Sapp, P., Hentati, A., Donaldson, D., Goto, J., O'Regan, J. P., Deng, H.-X., Rahmati, Z., Krizus, A., McKenna-Yasek, D., Cayabyab, A., Gaston, S. M., Berger, R., Tanzi, R. E., Halperin, J. J., Herzfeldt, B., Van den Bergh, R., Hung, W.-Y., Bird, T., Deng, G., Mulder, D. W., Smyth, C., Laing, N. G., Sorlano, E., Pericak-Vance, M. A., Haines, J., Rouleau, G. A., Gusella, J. S., Horvitz, H. R., and Brown, R. H. (1993). Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature (London)* **362**, 59–62.

Rothstein, J. D., Martin, L. J., and Kuncl, R. W. (1992). Decreased glutamate transport by the brain and spinal cord in amyotrophic lateral sclerosis. *N. Engl. J. Med*. **326**, 1464–1468.

Rouleau, G. A., Clark, A. W., Rooke, K., Pramatarova, A., Krizus, A., Suchowersky, O., Julien, J.-P., and Figlewicz, D. (1996). SOD1 mutations is associated with accumulation of neurofilaments in amyotrophic lateral sclerosis. *Ann. Neurol*. **39**, 128–131.

Sasaki, S., Ohsawa, Y., Yamane, K., Sakuma, H., Shibata, N., Nakano, R., Kikugawa, K., Mizutani, T., Tsuji, S., and Iwata, M. (1998). Familial amyotrophic lateral sclerosis with widespread vacuolation and hyaline inclusions. *Neurology* **51**, 871–873.

Schenk, D., Barbour, R., Dunn, W., Gordon, G., Grajeda, H., Guido, T., Hu, K., Huang, J., Johnson-Wood, K., Khan, K., Kholodenko, D., Lee, M., Liao, Z., Lieberburg, I., Motter, R., Mutter, L., Soriano, F., Shopp, G., Vasquez, N., Vandervert, C., Walker, S., Wogulis, M., Yednock, T., Games, D., and Seubert, P. (1999). Immunization with amyloid-β attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature (London)* **400**, 173–177.

Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T. D., Hardy, J., Hutton, M., Kukull, W., Larson, E., Levy-Hahad, E., Viitanen, M., Peskind, E., Poorkaj, P., Schellenberg, G., Tanzi, R., Wasco, W., Lannfelt, L., Selkoe, D., and Younkin, S. (1996). Secreted amyloid β-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat. Med*. **2**, 864–870.

Schmechel, D. E., Saunders, A. M., Strittmatter, W. J., Crain, B. J., Hulette, C. M., Joo, S. H., Pericak-Vance, M. A., Goldgaber, D., and Roses, A. D. (1993). Increased amyloid β-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A*. **90**, 9649–9653.

Schubert, W., Prior, R., Weidemann, A., Dircksen, H., Multhaup, G., Masters, C. L., and Beyreuther, K. (1991). Localization of Alzheimer beta A4 amyloid precursor protein at central and peripheral synaptic sites. *Brain Res*. **563**, 184–194.

Seabrook, G. R., and Rosahl, T. W. (1999). Transgenic animals relevant to Alzheimer's disease. *Neuropharmacology* **38**, 1–17.

Seabrook, G. R., Smith, D. W., Bowery, B. J., Easter, A., Reynolds, T., Fitzjohn, S. M., Morton, R. A., Zheng, H., Dawson, G. R., Sirinathsinghji, D. J., Davies, C. H., Collingridge, G. L., and Hill, R. G. (1999). Mechanisms contributing to the deficits in hippocampal synaptic plasticity in mice lacking amyloid precursor protein. *Neuropharmacology* **38**, 349–359.

Selkoe D. J. (1991). The molecular pathology of Alzheimer's disease. *Neuron* **6**, 487–498.

Selkoe, D. J., Bell, D. S., Podlisny, M. B., Price, D. L., and Cork, L.C. (1987). Conservation of brain amyloid proteins in aged mammals and humans with Alzheimer's disease. *Science* **235**, 873–877.

Seubert, P., Vigo-Pelfrey, C., Esch, F., Lee, M., Dovey, H., Davis, D., Sinha, S., Schlosmacher, M., Whaley, J., Swindlehurst, C., McCormack, R., Wolfert, R., Selkoe, D., Lieberburg, I., and Schenk, D. F. (1992). Isolation and quantification of soluble Alzheimer's beta-peptide from biological fluids. *Nature (London)* **359**, 325–327.

Sheng, H., Laskowitz, D. T., Bennett, E., Schmechel, D. E., Bart, R. D., Saunders, A. M., Pearlstein, R. D., Roses, A. D., and Warner, D. S. (1998).

Apolipoprotein E isoform-specific differences in outcome from focal ischemia in transgenic mice. *J. Cereb. Blood Flow Metab.* **18**, 361–366.

Shoji, M., Golde, T. E., Ghiso, J., Cheung, T. T., Estus, S., Shaffer, L. M., Cai, X. D., McKay, D. M., Tintner, R., Frangione, B., and Younkin, S. G. (1992). Production of the Alzheimer amyloid β protein by normal proteolytic processing. *Science* **258**, 126–129.

Sisodia, S. S., Koo, E. H., Beyreuther, K., Unterbeck, A., and Price, D. L. (1990). Evidence that beta-amyloid protein in Alzheimer's disease is not derived by normal processing. *Science* **248**, 492–495.

Smith, D. H., Nakamura, M., McIntosh, T. K., Wang, J., Rodriguez, A., Chen, X. H., Raghupathi, R., Saatman, K. E., Clemens, J., Schmidt, M. L., Lee, V. M., and Trojanowski, J. Q. (1998). Brain trauma induces massive hippocampal neuron death linked to a surge in β-amyloid levels in mice overexpressing mutant amyloid precursor protein. *Am. J. Pathol*. **153**, 1005–1010.

Spittaels, K., Van den Haute, C., Van Dorpe, J., Bruynseels, K., Vandezande, K., Laenen, I., Geerts, H., Mercken, M., Sciot, R., Van Lommel, A., Loos, R., and Van Leuven, F. (1999). Prominent axonopathy in the brain and spinal cord of transgenic mice overexpressing four-repeat human tau protein. *Am. J. Pathol*. **155**, 2153–2165.

Sturchler-Pierrat, C., Abramowski, D., Duke, M., Wiederhold, K. H., Mistl, C., Rothacher, S., Ledermann, B., Burki, K., Frey, P., Paganetti, P. A., Waridel, C., Calhoun, M. E., Jucker, M., Probst, A., Staufenbiel, M., and Sommer, B. (1997). Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. *Proc. Natl. Acad. Sci. U.S.A*. **9**, 13287–13292.

Tang, M. X., Stern, Y., Marder, K., Bell, K., Gurland, B., Lantigua, R., Andrews, H., Feng, L., Tycko, B., and Mayeux, R. (1998). The APOE-epsilon4 allele and the risk of Alzheimer disease among African Americans, whites, and hispanics. *JAMA, J. Am. Med. Assoc*. **279**, 751–755.

Teasdale, G. M., Nicoll, J. A. R., Murray, G., and Fiddes, M. (1997). Association of apolipoprotein E polymorphism with outcome after head injury. *Lancet* **350**, 1069–1071.

Tekirian, T. L., Cole, G. M., Russell, M. J., Yang, F., Wekstein, D. R., Patel, E., Snowdon, D. A., Markesbery, W. R., and Geddes, J. W. (1996). Carboxy terminal of beta-amyloid deposits in aged human, canine, and polar bear brains. *Neurobiol. Aging* **17**, 249–257.

Terry, R. D. (1996). The pathogenesis of Alzheimer disease: An alternative to the amyloid hypothesis. *J. Neuropathol. Exp. Neurol*. **55**, 1023–1025.

Tremml, P., Lipp, H. P., Müller, U., Ricceri, L., and Wolfer, D. P. (1998). Neurobehavioral development, adult openfield exploration and swimming navigation learning in mice with a modified β-amyloid precursor protein gene. *Behav. Brain Res*. **95**, 65–76.

Troost, D., Sillevis-Smitt, P. A,. de Jong, J. M., and Swaab, D. F. (1992). Neurofilament and glial alterations in the cerebral cortex in amyotrophic lateral sclerosis. *Acta Neuropathol*. **84**, 664–673.

Tu, P.-H., Raju, P., Robinson, K. A., Gurney, M. E., Trojanowski, J. Q., and Lee, V. M.-Y. (1996). Transgenic mice carrying a human mutant superoxide dismutase transgene develop neuronal cytoskeletal pathology resembling human amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. U.S.A*. **93**, 3155–3160.

Tu, P.-H., Robinson, K. A., de Snoo, F., Eyer, J., Peterson, A., Lee, V. M.-Y., and Trojanowski, J. Q. (1997). Selective degeneration of purkinje cells with Lewy body-like inclusion in aged NFHLACZ transgenic mice. *J. Neurosci*. **17**, 1064–1074.

Van Broeckhoven, C., Haan, J., Bakker, E., Hardy, J. A., Van Hul, W., Wehnert, A., Vegter-Van der Vlis, M., and Roos, R. A. (1990). Amyloid beta protein precursor gene and hereditary cerebral hemorrhage with amyloidosis (Dutch). *Science* **248**, 1120–1122.

Vechio, J. D., Bruijn, L. I., Xu, Z., Brown, R. H., Jr., and Cleveland, D. W. (1996). Sequence variants in human neurofilament proteins: Absence of linkage to familial amyotrophic lateral sclerosis. *Ann. Neurol*. **40**, 603–610.

Veinbergs, I., Mallory, M., Mante, M., Rockenstein, E., Gilbert, J. R., and Masliah, E. (1999). Differential neurotrophic effects of apolipoprotein E in aged transgenic mice. *Neurosci. Lett.* **265**, 218–222.

Vickers, J. C. (1997). Commentary: A cellular mechanism for the neuronal changes underlying Alzheimer's disease. *Neuroscience* **78**, 629–639.

Vickers, J. C., Delacourte, A., and Morrison, J. H. (1992). Progressive transformation of the cytoskeleton associated with normal aging and Alzheimer's disease. *Brain Res.* **594**, 273–278.

Vickers, J. C., Morrison, J. H., Friedrich, V. L., Jr., Elder, G. A., Perl, D. P., Katz, R. N., and Lazzarini, R. A. (1994). Age-associated and cell type-specific neurofibrillary pathology in transgenic mice expressing the human mid-sized neurofilament subunit. *J. Neurosci.* **14**, 5603–5612.

Vickers, J. C., Lazzarini, R. A., Riederer, B. M., and Morrison, J. H. (1995a). Intraperikaryal neurofilamentous accumulations in a subset of retinal ganglion cells in aged mice that express a human neurofilament gene. *Exp. Neurol.* **136**, 266–269.

Vickers, J. C., Schumer, R. A., Podos, S. M., Wang, R. F., Riederer, B. M., and Morrison, J. H. (1995b). Differential vulnerability of neurochemically identified subpopulations of retinal neurons in a monkey model of glaucoma. *Brain Res*. **680**, 23–25.

Vickers, J. C., Huntley, G. W., and Morrison, J. H. (1995c). Excitatory amino acids and neurotoxicity in the human neocortex. *In* "Neurotransmitters in the Human Brain" (D. Tracey, J. Stone, and G. Paxinos, eds.), pp. 79–99. Plenum, New York.

Vickers, J. C., Dickson, T. C., Adlard, P. A., Saunders, H. L., King, C. E., and McCormack, G. (2000). The cause of neuronal degeneration in Alzheimer's disease. *Prog. Neurobiol*. **60**, 139–165.

Walker, L. C., Kitt, C. A., Schwam, E., Buckward, F., Garcia, F., Sepinwall, J., and Price, D. L. (1987). Senile plaques in aged squirrel monkeys. *Neurobiol. Aging* **8**, 291–296.

Warita, H., Itoyama, Y., and Abe, K. (1999). Selective impairment of fast anterograde axonal transport in the peripheral nerves of asymptomatic transgenic mice with a G93A mutant SOD1 gene. *Brain Res*. **819**, 120–131.

White, A. R., Zheng, H., Galatis, D., Maher, F., Hesse, L., Multhaup, G., Beyreuther, K., Masters, C. L., and Cappai, R. (1998). Survival of cultured neurons from amyloid precursor protein knock-out mice against Alzheimer's amyloid-β toxicity and oxidative stress. *J. Neurosci*. **18**, 6207–6217.

Williamson, T. L., and Cleveland, D. W. (1999). Slowing of axonal transport is a very early event in the toxicity of ALS-linked SOD1 mutants to motor neurons. *Nat. Neurosci.* **2**, 50–56.

Williamson, T. L., Bruijn, L. I., Zhu, Q., Anderson, K. L., Julien, J.-P., and Cleveland, D. W. (1998). Absence of neurofilaments reduces the selective vulnerability of motor neurons and slows disease caused by a familial amyotrophic lateral sclerosis-linked superoxide dismutase 1 mutant. *Proc. Natl. Acad. Sci. U.S.A*. **95**, 9631–9636.

Wirak, D. O., Bayney, R., Ramabhadran, T. V., Fracasso, R. P., Hart, J. T., Hauer, P. E., Hsiau, P., Pekar, S. K., Scangos, G. A., Trapp, B. D., and Unterbeck, A. J. (1991). Deposits of amyloid β protein in the central nervous system of transgenic mice. *Science* **253**, 323–325.

Wirak, D. O., Bayney, R., Ramabhadran, T. V., Fracasso, R. P., Hart, J. T., Hauer, P. E., Hsiau, P., Pekar, S. K., Scangos, G. A., Trapp, B. D., and Unterbeck, A. J. (1992). Comment. *Science* **255**, 1445.

Wischik, C. M., Novak M., Thogersen, H. C., Edwards, P. C., Runswick, M. J., Jakes, R., Walker, J. E., Milstein, C., Roth, M., and Klug, A. (1988). Isolation of a fragment of tau derived from the core of the paired helical filament of Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A*. **85**, 4506–4510.

Wisniewski, H. M., Ghetti, B., and Terry, R. D. (1973). Neuritic (senile) plaques and filamentous changes in aged rhesus monkeys. *J. Neuropathol. Exp. Neurol*. **32**, 566–584.

Wisniewski, K. E., Wisniewski, H. M., and Wen, G. Y. (1985). Occurrence of neuropathological changes and dementia of Alzheimer's disease in Down's syndrome. *Ann. Neurol*. **17**, 278–282.

Wisniewski T., and Frangione B. (1992). Apolipoprotein E: A pathological chaperone protein in patients with cerebral and systemic amyloid. *Neurosci. Lett*. **135**, 235–238.

Wolfe, M. S., Xia, W., Ostaszewski, B. L., Diehl, T. S., Kimberly, W. T., and Selkoe, D. J. (1999). Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. *Nature (London)* **398**, 513–517.

Wong, P. C., Pardo, C. A., Borchelt, D. R., Lee, M. K., Copeland, N. G., Jenkins, N. A., Sisodia, S. S., Cleveland, D. W., and Price, D. L. (1995). An adverse property of a familial ALS-liked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. *Neuron* **14**, 1105–1116.

Wong, P. C., Zheng, H., Chen, H., Becher, M. W., Sirinathsinghji, D. J., Trumbauer, M. E., Chen, H. Y., Price, D. L., Van der Ploeg, L. H., and Sisodia, S. S. (1997). Presenilin 1 is required for *Notch1* and *DII1* expression in the paraxial mesoderm. *Nature (London)* **387**, 288–292.

Wyss-Coray, T., Masliah, E., Mallory, M., McConlogue, L., Johnson-Wood, K., Lin, C., and Mucke, L. (1997). Amyloidogenic role of cytokine TGF-β1 in transgenic mice and in Alzheimer's disease. *Nature (London)* **389**, 603–606.

Xu, Z., Cork, L. C., Griffin, J. W., and Cleveland, D. W. (1993). Increased expression of neurofilament subunit NF-L produces morphological alterations that resemble the pathology of human motor disease. *Cell (Cambridge, Mass.)* **73**, 23–33.

Zhang, B., Tu, P., Abtahian, F., Trojanowski, J. Q., and Lee, V. M. (1997). Neurofilaments and orthograde transport are reduced in ventral root axons of transgenic mice that express human SOD1 with a G93A mutation. *J. Cell Biol.* 139, 1307–1315.

Zheng, H., Jiang, M., Trumbauer, M. E., Sirinathsinghji, D. J., Hopkins, R., Smith, D. W., Heavens, R. P., Dawson, G. R., Boyce, S., Conner, M. W., Stevens, K. A., Slunt, H. H., Sisodia, S. S., Chen, H. Y., and Van der Ploeg, L. H. T. (1995). β-amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity. *Cell (Cambridge, Mass.)* **81**, 525–531.

Mark G. Baxter

26

Cognitive Aging in Nonhuman Primates

The study of cognitive aging in nonhuman primates provides a model system for understanding normal cognitive aging in humans, as well as the ability to examine specific potential neurobiological substrates of age-related cognitive impairment. This chapter reviews the evidence for age-related changes in five cognitive domains in nonhuman primates: visual recognition memory, spatial memory, stimulus–reward associative learning, relational memory, and attention/executive function. The effects of damage to specific neural systems on the performance of the particular behavioral tasks used to assess each of these domains are compared to the effects of aging on these tasks. The behavioral profile of aged monkeys is consistent with dysfunction of an inferior temporal-ventromedial prefrontal system for recognition memory and stimulus–reward associative learning, as well as deficits in dorsolateral prefrontal and/or hippocampal function contributing to spatial memory impairment, hippocampal system function contributing to relational memory impairment, and frontal lobe function contributing to executive system dysfunction. The declines in these systems seem to be at least partially independent of each other with increasing age. A focus on individual differences in the profiles of cognitive impairment in aged monkeys, and examination of relationships between behavioral and neurobiological parameters independently of main effects of age on these measures, might provide further insight into the biological basis of cognitive aging.

I. Introduction

Aged monkeys provide an extremely useful model system for understanding both the nature of normal cognitive aging in humans and the neurobiological basis of these cognitive impairments, due to their well-characterized behavioral repertoires, similarity in brain structure to humans, and lack of age-related neuropathological conditions such as Alzheimer's disease (Peters *et al*., 1996). There is a striking similarity between the profile of cognitive impairment in aged nonhuman primates and that of aged humans that are not affected by age-related neuropathological conditions such as Alzheimer's disease (Voytko, 1997).

Five general domains of cognitive function can be identified that have been examined in aged nonhuman primates: visual recognition memory, spatial memory, stimulus–reward associative learning, relational memory, and attentional processing/executive function. The general approach to be taken with each domain of cognitive function is to consider the evidence for impairments in a particular domain in aged nonhuman primates and then review the neuropsychological evidence from young animals linking each domain to the function of particular neural circuits. Much research in this area has been guided by the thesis that a particular behavioral task, known to be sensitive to damage to a particular neural system in young monkeys, should provide a functional index of the integrity of that neural system in aging. Accordingly, the approach taken in this chapter is task oriented, within the broad category of each particular domain of cognitive function. The vast majority of studies of cognitive aging in nonhuman primates have examined rhesus monkeys (*Macaca mulatta*). This is advantageous for the current discussion because most neuropsychological studies in nonhuman primates have also been done in macaques, providing a ready comparison to the effects of aging on cognition. (Although some experimenters have examined the performance of other species of aged nonhuman primates on various cognitive tasks, these experiments are generally consistent with the results in macaques and will not be discussed in this chapter unless they provide data unavailable from studies with rhesus monkeys.)

When data are available, the relationship of performance on different tasks by the same monkeys will be discussed. Identifying dissociations between impairments in cognitive function has been the most productive strategy in understanding the organization of cognitive systems (Olton, 1991). If impairments in two different tasks tend to be correlated across a group of monkeys, a common neural substrate for that impairment might be hypothesized. In contrast, if a group of aged monkeys is impaired on one task but performs normally on another, or if impairment on two different tasks is unrelated, it might be supposed that different neural systems might underlie the different impairments. Also, when data are available, the apparent time course of age-related cognitive decline will be discussed, although these observations are usually based on

cross-sectional comparisons of different groups of aged monkeys (but see Moss, 1993). Possible neural substrates for impairments in particular cognitive domains are noted when neurobiological and behavioral markers are determined in the same subjects; the reader is referred to other chapters in this text for a more comprehensive discussion of these age-related neurobiological changes.

II. Visual Recognition Memory

Behavioral tasks to measure stimulus recognition, literally ''knowing the stimulus again'' (Mishkin and Murray, 1994), were developed as part of an effort to develop an animal model of human amnesia (Correll and Scoville, 1965; Mishkin, 1978). In terms of the overall neuropsychological profile of the aged monkey, deficits in spatial working memory are associated much more consistently with advanced age (see Section III), but because of the intense focus on stimulus recognition memory in neuropsychological studies of nonhuman primates, and the converging interest in examining dysfunction of medial temporal lobe structures as a neural substrate for age-related cognitive decline, we will begin the discussion of cognitive aging in nonhuman primates with this domain of cognitive function.

The most common test of stimulus recognition memory, visual delayed nonmatching-to-sample (DNMS), was developed in its standard form, using trial-unique objects and a performance test that increases memory demand by lengthening the delay between sample and choice, and presenting lists of sample items, by two different research groups in the mid-1970s (Gaffan, 1974; Mishkin and Delacour, 1975). This test capitalizes on the monkey's natural propensity to explore novel objects. In the first phase of each DNMS trial, the monkey is allowed to view a sample object that covers the central well of a three-well test tray. The monkey displaces the object and obtains a food reward hidden underneath the object. An opaque screen is then lowered that occludes the monkey's view of the test tray. In the second phase of each trial, which takes place after a delay ranging from seconds to minutes, the opaque screen is raised and the monkey is allowed to choose between the sample object and a novel object, one covering each of the lateral wells of the test tray. The monkey can obtain another food reward by displacing the novel object. Ordinarily, a very large pool of objects (hundreds to thousands) are available for use as stimuli so the objects the monkey encounters are functionally ''trial unique'' and repeated only rarely throughout testing.

Because of the large number of studies exploring the effects of selective ablations of different brain areas on DNMS performance, this task provides a potentially rich source of information about the nature of functional impairment in aged monkeys; different brain areas may be involved in acquisition of the DNMS rule and in maintaining memory of the objects across delays (see Section II,B). In detecting a memory deficit in such tasks, particular emphasis is placed on the presence of a statistical interaction between condition (age or lesion) and delay: if the effect of condition increases with extended delays, the impairment is interpreted as one of memory because forgetting is occurring more rapidly in the impaired group. [This analysis is not universally accepted; see Murray (1990) and Ringo (1991)]. If there is no such interaction, the impairment could be one of sensory processing, attention, motivation, or some other nonmnemonic factor.

A. Effects of Aging

Aged monkeys (22–29 years old across the different studies) as a group show impairments in acquisition of the DNMS rule as well as impaired performance when the delay interval between sample and choice is increased (Presty *et al*., 1987; Moss *et al*., 1988; Rapp and Amaral, 1989). These initial studies noted some individual differences in the performance of aged monkeys: certain aged monkeys acquired the DNMS rule as efficiently as young monkeys and performed as well across delays as young monkeys. Notably, however, there was no interaction between age and delay in the scores on the DNMS performance test, casting doubt on the interpretation of the DNMS impairment as one of memory.

Although various explanations for this were suggested at the time, Rapp and Amaral (1991), after testing additional aged monkeys, offered an analysis based on classifying the mean performance of aged (22–27 years old) monkeys across all delays as ''impaired'' or ''unimpaired,'' depending on whether the score of each individual aged monkey fell within the range of young performance or not. (A 33-year-old monkey also tested as part of this study could not acquire the DNMS rule at all, and testing of this subject was discontinued after 3000 trials.) The two subgroups of aged monkeys in their sample were of similar chronological age. The subpopulation of aged monkeys who were ''impaired'' (2/3 of their sample) indeed demonstrated differentially impaired performance across longer delays (i.e., an age by delay interaction) that was not observed when the entire sample of aged monkeys was compared as a single group with the young monkeys. Interestingly, there was not a consistent relationship in this study population between impairment in delay performance and impairment in acquisition of the DNMS rule—both subgroups of aged monkeys demonstrated impairments in acquisition of the DNMS rule relative to the comparison group of young monkeys. More recent studies using larger samples of aged monkeys indicate that the incidence of impairment in DNMS acquisition seems to increase with advancing age, although there is no apparent relationship between chronological age and delay performance when aged monkeys are considered as a whole (Herndon *et al*., 1997; Moss *et al*., 1999; Killiany *et al*., 2000).

Rapp and Amaral (1989) also discovered that an alteration in the task demands of the DNMS task could reveal an impairment in performance—testing DNMS performance using only a single pair of objects, rather than trial-unique objects, revealed a dramatic impairment in the aged monkeys. They suggested that while object recognition memory was relatively unimpaired in aged monkeys, memory for temporal order (which of two familiar objects was presented most recently) was affected profoundly by aging. These two cognitive processes have distinct neural substrates (see Sections II,B and III,B).

The level of DNMS impairment seems to increase with advancing age; e.g., monkeys 25–29 years old were numerically more impaired on DNMS than monkeys 20–24 years

old (Presty *et al*., 1987). Inspection of data presented by Herndon *et al*. (1997) suggests that DNMS acquisition impairments are not reliably observed in monkeys younger than 25 years of age, but that impaired delay performance is present in monkeys 19 years of age and older (although, again, there are substantial individual differences, with even very old monkeys demonstrating delay performance in the range of the young monkeys).

Another procedure for assessing stimulus recognition memory, similar to DNMS, is the delayed recognition span task (DRST) developed by Moss and colleagues (1997). In the object and color conditions of this task, either objects or colored disks are placed one by one on a test tray. Each object or colored disk covers a food well. When a new object or colored disk is placed on the test tray, it covers a food reward; the old stimuli are moved to new locations on the test tray but are not rebaited. Hence, the monkey must remember on each trial which stimuli it has seen previously in order to choose the new object or colored disk and obtain a food reward. New objects or colored disks are added until the monkey makes a mistake. Monkeys older than 19 years of age appear to be impaired on the DRST–object condition, whereas impairment on the DRST–color condition does not appear to emerge until 25 years of age (Herndon *et al*., 1997; Moss *et al*., 1997, 1999; Killiany *et al*., 2000).

B. Neural Basis

Since the initial demonstration of an impairment in DNMS produced by combined aspiration lesions of the amygdala and hippocampus in monkeys (Mishkin, 1978), approximating the severe anterograde amnesia produced by a similar neurosurgical operation in human patient H.M. (Corkin, 1984; Corkin *et al*., 1997), considerable effort has been devoted to fractionating the contributions of different neural structures to the performance of this task, particularly within the medial temporal lobe. Of the tasks used to investigate the neuropsychology of memory function in nonhuman primates, DNMS is probably the most widely investigated in terms of the particular neural systems responsible for good performance. Hence, a fairly rich data base exists for the purpose of comparing the effects of aging on DNMS performance with the effects of selective damage to particular brain regions.

DNMS performance seems to involve the interaction of a network of three basic neural structures: the inferior temporal cortex, the mediodorsal thalamus, and the ventromedial prefrontal cortex, with a possible modulatory effect of the basal forebrain cholinergic system (Mishkin and Appenzeller, 1987; Mishkin and Murray, 1994). Within the inferior temporal cortex, the perirhinal cortex seems to be the single most important component for supporting good recognition memory performance, with combined damage to the entorhinal and perirhinal cortex producing an even more severe deficit (Meunier *et al*., 1993; Murray, 1996). Lesions of the entorhinal cortex alone produce only a mild deficit that appears to be transient (Meunier *et al*., 1993; Leonard *et al*., 1995). These deficits are characterized by an impairment in reacquiring the DNMS rule as well as more rapid forgetting across increased delays between sample and choice. Damage limited to the hippocampus produces little or no deficit in performance of this task (Alvarez *et al*., 1995; Murray and Mishkin, 1998; but see Beason-Held *et al*., 1999); similarly, the amygdala appears to make no contribution to performance of this task in its standard form (Zola-Morgan *et al*., 1989b; Murray and Mishkin, 1998; for review, see Baxter and Murray, 2000).

Less information is available about the contribution of specific thalamic and prefrontal structures to this task. Lesions of the mediodorsal thalamus also produce a severe impairment in DNMS performance, with an approximately equal contribution of anterior and posterior groups of thalamic nuclei (Aggleton and Mishkin, 1983a,b; Zola-Morgan and Squire, 1985a). Lesions of the ventromedial prefrontal cortex produce a profound impairment in DNMS performance as well (Bachevalier and Mishkin, 1986). Interestingly, lesions restricted to the inferior prefrontal convexity (ventral prefrontal cortex) appear to produce a selective deficit in reacquisition of the DNMS rule, without an impairment in memory across increasing delays (Kowalska *et al*., 1991), whereas damage to the orbital frontal cortex produces an impairment in memory across increasing delays with less of an impairment in reacquisition of the DNMS rule (Meunier *et al*., 1997). Direct interaction between the frontal cortex and the perirhinal cortex, and between the mediodorsal thalamus and the perirhinal cortex, is critical for visual recognition memory (Parker and Gaffan, 1998). Lesions of basal forebrain cholinergic neurons have little effect on performance of this task, although peripheral administration of a muscarinic receptor antagonist produces DNMS impairments (Aigner and Mishkin, 1986; Aigner *et al*., 1991; Voytko *et al*., 1994).

The overlap between deficits produced by different lesions makes it difficult to draw conclusions about defects in particular neural systems in aging based solely on the pattern of DNMS performance. Decreased performance across delays, as well as impaired acquisition of the DNMS rule, is associated with damage to a number of different neural structures [although the relative magnitude of such deficits can sometimes be dissociated; see, for example, Meunier *et al*. (1997)]. There is a lack of consensus on the relationship between DNMS acqusition and delay performance deficits in aged monkeys; some studies with relatively small numbers of subjects see little association between acquisition of the task and delay performance (Rapp and Amaral, 1991), which would suggest that these two deficits must have their origins in different neural systems. However, a study with a larger sample size reported that acquisition and delay performance loaded onto the same factor in a principal components analysis, suggesting that age-related declines in these two processes are linked (Herndon *et al*., 1997). Finally, an analysis of published data from the first two studies of DNMS performance in aged monkeys (Presty *et al*., 1987; Moss *et al*., 1988), which used similar testing protocols, reveals an association between acquisition of the DNMS rule and delay performance, although again individual differences may exist in this association (see Fig. 26.1). A similar observation has been made with respect to the performance of monkeys with medial temporal lobe lesions (Murray and Mishkin, 1984).

This correlated impairment in DNMS rule acquisition and delay performance is consistent with a dysfunction in the inferior temporal-prefrontal system that subserves visual recognition memory in aging, affecting both DNMS learning and

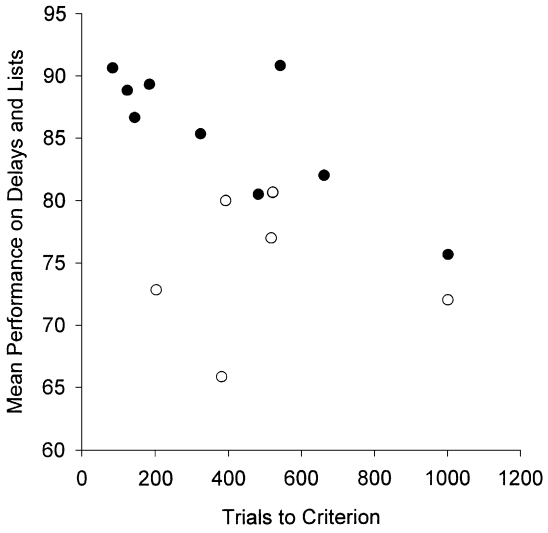

FIG. 26.1. Relationship between trials to criterion in DNMS acquisition and performance across delays (ranging from 30 to 120 sec) and lists of objects (3, 5, and 10 objects) from two studies of aged monkeys that used very similar testing protocols (Moss *et al*., 1988; Presty *et al*., 1987). The filled symbols are subjects from the study by Presty *et al*. (1987) that were older than 20 years (excluding the two aged monkeys with a history of behavioral testing), and the open symbols are subjects from the study by Moss *et al*. (1988). Overall, there appears to be a relationship between DNMS acquisition and delay performance, although the correlation between these variables does not reach statistical significance ($r = -0.48$, $p = 0.07$). However, 2 subjects in the study by Moss *et al*. (1988) show extremely impaired delay performance despite relatively intact acquisition of the DNMS rule, suggestive of individual differences in the relationship between these variables. If these 2 subjects are removed from the analysis, the correlation between these variables from the remaining 13 subjects reaches statistical significance ($r = -0.81$, $p = 0.0008$). Interestingly, this correlation remains even if age is partialled out ($pr = -0.76$, $p = 0.004$), indicating that this relationship is not due to a common main effect of age on DNMS acquisition and delay performance (see Section VII).

delay performance, a hypothesis that would also account for age-related deficits in stimulus–reward associative learning (see Section IV). However, impairments in learning the DNMS rule, without impairments in performance across increasing delays [as was reported for some of the subjects in Rapp and Amaral (1991)] might be suggestive of a selective impairment in prefrontal function, specifically the ventral prefrontal cortex (Kowalska *et al*., 1991).

Performance on DNMS with a single pair of objects appears to be more sensitive to aspects of prefrontal cortex function, likely tapping into similar cognitive processes that underlie performance on the delayed response task (see Section III,B). Monkeys with lesions of the hippocampus are not impaired on performance of DNMS with a small set of objects (Murray and Mishkin, 1984) and indeed monkeys with these lesions are facilitated in learning DNMS with a single pair of objects (M. Mishkin and J. L. Oubre, unpublished observations, as cited in Murray and Mishkin, 1984). Damage to the anterior rhinal cortex produces an impairment in relearning DNMS with a single pair of objects (Murray *et al*., 1996), but monkeys with lesions of the rhinal cortex can perform DMS with a single pair of objects well, even across extended delays, once the rule is relearned (Eacott *et al*., 1994). However, monkeys with damage to the ventral prefrontal cortex are impaired at relearning DMS with a single pair of objects or color stimuli (Passingham, 1975; Mishkin and Manning, 1978), although this impairment may be related to rule learning and not stimulus memory per se (Rushworth *et al*., 1997).

There is little positive evidence linking age-related impairments in DNMS performance to dysfunction of specific neural systems. Hypertrophy of cholinergic neurons in the rostral medial septal nucleus appears to distinguish aged monkeys with DNMS impairment from those with intact performance (Stroessner-Johnson *et al*., 1992), although no such association is seen at more caudal levels of the medial septal nucleus (Stroessner-Johnson *et al*., 1992) or at anterior or intermediate levels of the nucleus basalis (Voytko *et al*., 1995). Amyloid plaque burden in cortical areas is uncorrelated with a global index of cognitive performance that includes DNMS performance (Sloane *et al*., 1997). Similarly, no cell loss has been observed in the hippocampus or in layer II of the entorhinal cortex of monkeys behaviorally characterized on this task (Amaral, 1993); although it might be expected that cell loss in the perirhinal cortex would be linked more closely to DNMS impairment, this has not yet been evaluated. No studies have focused specifically on thalamic or prefrontal regions implicated in DNMS performance. Two studies have noted relationships between abnormalities in area 46 of the dorsolateral prefrontal cortex and impairments in DNMS performance based on white matter pathology in this cortical area (Peters *et al*., 1994) and thickness of layer I of area 46 (Peters *et al*., 1998; but see O'Donnell *et al*., 1999). The significance of these observations is unclear given the lack of effect of lesions of this area on DNMS performance in young monkeys (Bachevalier and Mishkin, 1986); it is also possible that some of these findings are related to common age effects on behavioral performance and neurobiological markers (see Section VII).

III. Spatial Memory

A. Effects of Aging

Medin (1969) first reported an impairment in short-term memory in a group of aged rhesus monkeys (17–18 years old) compared to a group of middle-aged rhesus monkeys (mean age 10 years) as part of a study on pattern reproduction by monkeys. All of these monkeys were experimentally sophisticated, and the magnitude of the difference between the groups was not large. This suggestive initial observation was replicated by Bartus and colleagues (1978), who reported a dramatic impairment of short-term memory for spatial locations in aged rhesus monkeys tested in a similar task. Monkeys were tested in an automated apparatus and were required to remember the location of an illuminated panel (chosen randomly from a matrix of nine locations) across a delay of 0 to 30 sec. Although both young (3–5 years old) and aged (18–23 years old) monkeys performed well at a 0 sec retention interval (demonstrating motivation and mastery of the task principle), aged monkeys were severely impaired at 15 and 30 sec retention intervals. Indeed, there was no overlap between the scores of young and aged monkeys at these two retention intervals, suggesting that aged monkeys were uni-

formly impaired on this task. Further control procedures suggested that these deficits were not due to an impairment in sensory processing in the aged monkeys.

In a more commonly used procedure to assess spatial working memory, the spatial delayed response (DR) task, the monkey is allowed to watch while one of two food wells is baited with a reward. Then an opaque screen is lowered between the monkey and the food wells, and the wells are covered with identical gray plaques. The screen is then raised after some retention interval and the monkey is allowed to displace one of the plaques in an attempt to gain the food reward. The food is not moved in the retention interval, so the monkey must simply remember which well it saw baited and displace the plaque covering that well to obtain the food reward.

Aged monkeys also demonstrate impairments on this more classical short-term spatial memory task (Arnsten and Goldman-Rakic, 1985a; Arnsten *et al*., 1988; Rapp and Amaral, 1989; Bachevalier *et al*., 1991; Voytko, 1993; Roberts *et al*., 1997; O'Donnell *et al*., 1999). It is interesting that aged monkeys appear to demonstrate more individual differences in performance in the DR task than in the more complex automated spatial working memory task originally employed by Bartus. As mentioned, all of the monkeys in Bartus's study (six female rhesus monkeys estimated to be 18–23 years old) were markedly impaired in short-term memory for spatial location (no overlap between scores of young and aged monkeys). In contrast, older monkeys in studies using the DR task occasionally show intact performance; e.g., a 30-year-old male rhesus monkey in the study by Bachevalier *et al*. (1991) performed as well on DR as any of the young monkeys tested in that study, and a wide range of performance is seen in the large sample of aged female rhesus monkeys studied by Roberts *et al*. (1997), from nearly perfect performance across retention intervals ranging from 5 to 60 sec to near-chance performance. Although it is difficult to make definitive conclusions because of the relatively small sample sizes involved in these studies, it is tempting to speculate that the DR task may be less sensitive to age-related memory impairments than the version employed by Bartus; perhaps the increased number of possible locations for responding increases the difficulty of the test. In contrast, however, interference will be higher in the DR task compared to Bartus's task because there are only two possible responses. Indeed, interference, rather than memory, may be a critical factor for age-related impairments in performance on these types of tasks (Rapp and Amaral, 1989), although an increased impairment in performance of aged monkeys with increasing delays in the DR task does reflect an impairment of memory (e.g., O'Donnell *et al*., 1999). It would be of interest to parametrically manipulate both of these factors (number of spatial locations for responding, and interference levels) within a single experiment.

Another related task is the spatial condition of the DRST developed by Moss and colleagues (1997). In this task the monkey is faced with a 3×6 array of wells on a test board. To begin a trial sequence, a brown disk covers a food reward placed in one well of the test board. The monkey displaces the disk and is allowed to obtain the food reward. An opaque screen is lowered between the monkey and the test board; the first disk is returned to its original position, now covering an unbaited well, and a new disk (visually identical to the first) is added, covering a food reward. The screen is then raised and the monkey is allowed to respond. The trial continues in this fashion, with one disk covering a new location and a food reward being added after each response, until the monkey makes a mistake. Thus the monkey must maintain in memory the locations that have already been chosen in order to correctly identify the new location on the test tray and obtain the food reward.

Rhesus monkeys demonstrate impairment on this task as early as 19 years of age (Killiany *et al*., 2000); monkeys 19–24 years of age are impaired on this task as a group, with a mean recognition span of 2.07 compared to a mean span of 2.57 for young monkeys 6–14 years of age (Killiany *et al*., 1999). Although this level of impairment is similar to a group of monkeys 25–27 years of age (mean spatial recognition span of 2.05) (Moss *et al*., 1997) as well as monkeys 30–35 years of age (mean recognition span of 1.83) (Moss *et al*., 1999), there appears to be a robust linear relationship between chronological age and DRST–spatial performance (see Herndon *et al*., 1997). Thus, the pattern of performance in this task is quite similar to that from the classic DR task—an impairment in spatial working memory that increases with advanced age. Notably, within the relatively large sample studied by these authors, few monkeys of advanced age perform as well as young monkeys. Compared with the findings of Bachevalier *et al*. (1991) and Roberts *et al*. (1997) of greater individual differences in DR performance of aged monkeys, this might suggest that the DRST is more sensitive to an aspect of cognitive function that is impaired more consistently in advanced age (possibly, as mentioned before, the ability to monitor many spatial locations instead of just two), but investigation of this possibility awaits a direct test.

The foregoing tests measure memory for locations on a test board, which is placed in a fixed position in front of the monkey. These tests are unable to distinguish between memory for allocentric (environment-centered) and egocentric (subject-centered) space—the monkey may be remembering the absolute position of the response locations within the testing environment or may be remembering its own movements relative to the test board (reaching left or right). Aged monkeys have also been tested in a task that tests allocentric spatial memory, very similar to tests used classically to test spatial memory in the rat. In this task, the monkey is allowed to move around on a large octagonal platform. At the center of each edge of the platform is a small well containing a food reward. The monkey is allowed to retrieve the rewards in any order, provided it returns to the center of the platform between choices (to prevent it from simply running around the edge to collect all the food rewards). This remarkable procedure (Rapp *et al*., 1997) is quite like the radial arm maze task used to test spatial memory in rats (Olton and Samuelson, 1976; Olton *et al*., 1982). Like rats, young monkeys employ the position of distal cues placed within the test room to locate the rewards (Rapp *et al*., 1997). Aged monkeys (23–33 years old) were impaired in acquiring this task and performed very poorly when a delay was interposed between the first four choices and the last four choices. Interestingly, the same monkeys that showed impaired allocentric spatial memory were unimpaired on standard DNMS testing, suggesting a distinct neural substrate for the spatial memory impairment.

B. Neural Basis

Impairments in spatial working memory (and working memory function more generally) are associated with damage to the dorsolateral prefrontal cortex (area 46) (Goldman and Rosvold, 1970; Bachevalier and Mishkin, 1986). Monkeys with lesions of the dorsolateral prefrontal cortex are unable to acquire the DR task. The integrity of catecholaminergic projections to the prefrontal cortex appears critical for normal spatial working memory (Brozoski *et al*., 1979), and dysfunction of these projections has been suggested as a neural substrate for the age-related impairment in DR performance (Arnsten and Goldman-Rakic, 1985a,b; Arnsten *et al*., 1988, 1995; Arnsten, 1993). Bartus noted the similarity between the short-term memory impairment in aged monkeys (Bartus *et al*., 1978) and the impairment in short-term memory in young monkeys induced by the administration of scopolamine, an antagonist of muscarinic cholinergic receptors (Bartus and Johnson, 1976). Alterations in the function of brain cholinergic systems are unlikely to be the neural substrate of age-related spatial memory decline; lesions of basal forebrain cholinergic input to neocortex in the rhesus monkey are without effect on DR performance (Voytko *et al*., 1994). Frank neuronal or volumetric loss in area 46 does not appear to occur in aging (Peters *et al*., 1994), nor is the volume of this cortical area related to impairments in DR performance in aged monkeys (O'Donnell *et al*., 1999).

Hippocampal damage produces no effect on acquisition of the DR task (Murray and Mishkin, 1986; Zola-Morgan *et al*., 1989a) but does induce a deficit when longer retention intervals are interposed (Zola-Morgan *et al*., 1989a), as does combined ablation of the amygdala and hippocampus, including the subjacent rhinal cortex (Zola-Morgan and Squire, 1985b). Thus, a contribution of impaired hippocampal and/or medial temporal lobe function to impaired performance at longer delays in the DR task is possible. Indeed, an *in vivo* measure of hippocampal glucose metabolism correlated highly with aged monkeys' performance of the DR task (Eberling *et al*., 1997). However, lesions restricted to the hippocampus, made with stereotaxically placed injections of the neurotoxin ibotenic acid, do not impair performance on a closely related task, spatial delayed nonmatching-to-sample (Murray and Mishkin, 1998), which calls into question the specific involvement of the hippocampus in memory for spatial location. Lesions restricted to the hippocampus (produced by injections of ibotenic acid) have also been reported to produce an impairment in the DRST–spatial task (Beason-Held *et al*., 1999). However, the monkeys in that study were also impaired in DNMS performance, a finding that has not been replicated by other investigators (Murray and Mishkin, 1998), so the impairment in DRST–spatial performance by these monkeys must be interpreted with caution. No lesion data are currently available for the allocentric spatial memory task of Rapp and colleagues (1997), but the intact DNMS performance in these monkeys suggests that the locus of this deficit must lie outside of the inferior temporal-medial thalamic-ventromedial prefrontal system (see Section II,B). Certainly, based on ample evidence in rats for hippocampal involvement in allocentric spatial memory (Olton *et al*., 1982), this task would be expected to depend on intact hippocampal function.

IV. Stimulus–Reward Associative Learning

A. Effects of Aging

Stimulus–reward associative learning tasks include classic object and spatial discrimination tasks, in which one of two (or more) stimuli is consistently associated with a reinforcer. Tasks of this nature can be varied to examine a number of different aspects of cognitive function by varying the rate of presentation of individual discrimination problems or by reversing the response–reinforcement contingencies once they are learned. These reversal problems test behavioral flexibility, a component of ''executive'' function, as well as the strength of stimulus–reinforcer bonds.

Monkeys trained on single object discrimination problems appear to show no impairment in the acquisition of individual problems or impairment in retention of these problems (Bartus *et al*., 1979; Rapp, 1990; Bachevalier *et al*., 1991; Lai *et al*., 1995), although aged monkeys tend to demonstrate impairments in pattern discrimination learning problems where the stimuli are black-and-white two-dimensional patterns that are less easy to discriminate (Rapp, 1990). In behaviorally sophisticated aged monkeys, Medin reported that aged monkeys appeared to show a reduced retention of object discrimination problems between the first and second day of testing on a list of 20 problems, each presented once per day (Medin *et al*., 1973), suggesting a selective age-related impairment in long-term memory for stimulus–reward associations. Bachevalier and colleagues reported a similar finding, although retention between the first and the second days of testing was not examined explicitly; their oldest group of monkeys (aged 28–31 years) took almost three times as many sessions to reach a criterion of 90% correct on a set of 20 object discrimination problems, each presented once per day (the ''24-hr intertrial interval (ITI)'' task; Bachevalier *et al*., 1991). This may reflect a selective impairment in a slow-learning, ''habit''-like system for forming stimulus-reward associations (see Section IV,B). Aged monkeys are not impaired on spatial discrimination learning (Lai *et al*., 1995; Voytko, 1999), an interesting contrast to their impairment in spatial working memory (see Section III).

Reversal of stimulus–reward associations is impaired markedly in aged monkeys, although the precise nature of this impairment is somewhat unclear. Impairments in object reversal learning (Bartus *et al*., 1979; Peters *et al*., 1996; Moss *et al*., 1999; Voytko, 1999) and spatial reversal learning (Lai *et al*., 1995; Peters *et al*., 1996; Moss *et al*., 1999) have been reported, as have intact spatial reversal learning (Voytko, 1999) and object reversal learning (Rapp, 1990; Lai *et al*., 1995). All of these studies used similar two-choice discrimination procedures. The discrepancies between these studies may be more apparent than real, however. It is interesting to note that in two of the studies that tested both spatial and object reversal learning, it was the first reversal task administered (whether spatial or object) that was impaired [spatial in the case of Lai *et al*. (1995), and object in the case of Voytko (1999)]. Additionally, Lai *et al*. (1995) noted a statistically significant increase in perseverative errors in both spatial and object reversals in their aged monkeys, although the overall number of errors in object reversal was not different between young and

aged groups. Additional studies suggest impairments on reversal learning after experimental ablation of particular cortical areas may be particularly apparent only the first time a reversal is encountered (see Section IV,B). Rapp (1990) observed no impairment in reversal of pattern discriminations (which were the first reversal problems administered in that study), but the difficulty experienced by the aged monkeys in learning to discriminate those stimuli initally could have confounded this result.

Relatively few experiments have focused on individual differences in aged monkeys performing discrimination and reversal tasks. Lai *et al*. (1995) noted that some of their aged monkeys performed within the range of young monkeys by the second reversal of the spatial discrimination. Voytko (1997, 1999) has noted that some aged monkeys perform object reversals as well as young monkeys, whereas other individuals are impaired dramatically in object reversal learning.

B. Neural Basis

The different forms of discrimination learning and reversal tasks seem to have distinct neural bases. Rapid acquisition of object discrimination problems seems to involve the inferotemporal cortex and ventral prefrontal cortex, as well as the mediodorsal thalamus (Gaffan and Murray, 1990; Gaffan *et al*., 1993; Baxter and Murray, 2000). The entorhinal and perirhinal cortices seem to be required for rapid learning of visual discrimination problems only to the extent that these cortical areas are involved in identification of the stimuli to be discriminated (see discussion in Baxter *et al*., 1999). Other medial temporal lobe structures may be involved in retention of these rapidly learned discrimination problems (Zola-Morgan *et al*., 1994; Alvarez *et al*., 1995). In contrast, slow learning of object discrimination problems (e.g., in the ''24-hr ITI'' task) is independent of limbic structures (amygdala and hippocampus) but does require inferior temporal cortical area TE (Phillips *et al*., 1988) and again the entorhinal and perirhinal cortices only to the extent that object identification is taxed (Buckley and Gaffan, 1997; Thornton *et al*., 1998); as well as the ventral and orbital frontal cortices (Murray and Wise, 1997) and the tail of the caudate nucleus (Wang *et al*., 1990). The commonality between cortical systems required for learning of visual discrimination problems and visual recognition memory is notable (see Section II,B). Interestingly, individual monkeys who are impaired on DNMS performance also appear to demonstrate an impairment in object discrimination learning (Rapp, 1993), suggesting a common neural substrate for these deficits, likely inferior temporal-prefrontal connections (see Section II,B).

Spatial discrimination learning appears to involve hippocampal formation and dorsolateral prefrontal cortex. Monkeys with dorsolateral prefrontal cortex lesions are markedly impaired on acquisition of a spatial discrimination problem (Pohl, 1973), as are monkeys with aspiration lesions of the hippocampus that include the overlying parahippocampal cortex (Jones and Mishkin, 1972). The contribution of the hippocampus proper to spatial discrimination learning is unclear, as monkeys with neurotoxic hippocampal lesions (sparing the parahippocampal cortex) are not impaired on this particular spatial discrimination learning task (Murray *et al*., 1998).

Reversal learning appears to recruit additional neural systems. Lesions that impair the initial discrimination also produce impairment in subsequent reversals of that discrimination. However, lesions of the orbitofrontal cortex, which produce no impairment in initial acquisition of a spatial discrimination, produce a marked impairment in reversal of that discrimination (Jones and Mishkin, 1972). Similarly, rhinal cortex lesions, which have little effect on object discriminations, produce an impairment in subsequent reversals of that discrimination (Murray *et al*., 1998). Lesions restricted to the hippocampus also appear to produce an impairment in object reversals, milder than that produced by rhinal cortex lesions (Mahut, 1971; Murray *et al*., 1998). Of note is the pattern of errors made during reversal learning: Jones and Mishkin (1972) analyzed errors in reversal learning based on whether the monkey was performing below chance (stage 1), at chance (stage 2), or above chance but not yet at criterion (stage 3). Damage to the orbitofrontal cortex is characterized by a marked increase in stage 1 errors, suggestive of perseverative behavior (Jones and Mishkin, 1972). Rhinal cortex lesions produce similar perseverative behavior (Murray *et al*., 1998). Hence, perseverative behavior on reversals may suggest damage to the prefrontal cortex, rhinal cortex, or both. It is also worth noting that impairments in reversal learning may be limited to the first time a particular type of reversal problem is encountered (Dias *et al*., 1997); this may explain why a lack of impairment on reversal learning is observed when the monkeys have previously performed reversals in a different stimulus domain (e.g., testing on spatial reversals after having performed object reversals).

V. Relational Memory

A. Effects of Aging

In an attempt to probe an aspect of memory function that might be related more specifically to the functional integrity of the hippocampus and its associated cortical regions (see Section V,B), some investigations have looked specifically at relational memory processing in aged monkeys.

One task of interest tests transitive inference ability. The task takes the form of a set of ordered object discriminations, such that the stimulus that is rewarded on a particular trial depends on what other stimulus is present on that trial. First the monkey is taught a discrimination between two objects, A and B, where A is rewarded and B is not (A+ B−). On the next discrimination problem, B is rewarded and a new object (C) is not (B+ C−). Additional problems are learned (C+ D− and D+ E−) and then the problems are presented in a randomly intermixed fashion within a test session. The ordering of the discriminations sets up a hierarchy among the different objects: A > B > C > D > E, so that the object highest in position in the hierarchy is chosen on any particular individual trial. It is worth noting that each of the objects in the middle of the list (B, C, D) are each paired with reward 50% of the time, making it difficult to use the associative history of a particular object to guide choices.

Once these problems (referred to as ''premise problems'') are learned, relational probe trials are introduced. On these trials, two objects that have not been paired during training

are presented simultaneously. One such pair (AE) can be solved purely on the basis of reward histories of the individual objects; A is always reinforced during training and E is never reinforced, so no inference is required, but if objects B and D are paired, each has been paired with reward 50% of the time, so this cannot guide the monkey's choice. Understanding of the relationship between the objects in the hierarchy—that B occupies a higher position than D—would produce a choice of B more often than D on such trials.

Aged monkeys trained on this task acquire the premise problems comparably to young monkeys and demonstrate an equivalent level of inferential responding on BD trials, i.e., they choose B over D about 80% of the time (Rapp *et al*., 1996). Extension of the series of discriminations to include an additional two objects (so the objects are ordered such that A > B > C > D > E > F > G) permits testing of "symbolic distance" effects: items farther apart in the list should elicit greater degrees of inferential responding. For example, inferential responding on BF trials (separated by three objects in the list) should be greater than inferential responding on CE or DF trials (separated by one object in the list). Such effects were observed with both young and aged rhesus monkeys, and there was no age effect on the magnitude of the symbolic distance effect (Fig. 26.2A; Rapp *et al*., 1996).

However, despite the preserved choice behavior in inferential probe trials, measures of response time on these trials suggested that the aged monkeys were using an alternative strategy to make these choices. Young monkeys took longer to make responses on inferential probe trials (by about 15% on average); indeed, this effect varied with symbolic distance, with longer response times being elicited on probe trials with shorter symbolic distances. These response time phenomena are predicted by theories of performance in this task, which emphasize cognitive representations of the ordering among the objects (McGonigle and Chalmers, 1992). Aged monkeys, despite levels of choice behavior on inferential probe trials equivalent to young monkeys, did not show these response time signatures of transitive inference (Fig. 26.2B; Rapp *et al*., 1996). Such behavior would be consistent with guiding of choice behavior by reinforcement histories of the individual objects, without recourse to a cognitive representation of the ordering of the objects (von Fersen *et al*., 1991). Hence, aged monkeys may have impairments in forming a cognitive representation of the ordered relationship between these objects or in accessing this representation to guide choice behavior. Nevertheless, other behavioral mechanisms that do not involve formation of an ordered representation presumably remain intact to support accurate inferential choice behavior.

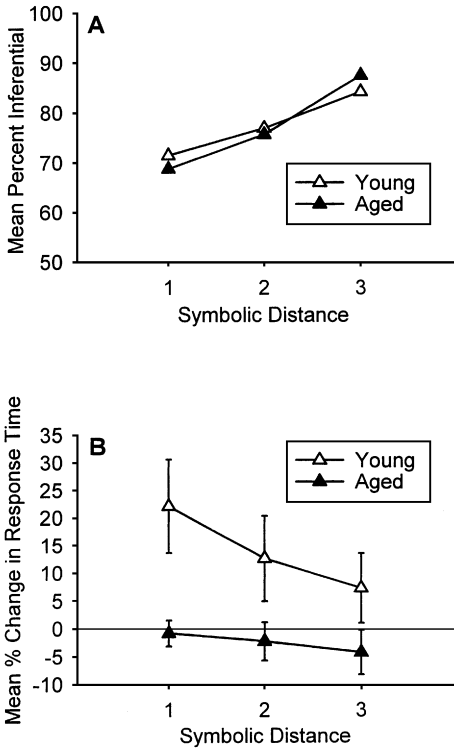

FIG. 26.2. Performance of young and aged monkeys on a relational memory task (Rapp *et al*., 1996). (A) Aged and young monkeys exhibit identical levels of inferential responding on probe trials with novel pairings of elements from the premise problems. Elements farther apart in the series (larger symbolic distance) result in higher levels of inferential responding. (B) Reaction times of young and aged monkeys on the inferential probe trials. Young monkeys exhibit a reaction time signative of inferential processing, longer reaction times on trials where the symbolic distance is lower. Aged monkeys do not display this change in reaction time, suggesting that they are solving the inferential probe trials by a nonrelational strategy. Figures reproduced from Rapp *et al*. (1996) © the American Psychological Association, by permission.

B. Neural Basis

There is currently little information available about the effects of selective brain lesions in monkeys on relational memory tasks. In particular, no lesion data exist for the transitive inference task described earlier. However, rats with damage to the perirhinal/entorhinal cortex, or the fornix, fail to demonstrate inferential choice behavior on a closely related task that used olfactory stimuli (Dusek and Eichenbaum, 1997). Notably, lesions of the orbital frontal cortex do not impair inferential choice behavior in this task (Dusek *et al*., 1997), suggesting that inferential choice behavior in this task may be a particularly sensitive probe of the integrity of the hippocampal system. Hence, the impairment of the aged monkeys is difficult to interpret: they displayed normal inferential choice behavior, but did not demonstrate the response time signatures of inferential processing, whereas rats with damage to the hippocampal system did not display correct inferential choice behavior. Lesion studies in young monkeys will be required to determine whether monkeys with hippocampal system damage show the same kind of deficit as aged monkeys, or a qualitatively different one. Monkeys with entorhinal cortex lesions are unable to make transitive inferences in a similar task (Buckmaster *et al*., 1998), modeled after one used in rats that is impaired by neurotoxic hippocampal lesions (Bunsey and Eichenbaum, 1996), providing support for the notion that the hippocampal formation in monkeys, like in rats, is involved in relational memory processing. Certainly, the sensitivity of the transitive inference task to age-related impairments suggests that this may be a productive line of investigation for testing the cognitive function of aged nonhuman primates.

VI. Attention/Executive Function

A. Effects of Aging

Tasks that examine attention and executive function have not been widely studied in aged nonhuman primates. Reversal learning is sometimes considered to be an indicator of executive function (Lai *et al*., 1995), primarily because of the extent to which it requires flexibility in behavioral performance and the ability to rapidly change responses based on reinforcement contingencies (see Section IV). However, a variety of other tasks have been devised to assess these domains in nonhuman primates, but few have been examined in aging.

The first explicit investigation of attentional processing in aged monkeys examined the performance of adult and aged monkeys on a test of spatial orienting of attention (Baxter and Voytko, 1996). In this task, the monkey must depress and hold the center panel in an array of three panels. A brief peripheral cue illuminates over one of the side panels; during this time the monkey continues to depress the center panel. One of the side panels then illuminates, and the monkey releases the center panel and depresses the illuminated side panel in order to obtain a reward. On most trials, the illuminated side panel (the ''target'') appears on the same side where the cue light appeared; these trials are referred to as ''valid'' trials. On a small fraction of the trials, the target appears on the opposite side from the cue light; these trials are referred to as ''invalid'' trials. The detection of the target is facilitated on valid trials and is delayed slightly on invalid trials, as reflected in the response time of the monkey to release the center panel in order to depress the side panel. The difference in reaction time between valid and invalid trials is an index of shifting or orienting of attention.

Aged monkeys performed identically to adult monkeys when tested in this task, showing no indication of impaired orienting of spatial attention. Furthermore, when ''neutral'' trials were included that permitted separate analysis of costs and benefits of cueing (on neutral trials, both cue lights are illuminated, so no information about the target location is provided), aged monkeys showed similar costs and benefits of cueing compared to adult monkeys (Baxter and Voytko, 1996). These observations are consistent with studies showing preserved spatial orienting of attention in normal aged humans (Greenwood *et al*., 1993), but not in aged humans with Alzheimer's disease (Parasuraman *et al*., 1992). They also suggest that impairments in spatial attention are unlikely to contribute to age-related impairments in the performance of spatial memory tasks such as delayed response (see Section III).

Three other studies have examined attentional function in aged monkeys. One tested aged bonnet macaques (*Macaca radiata*) on a divided attention task that required tracking of multiple moving visual targets on a display. Aged monkeys performed well on the divided attention task and were not impaired relative to the young monkeys in this study, although the small sample size limits the generality of these findings (O'Neill *et al*., 1999). A pair of preliminary reports examined the performance of aged monkeys on visual search and attentional set-shifting tasks (Killiany *et al*., 1998; Moore *et al*., 1998). Aged rhesus monkeys showed increased reaction times in the visual search task, but not an increased number of errors, nor an impairment in orienting of attention or vigilance. In the set-shifting task, monkeys were confronted with an array of stimuli that differed across two dimensions (color and shape). For a block of consecutive trials, one stimulus type was correct (e.g., the red stimulus); then after 10 consecutive correct trials the correct response was switched (e.g., now a response to a triangle was correct, regardless of color). Aged monkeys were characterized by an increased number of perseverative errors and a greater tendency to break set (making six to nine consecutive correct responses and then an error).

B. Neural Basis

The cued reaction time task that tests spatial orienting of attention is sensitive to damage to the basal forebrain in monkeys (Voytko *et al*., 1994) and to parietal cortex damage in humans (Posner *et al*., 1984). Basal forebrain lesions and damage to the parietal cortex increase the ''cost'' of invalid trials, an effect interpreted as an inability to shift attention from the invalidly cued location. The lack of impairment of aged monkeys in this task (Baxter and Voytko, 1996) suggests that the neural systems that underlie this form of attentional processing are functionally intact in aging, although the small sample size in that study precluded an analysis of individual differences in performance.

Little or no data are available for the effects of selective brain lesions on divided attention, visual search, or attentional set-shifting in rhesus monkeys. Compounds that enhanced cholinergic function appeared to produce some improvement in divided attention function in young and aged bonnet macaques (O'Neill *et al*., 1999), suggesting involvement of the cholinergic system in this aspect of attentional processing as well. Damage to the lateral prefrontal cortex of marmosets impairs shifting of attention to different stimulus dimensions (Dias *et al*., 1997) in a similar type of task to that used by Killiany *et al*. (1998), but rhesus monkeys with prefrontal cortex lesions have not been tested on these tasks.

VII. Integration and Conclusions about Neuropsychological Profile of Aged Nonhuman Primates

Aged monkeys generally appear to have a profile of impairments indicative of temporal lobe and frontal lobe function, evidenced by difficulties in recognition memory, spatial memory, stimulus–reward associative learning, and attentional set-shifting, with some attentional capacities intact. To the extent that individual differences in performance have been examined, most of these tasks demonstrate individual differences to some degree—advanced chronological age does not necessarily imply that a particular aged monkey will be impaired on a particular task, although the incidence of age-related impairment on some tasks appears to be relatively high.

Beyond this global assessment, conclusions about altered function in specific cortical areas remain uncertain. Particularly, the wealth of data available from standard behavioral assessments has provided relatively little information about the possible locus of neurobiological alterations that may be responsible for age-related cognitive impairment. Tests of rela-

tional memory and allocentric spatial memory may be particularly useful in detecting age-related impairments in hippocampal function, although the absence of lesion data in monkeys on these tasks limits their usefulness as neuropsychological probes. Despite impairments in a wide array of tasks sensitive to prefrontal cortex function, many of these tasks—learning of the DNMS rule, delayed response, and reversal learning—are also impaired by damage to temporal cortical structures, and the pattern of impairments is difficult to distinguish from those produced by prefrontal cortex damage. Tests of attentional set-shifting might be useful to pursue as a selective index of prefrontal cortex function, although monkeys with temporal cortex damage have not been tested on these tasks, so a potential contribution of these structures to attentional set-shifting remains to be evaluated, and performance of DNMS with a single pair of objects appears to be independent of medial temporal lobe structures. It may be particularly challenging to find behavioral tasks that are affected by rhinal cortex damage but not prefrontal damage, although investigations of object perception and identification following damage to the rhinal cortex are promising candidates (Buckley and Gaffan, 1998; Bussey *et al*., 1999).

Relatively little data are available on the extent to which impairments in different functional domains may coassort with one another. Experiments that have examined this explicitly tend to find that impairments in different domains do not associate with one another, with a few exceptions. In one study population, monkeys that were impaired on DNMS were also impaired in acquiring object discrimination problems (Rapp, 1993), suggesting disruption of a temporal-prefrontal cortical network, although no such relationship was observed in another study (Bachevalier *et al*., 1991). In neither study were these impairments related to impaired delayed response performance, suggesting that these impairments are related to damage to different neural systems. Monkeys impaired on a test of allocentric spatial memory (presumably sensitive to hippocampal function) were not impaired on DNMS (Rapp *et al*., 1997), suggesting that impaired spatial memory in aged monkeys has a neurobiological substrate distinct from that mediating DNMS performance. Of course, an alternative interpretation of this latter finding is that allocentric spatial memory is simply a more sensitive indicator of medial temporal lobe dysfunction than DNMS performance; this question could be addressed by future studies that examine the relationship of DNMS performance to spatial maze performance in a population of aged monkeys that do show DNMS impairments and examining the correlation between performance in these behavioral domains.

Other studies place more of an emphasis on global age-related impairments in cognition (Herndon *et al*., 1997). These authors employed a principal components analysis to examine the pattern of variance in performance on a number of different behavioral tasks. Using data from 30 monkeys tested on six different behavioral tasks in their principal components analysis (DNMS acquisition, DNMS 120-sec delay performance, DRST–spatial, DRST–color, spatial reversal, and object reversal), the first component extracted accounted for 48% of the overall variance and was correlated significantly with age ($r = -0.74$). Using a larger set of monkeys (53 monkeys) who had only been tested on a subset of these tasks produced a similar result, from which the authors derived a composite "cognitive performance index" that was correlated substantially with age ($r = -0.78$). These types of analyses emphasize that overall levels of cognitive performance tend to decline with age, but they neglect the potential dissociations between performance in different domains. When aged monkeys are considered as a group, they frequently will be impaired relative to young monkeys on most cognitive tasks, which will be the component of variance extracted by this type of analysis. However, the most impaired individual aged monkey on one particular task will not necessarily be the most impaired monkey on another task. It is these patterns of dissociation (suggested by experiments that have focused on individual differences in performance) that may provide greater insight into the neurobiological substrates of cognitive aging. The observation that the prevalence of impairment in different cognitive tasks varies across age groups (Herndon *et al*., 1997) provides further support for the hypothesis that aging may differentially affect different neural systems and, by extension, different cognitive abilities.

Caution also needs to be taken when changes in neurobiological variables are related to changes in behavior, when there are common main effects of age on both (Breckler, 1993; Baxter and Gallagher, 1996). There are many instances in which behavioral and neurobiological changes are both correlated with age, creating the possibility for a common age effect and no unique relationship of biological marker to behavior. In these instances, the neurobiological marker serves as a proxy for age when the common relationship with age is not considered in the analysis. As one example, consider the relationship of thickness of layer I of cortical area 46 to cognitive ability in aged monkeys (Peters *et al*., 1998). This study reports a significant relationship between cognitive impairment and thickness of layer I, as well as the density of synapses in the different divisions of layer I. However, all of these morphological variables are correlated significantly with age (r ranging from -0.78 to -0.93) as are the cognitive measures under consideration (r ranging from 0.64 to 0.74). The authors reported a statistically significant correlation of 0.60 between thickness of layer I and performance of monkeys on DNMS at a 120-sec delay. However, when the common relationship of DNMS performance and layer I thickness is partialled out, no significant correlation remains ($pr = -0.11$, $p = 0.73$). (Correlations between other morphological parameters and behavioral performance similarly lose statistical significance when the effect of age is partialled out.) Hence, there appears to be no unique relationship of layer I thickness to behavioral performance, independent of a common effect of age on both. This type of analysis has been useful in identifying neurobiological parameters that are uniquely related with behavioral decline, independently of common main effects of age, in nonhuman primates (Gorman, 1993; Wagster, 1993; Voytko *et al*., 1995) and may provide more informative results.

The complex pattern of age-related deficits in nonhuman primates will likely preclude the identification of a single neurobiological substrate, or even a relatively limited number of substrates, for cognitive impairment in aging. In particular, the behavioral impairments of aged monkeys resemble no lesion of a single cortical region. This is consistent with neurobiological investigations in both rodents and monkeys, which

have found little support for the idea that frank neuronal loss is responsible for age-related cognitive impairment (for review, see Gallagher and Rapp, 1997). Instead, these observations seem to be consistent with alterations in cortical connectivity and more subtle alterations in neural systems with aging that may impair information-processing capacity in less obvious ways (e.g., Gazzaley *et al*., 1996, 1997; O'Donnell *et al*., 1999; Rapp *et al*., 1999).

Despite the complexity of the neuropsychological profile of aged nonhuman primates, these behavioral studies have provided a strong foundation for efforts to define the particular neurobiological substrates of particular aspects of age-related cognitive decline. The development of neuropsychologically characterized behavioral tasks for further probing the functional status of different brain systems in aged monkeys, combined with a focus on individual differences and relationships between performance in different cognitive domains, promises further advances in understanding the biological factors underlying cognitive impairments in aging.

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References

Aggleton, J. P., and Mishkin, M. (1983a). Memory impairments following restricted medial thalamic lesions in monkeys. *Exp. Brain Res.* **52**, 199–209.

Aggleton, J. P., and Mishkin, M. (1983b). Visual recognition impairment following medial thalamic lesions in monkeys. *Neuropsychologia* **21**, 189–197.

Aigner, T. G., and Mishkin, M. (1986). The effects of physostigmine and scopolamine on recognition memory in primates. *Behav. Neural Biol.* **45**, 81–87.

Aigner, T. G., Mitchell, S. J., Aggleton, J. P., DeLong, M. R., Struble, R. G., Price, D. L., Wenk, G. L., Pettigrew, K. D., and Mishkin, M. (1991). Transient impairment of recognition memory following ibotenic-acid lesions of the basal forebrain in macaques. *Exp. Brain Res.* **86**, 18–26.

Alvarez, P., Zola-Morgan, S., and Squire, L. R. (1995). Damage limited to the hippocampal formation produces long-lasting memory impairment in monkeys. *J. Neurosci.* **15**, 3796–3807.

Amaral, D. G. (1993). Morphological analyses of the brains of behaviorally characterized aged nonhuman primates. *Neurobiol. Aging* **14**, 671–672.

Arnsten, A. F. T. (1993). Catecholamine mechanisms in age-related cognitive decline. *Neurobiol. Aging* **14**, 639–641.

Arnsten, A. F. T., and Goldman-Rakic, P. S. (1985a). α_2-Adrenergic mechanisms in prefrontal cortex associated with cognitive decline in aged nonhuman primates. *Science* **230**, 1273–1276.

Arnsten, A. F. T., and Goldman-Rakic, P. S. (1985b). Catecholamines and cognitive decline in aged nonhuman primates. *Ann. N.Y. Acad. Sci*. **444**, 218–234.

Arnsten, A. F. T., Cai, J. X., and Goldman-Rakic, P. S. (1988). The alpha-2 adrenergic agonist guanfacine improves memory in aged monkeys without sedative or hypotensive side effects: Evidence for alpha-2 receptor subtypes. *J. Neurosci.* **8**, 4287–4298.

Arnsten, A. F. T., Cai, J. X., Steere, J. C., and Goldman-Rakic, P. S. (1995). Dopamine D2 receptor mechanisms contribute to age-related cognitive decline: The effects of quinpirole on memory and motor performance in monkeys. *J. Neurosci.* **15**, 3429–3439.

Bachevalier, J., and Mishkin, M. (1986). Visual recognition impairment follows ventromedial but not dorsolateral prefrontal lesions in monkeys. *Behav. Brain Res.* **20**, 249–261.

Bachevalier, J., Landis, L. S., Walker, L. C., Brickson, M., Mishkin, M., Price, D. L., and Cork, L. C. (1991). Aged monkeys exhibit behavioral deficits indicative of widespread cerebral dysfunction. *Neurobiol. Aging* **12**, 99–111.

Bartus, R. T., and Johnson, H. R. (1976). Short-term memory in the rhesus monkey: Disruption from the anticholinergic scopolamine. *Pharmacol. Biochem. Behav.* **5**, 31–39.

Bartus, R. T., Fleming, D., and Johnson, H. R. (1978). Aging in the rhesus monkey: Debilitating effects on short-term memory. *J. Gerontol.* **33**, 858–871.

Bartus, R. T., Dean, R. L., III, and Fleming, D. L. (1979). Aging in the rhesus monkey: Effects on visual discrimination learning and reversal learning. *J. Gerontol.* **34**, 209–219.

Baxter, M. G., and Gallagher, M. (1996). Neurobiological substrates of behavioral decline: Models and data analytic strategies for individual differences in aging. *Neurobiol. Aging* **17**, 491–495.

Baxter, M. G., and Murray, E. A., (2000). Reinterpreting the behavioral effects of amygdala lesions in nonhuman primates. *In* "The Amygdala: A Functional Analysis" (J. P., Aggleton, ed.), pp. 545–568. Oxford University Press, Oxford, UK.

Baxter, M. G., and Voytko, M. L. (1996). Spatial orienting of attention in adult and aged rhesus monkeys. *Behav. Neurosci.* **110**, 898–904.

Baxter, M. G., Hadfield, W. S., and Murray, E. A. (1999). Rhinal cortex lesions produce mild deficits in visual discrimination learning for an auditory secondary reinforcer in rhesus monkeys. *Behav. Neurosci.* **113**, 243–252.

Beason-Held, L. L., Rosene, D. L., Killiany, R. J., and Moss, M. B. (1999). Hippocampal formation lesions produce memory impairment in the rhesus monkey. *Hippocampos* **9**, 562–574.

Breckler, S. J. (1993). Age-related behavioral and neurochemical deficits: New data analytic strategies. *Neurobiol. Aging* **14**, 695–697.

Brozoski, T. J., Brown, R. M., Rosvold, H. E., and Goldman, P. S. (1979). Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. *Science* **205**, 929–932.

Buckley, M. J., and Gaffan, D. (1997). Impairment of visual object-discrimination learning after perirhinal cortex ablation. *Behav. Neurosci.* **111**, 467–475.

Buckley, M. J., and Gaffan, D. (1998). Perirhinal cortex ablation impairs visual object identification. *J. Neurosci.* **18**, 2268–2275.

Buckmaster, C. A., Eichenbaum, H., Amaral, D. G., Suzuki, W. A., and Rapp, P. R. (1998). Entorhinal cortex lesions severely impair flexible memory expression in monkeys. *Soc. Neurosci. Abstr.* **24**, 18.

Bunsey, M., and Eichenbaum, H. (1996). Conservation of hippocampal memory function in rats and humans. *Nature (London)* **379**, 255–257.

Bussey, T. J., Saksida, L. M., and Murray, E. A. (1999). Overgeneralization in monkeys with perirhinal cortex lesions. *Soc. Neurosci. Abstr.* **25**, 789.

Corkin, S. (1984). Lasting consequences of bilateral medial temporal lobectomy: Clinical course and experimental findings in H. M. *Semin. Neurol.* **4**, 249–259.

Corkin, S., Amaral, D. G., Gonzalez, R. G., Johnson, K. A., and Hyman, B. T. (1997). H. M.'s medial temporal lobe lesion: Findings from magnetic resonance imaging. *J. Neurosci.* **17**, 3964–3979.

Correll, R. E., and Scoville, W. B. (1965). Performance on delayed match following lesions of medial temporal lobe structures. *J. Comp. Physiol. Psychol.* **60**, 360–367.

Dias, R., Robbins, T. W., and Roberts, A. C. (1997). Dissociable forms of inhibitory control within prefrontal cortex with an analog of the Wisconsin Card Sort Test: Restriction to novel situations and independence from "on-line" processing. *J. Neurosci.* **17**, 9285–9297.

Dusek, J. A. and Eichenbaum, H. (1997). The hippocampus and memory for orderly stimulus relations. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 7109–7114.

Dusek, J., Sadalge, A., Kirchhoff, B., and Eichenbaum, H. (1997). Prefrontal cortex damage delays the acquisition of overlapping odor discriminations, but does not affect the expression of transitive inferences. *Soc. Neurosci. Abstr.* **23**, 1604.

Eacott, M. J., Gaffan, D., and Murray, E. A. (1994). Preserved recognition memory for small sets, and impaired stimulus identification for large sets, following rhinal cortex ablations in monkeys. *Eur. J. Neurosci.* **6**, 1466–1478.

Eberling, J. L., Roberts, J. A., Rapp, P. R., Tuszynski, M. H., and Jagust, W. J. (1997). Cerebral glucose metabolism and memory in aged rhesus macaques. *Neurobiol. Aging* **18**, 437–443.

Gaffan, D. (1974). Recognition impaired and association intact in the memory of monkeys after transection of the fornix. *J. Comp. Physiol. Psychol.* **86**, 1100–1109.

Gaffan, D., and Murray, E. A. (1990). Amygdalar interaction with the mediodorsal nucleus of the thalamus and the ventromedial prefrontal cortex in stimulus-reward associative learning in the monkey. *J. Neurosci.* **10**, 3479–3493.

Gaffan, D., Murray, E. A., and Fabre-Thorpe, M. (1993). Interaction of the amygdala with the frontal lobe in reward memory. *Eur. J. Neurosci.* **5**, 968–975.

Gallagher, M., and Rapp, P. R. (1997). The use of animal models to study the effects of aging on cognition. *Annu. Rev. Psychol.* **48**, 339–370.

Gazzaley, A. H., Siegel, S. J., Kordower, J. H., Mufson, E. J., and Morrison, J. H. (1996). Circuit-specific alterations of *N*-methyl-D-aspartate receptor subunit 1 in the dentate gyrus of aged monkeys. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 3121–3125.

Gazzaley, A. H., Thakker, M. M., Hof, P. R., and Morrison, J. H. (1997). Preserved number of entorhinal layer II neurons in aged macaque monkeys. *Neurobiol. Aging* **18**, 549–553.

Goldman, P. S., and Rosvold, H. E. (1970). Localization of function within the dorsolateral prefrontal cortex of the rhesus monkey. *Exp. Neurol.* **27**, 291–304.

Gorman, L. K. (1993). Age-related behavioral and neurochemical deficits: The cholinergic system revisited. *Neurobiol. Aging* **14**, 699–702.

Greenwood, P. M., Parasuraman, R., and Haxby, J. V. (1993). Changes in visuospatial attention over the adult lifespan. *Neuropsychologia* **31**, 471–485.

Herndon, J. G., Moss, M. B., Rosene, D. L., and Killiany, R. J. (1997). Patterns of cognitive decline in aged rhesus monkeys. *Behav. Brain Res.* **87**, 25–34.

Jones, B., and Mishkin, M. (1972). Limbic lesions and the problem of stimulus-reinforcement associations. *Exp. Neurol.* **36**, 362–377.

Killiany, R. J., Moore, T., Rosene, D. L., and Moss, M. B. (1998). An age-related deficit of attention in the rhesus monkey. *Soc. Neurosci. Abstr.* **24**, 1764.

Killiany, R. J., Moss, M. B., Rosene, D. L., and Herndon, J. (2000). Recognition memory function in early senescent rhesus monkeys. *Psychobiology* **28**, 45–56.

Kowalska, D. M., Bachevalier, J., and Mishkin, M. (1991). The role of the inferior prefrontal convexity in performance of delayed nonmatching-to-sample. *Neuropsychologia* **29**, 583–600.

Lai, Z. C., Moss, M. B., Killiany, R. J., Rosene, D. L., and Herndon, J. G. (1995). Executive system dysfunction in the aged monkey: Spatial and object reversal learning. *Neurobiol. Aging* **16**, 947–954.

Leonard, B. W., Amaral, D. G., Squire, L. R., and Zola-Morgan, S. (1995). Transient memory impairment in monkeys with bilateral lesions of the entorhinal cortex. *J. Neurosci.* **15**, 5637–5659.

Mahut, H. (1971). Spatial and object reversal learning in monkeys with partial temporal lobe ablations. *Neuropsychologia* **9**, 409–424.

McGonigle, B., and Chalmers, M. (1992). Monkeys are rational! *Q. J. Exp. Psychol.* **45**, 189–228.

Medin, D. L. (1969). Form perception and pattern reproduction by monkeys. *J. Comp. Physiol. Psychol.* **68**, 412–419.

Medin, D. L., O'Neil, P., Smeltz, E., and Davis, R. T. (1973). Age differences in retention of concurrent discrimination problems in monkeys. *J. Gerontol.* **28**, 63–67.

Meunier, M., Bachevalier, J., Mishkin, M., and Murray, E. A. (1993). Effects on visual recognition of combined and separate ablations of the entorhinal and perirhinal cortex in rhesus monkeys. *J. Neurosci.* **13**, 5418–5432.

Meunier, M., Bachevalier, J., and Mishkin, M. (1997). Effects of orbital frontal and anterior cingulate lesions on object and spatial memory in rhesus monkeys. *Neuropsychologia* **35**, 999–1015.

Mishkin, M. (1978). Memory in monkeys severely impaired by combined but not by separate removal of amygdala and hippocampus. *Nature (London)* **273**, 297–298.

Mishkin, M., and Appenzeller, T. (1987). The anatomy of memory. *Sci. Am.* **256**, 80–89.

Mishkin, M., and Delacour, J. (1975). An analysis of short-term visual memory in the monkey. *J. Exp. Psychol.: Anim. Behav. Process.* **1**, 326–334.

Mishkin, M., and Manning, F. J. (1978). Non-spatial memory after selective prefrontal lesions in monkeys. *Brain Res.* **143**, 313–323.

Mishkin, M., and Murray, E. A. (1994). Stimulus recognition. *Curr. Opin. Neurobiol.* **4**, 200–206.

Moore, T. L., Killiany, R. J., Rosene, D. L., and Moss, M. B. (1998). Executive system dysfunction in the aged rhesus monkey using an analog of the Wisconsin card sorting test. *Soc. Neurosci. Abstr.* **24**, 1764.

Moss, M. B. (1993). The longitudinal assessment of recognition memory in aged rhesus monkeys. *Neurobiol. Aging* **14**, 635–636.

Moss, M. B., Rosene, D. L., and Peters, A. (1988). Effects of aging on visual recognition memory in the rhesus monkey. *Neurobiol. Aging* **9**, 495–502.

Moss, M. B., Killiany, R. J., Lai, Z. C., Rosene, D. L., and Herndon, J. G. (1997). Recognition memory span in rhesus monkeys of advanced age. *Neurobiol. Aging* **18**, 13–19.

Moss, M. B., Killiany, R. J., and Herndon, J. G. (1999). Age-related cognitive decline in the rhesus monkey. *In* "Cerebral Cortex: Neurodegenerative and Age-Related Changes in Structure and Function of Cerebral Cortex" (A. Peters and J. H. Morrison, eds.), Vol. 14, pp. 21–47. Kluwer Academic/Plenum Publishers, New York.

Murray, E. A. (1990). Representational memory in nonhuman primates. *In* "Neurobiology of Comparative Cognition" (R. P. Kesner and D. S. Olton, eds.), pp. 127–155. Erlbaum, Hillsdale, NJ.

Murray, E. A. (1996). What have ablation studies told us about the neural substrates of stimulus memory? *Semin. Neurosci.* **8**, 13–22.

Murray, E. A., and Mishkin, M. (1984). Severe tactual as well as visual memory deficits follow combined removal of the amygdala and hippocampus in monkeys. *J. Neurosci.* **4**, 2565–2580.

Murray, E. A., and Mishkin, M. (1986). Visual recognition in monkeys following rhinal cortical ablations combined with either amygdalectomy or hippocampectomy. *J. Neurosci.* **6**, 1991–2003.

Murray, E. A., and Mishkin, M. (1998). Object recognition and location memory in monkeys with excitotoxic lesions of the amygdala and hippocampus. *J. Neurosci.* **18**, 6568–6582.

Murray, E. A., and Wise, S. P. (1997). Role of orbitoventral prefrontal cortex in conditional motor learning. *Soc. Neurosci. Abstr.* **23**, 11.

Murray, E. A., Gaffan, E. A., and Flint, R. W., Jr. (1996). Anterior rhinal cortex and amygdala: Dissociation of their contributions to memory and food preference in rhesus monkeys. *Behav. Neurosci.* **110**, 30–42.

Murray, E. A., Baxter, M. G., and Gaffan, D. (1998). Monkeys with rhinal cortex damage or neurotoxic hippocampal lesions are impaired on spatial scene learning and object reversals. *Behav. Neurosci.* **112**, 1291–1303.

O'Donnell, K. A., Rapp, P. R., and Hof, P. R. (1999). Preservation of prefrontal cortical volume in behaviorally characterized aged macaque monkeys. *Exp. Neurol.* **160**, 300–310.

Olton, D. S. (1991). Experimental strategies to identify the neurobiological bases of memory: Lesions. *In* "Learning and Memory: A Biological

View'' (J. L. Martinez, Jr., and R. P. Kesner, eds.), 2nd ed., pp. 441–466. Academic Press, San Diego, CA.

Olton, D. S., and Samuelson, R. J. (1976). Remembrance of places passed: Spatial memory in rats. *J. Exp. Psychol.: Anim. Behav. Process.* **2**, 97–116.

Olton, D. S., Walker, J. A., and Wolf, W. A. (1982). A disconnection analysis of hippocampal function. *Brain Res.* **233**, 241–253.

O'Neill, J., Fitten, L. J., Siembieda, D. W., Crawford, K. C., Halgren, E., Fisher, A., and Refai, D. (1999). Divided attention-enhancing effects of AF102B and THA in aging monkeys. *Psychopharmacology* **143**, 123–130.

Parasuraman, R., Greenwood, P. M., Haxby, J. V., and Grady, C. L. (1992). Visuospatial attention in dementia of the Alzheimer type. *Brain* **115**, 711–733.

Parker, A., and Gaffan, D. (1998). Interaction of frontal and perirhinal cortices in visual object recognition memory in monkeys. *Eur. J. Neurosci.* **10**, 3044–3057.

Passingham, R. (1975). Delayed matching after selective prefrontal lesions in monkeys (*Macaca mulatta*). *Brain Res.* **92**, 89–102.

Peters, A., Leahu, D., Moss, M. B., and McNally, K. J. (1994). The effects of aging on area 46 of the frontal cortex of the rhesus monkey. *Cereb. Cortex* **6**, 621–635.

Peters, A., Rosene, D. L., Moss, M. B., Kemper, T. L., Abraham, C. R., Tigges, J., and Albert, M. S. (1996). Neurobiological bases of age-related cognitive decline in the rhesus monkey. *J. Neuropathol. Exp. Neurol.* **55**, 861–874.

Peters, A., Sethares, C., and Moss, M. B. (1998). The effects of aging on layer 1 in area 46 of prefrontal cortex in the rhesus monkey. *Cereb. Cortex* **8**, 671–684.

Phillips, R. R., Malamut, B. L., Bachevalier, J., and Mishkin, M. (1988). Dissociation of the effects of inferior temporal and limbic lesions on object discrimination learning with 24-h intertrial intervals. *Behav. Brain Res.* **27**, 99–107.

Pohl, W. (1973). Dissociation of spatial discrimination deficits following frontal and parietal lesions in monkeys. *J. Comp. Physiol. Psychol.* **82**, 227–239.

Posner, M. I., Walker, J. A., Friedrich, F. J., and Rafal, R. D. (1984). Effects of parietal injury on covert orienting of attention. *J. Neurosci.* **4**, 1863–1874.

Presty, S. K., Bachevalier, J., Walker, L. C., Struble, R. G., Price, D. L., Mishkin, M., and Cork, L. C. (1987). Age differences in recognition memory of the rhesus monkey (*Macaca mulatta*). *Neurobiol. Aging* **8**, 435–440.

Rapp, P. (1993). Neuropsychological analysis of learning and memory in the aged nonhuman primate. *Neurobiol. Aging* **14**, 627–629.

Rapp, P. R. (1990). Visual discrimination and reversal learning in the aged monkey (*Macaca mulatta*). *Behav. Neurosci.* **104**, 876–884.

Rapp, P. R., and Amaral, D. G. (1989). Evidence for task-dependent memory dysfunction in the aged monkey. *J. Neurosci.* **9**, 3568–3576.

Rapp, P. R., and Amaral, D. G. (1991). Recognition memory deficits in a subpopulation of aged monkeys resemble the effects of medial temporal lobe damage. *Neurobiol. Aging* **12**, 481–486.

Rapp, P. R., Kansky, M. T., and Eichenbaum, H. (1996). Learning and memory for hierarchical relationships in the monkey: Effects of aging. *Behav. Neurosci.* **110**, 887–897.

Rapp, P. R., Kansky, M. T., and Roberts, J. A. (1997). Impaired spatial information processing in aged monkeys with preserved recognition memory. *NeuroReport* **8**, 1923–1928.

Rapp, P. R., Stack, E. C., and Gallagher, M. (1999). Morphometric studies of the aged hippocampus: I. Volumetric analysis in behaviorally characterized rats. *J. Comp. Neurol.* **403**, 459–470.

Ringo, J. L. (1991). Memory decays at the same rate in macaques with and without brain lesions when expressed in d' or arcsine terms. *Behav. Brain Res.* **42**, 123–134.

Roberts, J. A., Gilardi, K. V., Lasley, B., and Rapp, P. R. (1997). Reproductive senescence predicts cognitive decline in aged female monkeys. *NeuroReport* **8**, 2047–2051.

Rushworth, M. F., Nixon, P. D., Eacott, M. J., and Passingham, R. E. (1997). Ventral prefrontal cortex is not essential for working memory. *J. Neurosci.* **17**, 4829–4838.

Sloane, J. A., Pietropaolo, M. F., Rosene, D. L., Moss, M. B., Peters, A., Kemper, T., and Abraham, C. R. (1997). Lack of correlation between plaque burden and cognition in the aged monkey. *Acta Neuropathol.* **94**, 471–478.

Stroessner-Johnson, H. M., Rapp, P. R., and Amaral, D. G. (1992). Cholinergic cell loss and hypertrophy in the medial septal nucleus of the behaviorally characterized aged rhesus monkey. *J. Neurosci.* **12**, 1936–1944.

Thornton, J. A., Malkova, L., and Murray, E. A. (1998). Rhinal cortex ablations fail to disrupt reinforcer devaluation effects in rhesus monkeys (*Macaca mulatta*). *Behav. Neurosci.* **112**, 1020–1025.

von Fersen, L., Wynne, C. D. L., Delius, J. D., and Staddon, J. E. R. (1991). Transitive inference formation in pigeons. *J. Exp. Psychol.: Anim. Behav. Process.* **17**, 334–341.

Voytko, M. L. (1993). Cognitive changes during normal aging in monkeys assessed with an automated test apparatus. *Neurobiol. Aging* **14**, 643–644.

Voytko, M. L. (1997). Functional and neurobiological similarities of aging in monkeys and humans. *Age* **20**, 29–44.

Voytko, M. L. (1999). Impairments in acquisition and reversals of two-choice discriminations by aged rhesus monkeys. *Neurobiol. Aging* **20**, 617–627.

Voytko, M. L., Olton, D. S., Richardson, R. T., Gorman, L. K., Tobin, J. R., and Price, D. L. (1994). Basal forebrain lesions in monkeys disrupt attention but not learning and memory. *J. Neurosci.* **14**, 167–186.

Voytko, M. L., Sukhov, R. R., Walker, L. C., Breckler, S. J., Price, D. L., and Koliatsos, V. E. (1995). Neuronal number and size are preserved in the nucleus basalis of aged rhesus monkeys. *Dementia* **6**, 131–141.

Wagster, M. V. (1993). Changes in cholinergic receptor subtypes in behaviorally-tested, aged monkeys. *Neurobiol. Aging* **14**, 693–694.

Wang, J., Aigner, T., and Mishkin, M. (1990). Effects of neostriatal lesions on visual habit formation in rhesus monkeys. *Soc. Neurosci. Abstr.* **16**, 617.

Zola-Morgan, S., and Squire, L. R. (1985a). Amnesia in monkeys after lesions of the mediodorsal nucleus of the thalamus. *Ann. Neurol.* **17**, 558–564.

Zola-Morgan, S., and Squire, L. R. (1985b). Medial temporal lesions in monkeys impair memory on a variety of tasks sensitive to human amnesia. *Behav. Neurosci.* **99**, 22–34.

Zola-Morgan, S., Squire, L. R., and Amaral, D. G. (1989a). Lesions of the hippocampal formation but not lesions of the fornix or the mammilary bodies produce long-lasting memory impairment in monkeys. *J. Neurosci.* **9**, 898–913.

Zola-Morgan, S., Squire, L. R., and Amaral, D. G. (1989b). Lesions of the amygdala that spare adjacent cortical regions do not impair memory or exacerbate the impairment following lesions of the hippocampal formation. *J. Neurosci.* **9**, 1922–1936.

Zola-Morgan, S., Squire, L. R., and Ramus, S. J. (1994). Severity of memory impairment in monkeys as a function of locus and extent of damage within the medial temporal lobe memory system. *Hippocampus* **4**, 483–495.

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27

Brain Aging in Strepsirhine Primates

Among primates, the small nocturnal mouse lemur appears to be a promising species for aging research. With a body size of 60 to 130 g, the mouse lemur lives up to about 13 years in captivity. This primate offers a good model system for aging chronobiology because its life span can be changed by manipulating photoperiodic cycles. Age-related behavioral alterations as well as amyloid deposits, neurofibrillary degeneration, and iron and lipofuscin deposits, the hallmarks of normal and pathological human aging, have been observed in the mouse lemur brain. Cerebral atrophy also occurred in some aged animals. Magnetic resonance imaging allowed the evaluation of the iron content-based indice of aging and the detection, *in vivo*, of iron levels in basal forebrain. Comparative studies of aging should help dissect species-specific factors contributing to aging and to the etiology of dementing illnesses in humans. © 2001 Academic Press.

I. Introduction

A small, rapidly maturing, and short-lived primate model would provide significant advantages for the study of cerebral aging. The small nocturnal primate *Microcebus murinus* (mouse lemur) appears to be a promising species for aging research. This strepsirhine primate belongs to the family Cheirogaleidae, which includes the genera *Microcebus* (mouse lemurs), *Cheirogaleus* (dwarf lemurs), *Allocebus*, and *Phaner*. Strepsirhine primates and tarsiers are usually called prosimian primates, but taxonomy distinguishes strepsirhine primates and their sister group, the haplorhine primates, which includes tarsiers, monkeys, apes, and humans.

Age-specific mortality rate data from mouse lemur (Fig. 27.1) indicate that the longevity of this small primate is far longer that what would be expected for a mammal of that body size (60 to 130 g). Despite a life expectancy of about 5 years in the wild, the mouse lemur lives up to about 15 years in captivity (Hakeem *et al*., 1996). In comparison, the body size of *Rattus norvegicus* averages 265.5 g with a maximum longevity record of 37 months in captivity (Eisenberg, 1981). This situation is similar for the other cheirogaleid primates. The life span of the fat-tailed dwarf lemur, *Cheirogaleus medius* (body weight averaging at 179.0 g), in the wild is not known, but specimens up to 19 years old have been observed in captivity (Hakeem *et al*., 1996). Cheirogaleid primates have the body size as well as the energetic and environmental constraints of small mammals, but their longevity is comparable to larger mammals. In this sense, they are both atypical primates (in terms of body size) and atypical small mammals (in terms of longevity). It should be noted that other mammals, as some bats or the naked mole rat (Sherman *et al*., 1991), also combine small body size and a long life span.

In mouse lemur, puberty occurs by 1 year (Perret, 1992), and reproduction continues in females until about 6–8 years. Because of its small size, rapid maturity, fecundity, and relatively short life expectancy compared to other primates, the mouse lemur therefore constitutes a useful model system for the study of normal and pathological cerebral aging. This chapter summarizes studies on age-related changes in mouse lemur and explores some age-related features that could be studied *in vivo* in this primate.

II. Cognitive Function during Aging in Mouse Lemurs

The study of behavioral alterations is an important step for the evaluation of mouse lemurs as models for cerebral aging. This section focuses on age-related modifications of spontaneous social and sexual behavior, anxiety-related behaviors, and memory.

A. Spontaneous Social and Sexual Behavior

Mouse lemurs are tree-dwelling animals. The general strength and motor coordination of aged animals are fairly conserved. The only activities that become restricted are those requiring an extensive strength and/or good stretching capacities, such as jumps from the ground to an elevated platform placed 50 cm above the floor (Dhenain, 1998). Studies of locomotor behavior revealed heterogeneity in old animals. Some aged animals become more lethargic, e.g., walk instead of jumping and spend more time in their nests. This reduced activity is not the result of impaired physical shape. In contrast, some aged animals develop stereotyped hyperactive locomotor behaviors (Picq,

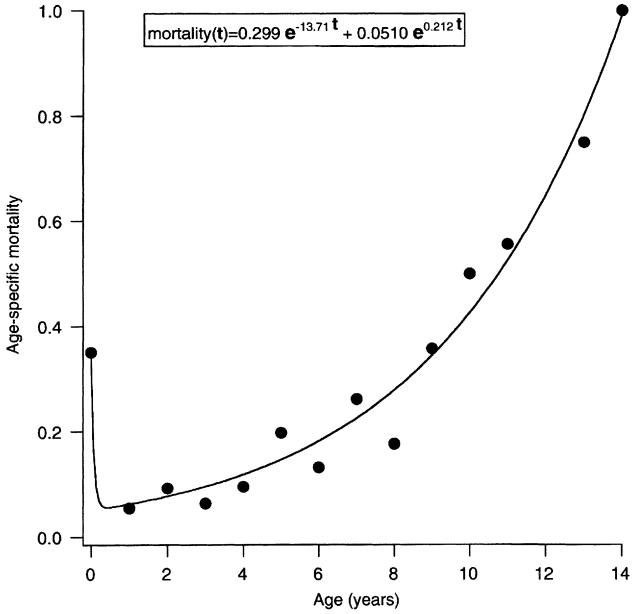

FIG. 27.1. Age-specific mortality data for *Microcebus murinus*. Data are fit by the equation shown in the box. The first term represents infant mortality and the second shows the effects of senescence. For this data set, $n = 140$. Data points represent rates for a population that had survived to a particular age. Unpublished data from Zachary Berger, John Allman, and Atiya Hakeem.

1993a; Dhenain *et al*., 1997c). Curiously, the latter animals are often the ones with the worst physical shape (Picq, 1993a).

In the wild, active animals, which are observed only during the night, are relatively solitary. They move around in well delimited but partly superposed domains. During the day, they gather to sleep in shelters (hollow trees, nests made of leaves, unstuck barks or wood nests in captivity). Mouse lemur meetings, whether they occur by night or by day, are often associated with peaceful tactile contacts called allogrooming, sound emissions, and scent marking. Aggressive behavior is seldom displayed except occasionally during estrus periods (Martin, 1972; Petter *et al*., 1977; Pages-Feuillade, 1988). Studies of social behavior from the observation of single-sex pairs of young and very old (9–12 years) captive animals showed that young animals seek positive social contacts and try to approach their partner to groom it. Older individuals may accept the contacts in a passive manner, but do not seek them. Sometimes, they even threaten or chase their partners in response to attempt of approach (Picq, 1992). This suggests that although they remain socially attractive partners, old mouse lemurs tend to withdraw from social interactions. Moreover, whether they are observed in social groups or alone, old mouse lemurs tend to increase the time spent in self-centered activities such as autogrooming (Picq, 1993a). This increase is at the expense of the time spent observing the environment. These modifications do not seem to be related to alteration of senses such as smell or sight (Picq, 1993a). The withdrawal of old animals from social interactions is similar to observations in other primates, such as macaques (Davis, 1978; Hauser and Tyrrel, 1984).

Mouse lemurs are strict seasonal breeders. During the breeding season, in the wild, males have to compete for the acquisition of large home ranges, which overlap several female home ranges. Laboratory studies suggested that the dominant status of males increases with age until the sixth to seventh breeding season. The dominant status only decreases in very old animals (10–13 breeding seasons) (Aujard and Perret, 1998). Young animals must display a high degree of aggressive behavior to reach a dominant rank and are contested routinely. Aged animals, however, do not need to display as much aggressive behavior to gain a dominant status and this status remains uncontested by other animals. Interestingly, when aged animals are paired with young animals, the aged animals must be more aggressive to obtain dominant status than when they are paired with other aged animals (Aujard and Perret, 1998). Observations of age-matched pairs of males after introduction of a preoestrous female in their cage revealed that old animals display less sexual behavior than young animals, but they are as efficient as young animals in term of reproduction. When old mouse lemurs are paired with young ones, the young animals become less active and display less sexual behavior than when they are paired with age-matched lemurs (Aujard and Perret, 1998). This might reflect the dominant status reached by old animals. This allows their priority access to estrous females and assures reproductive success.

B. Anxiety-Related Behaviors

Mouse lemurs are very sensitive to stress (Perret, 1982). Picq (1993a) described modifications of their behavior as a function of the surrounding stress. A low stress level provokes a rapid exploration of the environment, often characterized by oscillatory movements of the head and of the body. This exploration decreases rapidly in intensity and is replaced by behaviors such as resting, nutrition, or grooming. A moderate stress level brings very active exploratory behaviors. A stronger stress level produces long periods of visual explorations associated with alarm cries or aimless jumps. A very stressful situation gives rise to an absolute freezing where animals are literally petrified. The open-field test is a behavioral test, which consists of direct observation of animals when they are put in a new enclosure. It evaluates the spontaneous response to novelty and allows evaluation of anxiety levels (Halliday, 1966). Overall, there is a decrease of the sensitivity of animals to stress during aging. What slightly worries the young animal does not worry the old at all. What terrifies the young, just worries the old (Picq, 1993a; Dhenain, 1998). However, some aged animals are more stressed than young animals. The inter-individual heterogeneity is a common feature reported in many studies involving old mouse lemurs. It might reflect differences between normal and pathological aging. In a preliminary study, two out of two animals with abnormal sensitivity to stress had diffuse amyloid deposits in the brain (Dhenain, 1998) (Fig. 27.2). This suggests that the open-field test might have a predictive value to detect animals with abnormal aging.

C. Memory

For a long time, prosimian primates were not studied by specialists of animal intelligence because they were said to be timid and not cooperative. However, adaptation of experimental protocols to their sensory-motor capacities revealed very

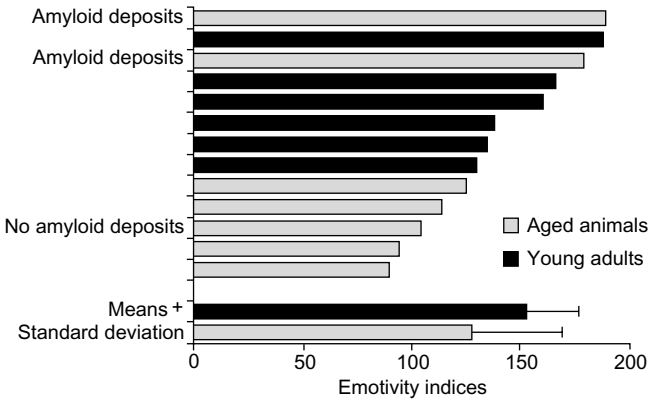

FIG. 27.2. Emotivity indices calculated for different animals. The higher the index, the higher the stress level. In this preliminary study, there was no statistical difference between young and aged animals (Mann–Whitney's $U = 11$, $p > 0.05$). The two aged animals with high stress levels had amyloid deposits in the brain. The brain of only one aged animal with a low stress level has been tested for amyloid deposits and had no amyloid.

good cognitive abilities (Ehrlich *et al*., 1976). In mouse lemurs, it is possible to assess memory in tasks based on spontaneous behaviors where animals do not have to learn any rule to perform the task. As an example, the free object exploration task (Picq and Dhenain, 1998) consists of an enclosure containing various objects placed in permanent positions. First, animals are allowed to visit the enclosure for repeated periods of time to make the environment and its objects familiar and also to suppress anxiety or fear responses. After the habituation period, the object exploration task is done during six tests of 8 min during 6 consecutive days (one test per day). During each test, new objects are added in new locations or familiar objects are displaced to new locations and replaced by new objects. Aged mouse lemurs were more active than young animals in the task (Picq and Dhenain, 1998). This feature is often characteristic of aged animals in behavioral tasks and rules out the possibility that lower performances are related to physical alterations. Aged lemurs tended to spend more time exploring the objects (new or familiar) than young animals, which suggested a higher curiosity or motivation. Young and old animals spent more time exploring new objects than visiting familiar ones. However, only young animals explored new or displaced objects in priority. They explored the new objects for a longer time than old lemurs. In contrast to old animals, young animals' interest for new objects diminished greatly on second contact with the objects (after 1 day). The behavior of young animals indicated that, like other primates (Vauclair, 1987), mouse lemurs are able to mentally ''map out'' the experimental enclosure. They are able to construct internal models of their environment, including the nature and the location of each object. The internal map can be retained for at least 24 hr and be revised after a few minutes of exploration if changes occur in the environment. The behavior of aged animals suggests that they did not detect the new or displaced objects as quickly as young animals. Moreover, the new objects were not perfectly memorized or included in the internal map during the first contact. This suggests an age-related alteration of spatial memory (Picq and Dhenain, 1998).

More subtle memory alterations can be tested by artificial tasks in which animals have to reach a goal to get a reward. In many species the usual rewards are alimentary. In mouse lemurs, most of the studies used the ''right to find refuge'' for approximately 2 min in a nest box as a positive reinforcement. The reason for this choice is that mouse lemurs are very sensitive to stress, and during a test situation, nothing is more attractive than returning to the nest. In rodents, behavioral tasks are often based on spatial paradigms [Olton's radial maze (Olton, 1979), Morris' water maze (Hsiao *et al*., 1996)]. Because of their small size, Olton's radial maze can be adapted readily to mouse lemurs (Picq, 1993b). The maze was made of a central platform from which radiated eight arms ending on a nest box. Each arm was obstructed by an opaque curtain. Four arms (''the blind arms'') were blocked by a door placed just behind the curtain and thus not visible from the entrance. Four arms (''the free arms'') with a free throughway were opened behind the curtain and might freely be explored right through the end where a transparent door blocked the access to the nest box. First, mouse lemurs were habituated to the enclosure. Thereafter, the test phase consisted of eight series of five trials (one series per day) during which the mouse lemur was placed in the central platform and then freely explored the radial maze. Animals had five trials to visit the four free arms. When they visited the fourth free arm, access to the nest box was allowed. Aged mouse lemurs did not visit the blind arms more often than the young. This suggested that their reference memory, which is the memory for data that are stable across trials in tests used in rodents (Olton and Papas, 1979), was not altered. However, during one set of five trials, old animals explored the same free arm several times, whereas young animals were able to visit a new free arm at every new trial. This suggests that old animals' memory for data, which varied from trial to trial (such as which free arms were already visited), was altered. This memory is called ''rodent type'' working memory (Olton and Papas, 1979) (working memory does not have the same meaning for human or other anthropoid primates and rodents).

The neuropsychological models used to assess memory involved in rodent and human tasks are different. This makes it difficult to compare alterations described in rodent tasks with alterations in humans. In anthropoid primates, several tasks, such as discrimination (Buerger *et al*., 1974; Zola-Morgan and Squire, 1984), delayed response (Fletcher, 1965), delayed nonmatching-to-sample (DNMS) (Mishkin and Appenzeller, 1987) or delayed alternation (DA) tasks (Mishkin and Pribram, 1955; Winocur, 1991), have been developed to study animal models of amnesia. They are conceptually very similar to tasks that can be used in humans. These tasks have also been used to study aging primates. Generally, they are based on the visual recognition of objects and on manipulation of the recognized objects. Mouse lemurs have a limited ability to handle objects; for this reason, Picq developed a spatial version of these tasks. His apparatus (Fig. 27.3) is composed of a starting box in which the animal is placed at the beginning of the test. This starting box gives access to a work chamber from which there are six corridors leading to a reinforcement chamber housing the mouse lemur's nest box. The corridors can be shut off at each end by sliding doors. They can be illuminated, and their relevance is based on whether they are lit. An opaque screen

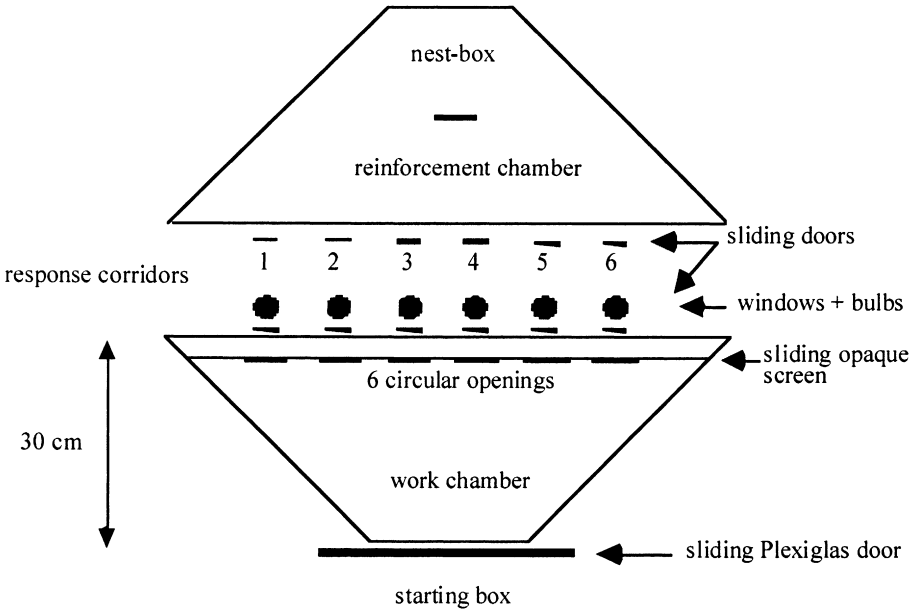

FIG. 27.3. Apparatus used in memory tasks in lemurs. The entire apparatus was made of plywood and was covered with a Plexiglas roof. It was lit by a 15 W red bulb. The mouse lemur's urge to find its nest box was the only motivation. No food reward was necessary.

can be slid between the corridors and the working chamber to impose delays between the visualization of illuminated corridors and the possibility to walk through them. A positively reinforced corridor gives access to the reinforcement chamber where the mouse lemur may rest in its nest box for 2 min. When a mouse lemur enters in a nonreinforced corridor, the exit door remains shut and the sliding entrance door is lowered behind it. The animal is immediately returned to the starting box for another trial.

Discrimination tasks have been performed with this apparatus. Mouse lemurs learned to go through corridors that were illuminated (which is against their natural instinct). This test required that animals associate the stimulus (illuminated corridor) to the response (walk through the corridor), which was associated to the reward (stay 2 min in the nest). This stimulus–response–reward test is a rule-based test (Ridley, 1991). Young and aged lemurs learned the task similarly (Picq, 1995). After 3 months without contact with the apparatus, they were both still able to perform the task (Picq, 1995). The apparatus can also be used to teach other rules to lemurs. For example, to teach a "nonmatching-to-sample" rule, one out of the six corridors was lit. The mouse lemur had to choose this corridor to access the reinforcement chamber. Five seconds later, the animal was put back in the starting box and two corridors were lit: the previous corridor and a new one. The animal had to go through the new corridor to get a reward. The task was performed similarly by young and old animals (Dhenain *et al*., 1998b). In humans, general concepts and rules about the world are stocked in semantic memory. This memory also refers to language and knowledge of facts (Tulving, 1972). In the context of animal experiments, Ridley (1991) regarded the rules as equivalent to knowledge about the "meaning of things," e.g., associations among stimuli, responses, and rewards. They may be regarded as an equivalent of semantic memory (Ridley, 1991), especially if the behavioral response to the "rule-based test" requires flexibility (Eichenbaum *et al*., 1992), as is the case for the DNMS test. In this context, rodent reference memory, which refers to data stable from one trial to another, is also comparable to semantic memory (Ridley, 1991). The performances of mouse lemurs during discrimination learning, nonmatching-to-sample learning, or reference memory tasks suggest that rule acquisition by semantic memory is not altered during aging and that this memory is stable for at least 3 months even in aged animals. In humans, semantic memory is not altered during normal aging (Nebes, 1992) but is altered during Alzheimer's disease (AD) (Nebes, 1989).

Once animals have learned the discrimination or the nonmatching-to-sample rules, delays can be added between the presentation of the stimulus (illuminated corridor) and the response (go in a corridor). Delayed response (DR) tasks have been performed with mouse lemurs by adding delays up to 60 sec between the presentation of one lit corridor out of four and access to go through the corridors. To get a reward, animals had to choose the corridor illuminated during the presentation phase (application of discrimination rule). Performances of young animals were decreasing slightly from zero to 15-sec delays and after remained stable. Old animals displayed severe time-related memory alterations. After 30 sec, they were performing at chance level (Picq, 1995). The time-related deficiency for delays inferior to 30 sec suggested a severe alteration of short-term memory in old mouse lemurs (Wright *et al*., 1985; Baddeley, 1998). Such an alteration is very similar to the short-term memory alteration described in other primates (Bartus, 1979; Rapp and Amaral, 1992) or reported by some authors in humans during normal aging (Craik, 1977) or AD (Nebes, 1989).

Delayed nonmatching-to-sample tasks have been performed with mouse lemurs by imposing 40 sec delays between the presentation and the choice phases of the nonmatching-to-sample task. Tests involving delays from 30 to 60 sec with intervening distracting events are known to assess long-term memory in animals (Ridley, 1991). The distracting event used in lemurs consisted of moving the animal from the reinforcement chamber to the work chamber. Two kinds of DNMS-related tasks exist (Ridley, 1991). If different pairs of objects are used for each trial, then the DNMS is a trial unique task because there is no interference between the current trial and former trials. However, if a very limited number of stimuli is used, the DNMS is a trial-dependent task; earlier trials provide interfering information and the task requires the memory of the event presentation (Rapp, 1993; Pertrides, 1994). The small number of corridors that mouse lemurs had to discriminate during the DNMS (six corridors) would make the task a trial-dependent task (see Dhenain *et al*., 1998b, for discussion). Good performance in the task depends on the integrity of temporomedian and prefrontal areas (Otto and Eichenbaum, 1992). The memory involved is similar to the human episodic memory, which is a memory for precise events, specific of every subject life and occurring in particular temporospatial contexts (Squire *et al*., 1993). Performances of aged mouse lemurs in the DNMS task were significantly poorer than those of young adults (Dhenain *et al*., 1998b). Interestingly, as described previously, aged mouse lemurs' memory is also impaired in the ''rodent'' working memory task of the radial maze, which is also a trial-dependent task. This suggests an alteration of the long-term episodic memory in aged mouse lemurs. This alteration is similar to episodic memory alteration described during human normal aging (Nebes, 1989) and also in AD (Baddeley *et al*., 1991).

Age-related behavioral alterations in mouse lemurs suggest that prefrontal areas are altered during aging. This is supported by the observed DNMS memory impairment and also by poor performances of aged mouse lemurs in other tasks known to involve this area, such as the DR task (Fletcher, 1965; Goldman and Rosvold, 1970). The perseverance tendency of aged animals in several tasks (Picq, 1993b) also favors this hypothesis. Alteration of the prefrontal area in mouse lemurs is similar to the one described in other primates (Rapp, 1993) and also in human during normal aging (Schacter *et al*., 1996; Petit-Taboue *et al*., 1998). The temporal lobe also seems altered during aging in mouse lemurs, as suggested by the poor performances in the DNMS and in spatial memory tasks such as the free object exploration task or the radial maze. The hippocampus in particular is the temporal region involved in spatial tasks (Olton and Papas, 1979). A medial temporal alteration has been described in a subpopulation of primates (although in a task more dependent on entorhinal cortex) (Rapp and Amaral, 1992; Rapp, 1993). Temporal alteration has been shown to be particularly severe in subjects with AD (Desgranges *et al*., 1998).

As a conclusion, mouse lemurs present behavioral alteration during aging. Actual studies described average behavioral changes in populations of aged animals. In these studies, individual heterogeneity has often been reported in aged animals. Future work must characterize the normal pattern of age-related behavioral changes so that pathological aging can be identified accurately by comparison.

III. Age-Related Cerebral Atrophy and Neuronal Alterations in Mouse Lemurs

Neuromorphological studies revealed ventricular enlargement associated to a severe cortical and hippocampal atrophy in a few aged specimens. Other regions, such as the corpus callosum, the fornix, the basal ganglia, the brain stem, and the cerebellum, are atrophied in some aged animals (Bons *et al*., 1991a,b). More recently, Dhenain *et al*. (2000) showed in a longitudinal magnetic resonance imaging (MRI) study that cerebral atrophy only occurs in aged mouse lemurs but that the aged animals are not all atrophied. Atrophy thus appears to be an age-related pathological condition and not an inevitable effect of age. The atrophy process evolves within a few months and starts between 5 and 8 years old. Several subgroups of animals can be distinguished on the base of regional atrophy. For example, some animals showed a severe atrophy of the temporal lobe, whereas others displayed diffuse cortical atrophy in addition to temporal atrophy. Further studies are necessary to determine the relationships between these different types as well as the origin of cerebral atrophy, which might be associated with the presence of neurites and neuronal degeneration (Bons *et al*., 1991b). The detection and follow-up of the atrophic process *in vivo* with MRI offer the possibility to search for the biological correlates of ongoing neurodegeneration.

IV. Amyloid Deposits, Amyloid Angiopathy, and Cytoskeletal Alterations

Senile plaques are extracellular β-amyloid deposits associated to reactive astrocytes, microglial cells, dystrophic neurites, and neurofibrillary tangles (NFT). Amyloid angiopathy is amyloid deposits surrounding blood vessels. NFT are neuronal accumulations of hyperphosphorylated tau proteins, and with senile plaques, and amyloid angiopathy, are the neuropathological hallmarks of AD (Wisniewski *et al*., 1989; Ball *et al*., 1997). They can also be present in nondemented aged subjects, although in lower density and in more restricted areas (Hyman *et al*., 1993).

A. Amyloid Deposits

The Aβ peptide sequence in mouse lemurs is homologous to the human Aβ (Silhol *et al*., 1996). In mouse lemurs older than 7 years old, histological studies (amyloid-specific silver method, histochemistry for Aβ 1–40 and APP, binding of the amyloid-indicating dyes, thioflavin and Congo red, and immunocytochemistry against Aβ) revealed the presence of Aβ protein aggregates in the cerebral cortex, the meninges, and the cerebral vasculature (amyloid angiopathy), of neuritic plaque-like deposits composed of degenerated neurites surrounding an amyloid deposit, and of neurofibrillary changes such as bundles of argyrophilic filaments in pyramidal neurons (Bons *et al*., 1991a,b, 1994, 1995b; Mestre-Francés *et al*., 1996) (Fig. 27.4). The temporal and parietal lobes are especially affected by Aβ deposition. In animals older than 7 years, Aβ protein deposits were observed in four forms, from small cloudy deposits to a dense core of Aβ surrounded by a halo

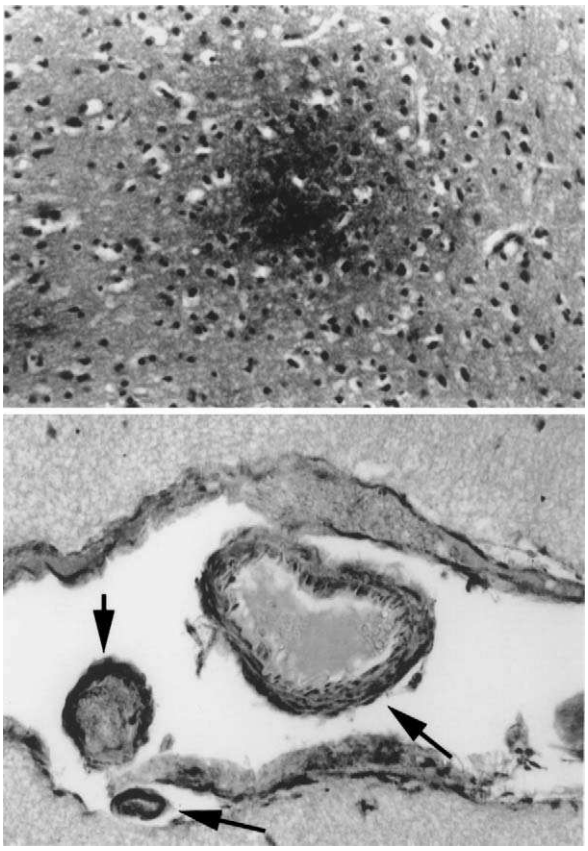

FIG. 27.4. Amyloid deposits in a 11.4 year old female mouse lemur cortex ($\times$20, top) and amyloid angiopathy in leptomeningeal vessels ($\times$20, arrows, bottom). Tissues were stained with antibody E50 against Aβ.

of amyloid fibrils (Mestre-Francés *et al*., 1996). These different forms may characterize different stages of maturation. Only a few animals over 7 years presented very numerous amyloid deposits, which seemed to represent a characterized pathology (Mestre-Francés *et al*., 1996). Diffuse amyloid deposits were also found in very young adults (1 year old).

B. Cytoskeletal Alterations

Tau is a cytoskeletal microtubule-associated protein. In humans, and NFT are neuronal accumulations of pathological hyperphosphorylated tau proteins that form the basic matrix of paired helical filaments (PHF) within degenerating neurons. The biochemical profile of tau proteins during cerebral aging has been characterized in the mouse lemur using immunoblotting and several probes directed against human tau proteins and PHF by Delacourte *et al*. (1995). The molecular weight of tau proteins increased during aging in mouse lemurs, reflecting age-related states of hyperphosphorylation (Bons *et al*., 1995b; Delacourte *et al*., 1995). The localization of abnormally phosphorylated and PHF-immunoreactive tau proteins has been studied in lemurs by immunohistochemistry (Bons *et al*., 1995a,b; Giannakopoulos *et al*., 1997). Antibodies directedagainst ''normal'' human tau proteins (Flament *et al*., 1989) revealed staining in almost all of the tested animals. Antibodies directed against both normal and abnormally phosphorylated tau proteins (961-S28T) (Delacourte *et al*., 1990) revealed tau proteins aggregated in thick granules in almost all of the tested animals (independent of their age). This particular form of tau proteins seems to be specific of lemurs and has not been described in humans (Bons *et al*., 1995b). Numerous aggregated tau proteins were always found in lemurs with numerous amyloid deposits. Lemurs without amyloid deposits either displayed numerous neurons with aggregated tau proteins or few labeled neurons (Bons *et al*., 1995a). Neocortical areas were frequently affected, even in young mouse lemurs, whereas the subiculum and entorhinal cortex were only involved occasionally in animals older than 8 years (Giannakopoulos *et al*., 1997). The fact that the hippocampus was relatively nonlabeled in lemurs, whereas it is one of the first structure to be involved by NFT formation in AD (Braak and Braak, 1991), could be explained by different characteristics of neuroprotection in nonhuman primate and human hippocampus. It could also be related to the nonspecificity of the antibodies used in the lemurs to detect abnormal tau proteins characteristic of AD. Finally, the use of antibodies directed against abnormally phosphorylated tau proteins revealed either no staining (Dhenain *et al*., 2000) or a weak immunolabeling in comparison to the one obtained in AD patients (adsorbed anti-PHF antibody; Bons *et al*., 1995a). Animals that displayed a weak immunolabeling with anti-PHF antibody labeling displayed numerous aggregated tau proteins with 961-S28T antibodies. This suggests that PHF-immunoreactive tau proteins are related to the presence of high number neurons with aggregated tau proteins. However, some animals showing numerous aggregated tau proteins with 961-S28T antibodies did not display labeling with anti-PHF antibodies.

In conclusion, alterations in tau metabolism occur during aging in lemurs and pathological tau metabolism might be related to amyloid deposits. However, the tau immunoreactivity that is displayed by plaque neurites in mouse lemurs can hardly be compared to the tau immunoreactivity seen in AD (it is weaker or absent) and PHF have never been described in lemurs. From these results, it appears that the production of highly specific immunological probes directed against specific tau protein isoforms is needed to evaluate better the tau pathology displayed by lemurs.

C. Genetic Origin of "Alzheimer-like" Lesions in Lemurs

In humans, mutations of different genes, such as the β-amyloid precursor protein (APP) gene (which codes for large glycoproteins, which can be metabolized to form the Aβ peptide) (Kang *et al*., 1987), presenillin 1 (PS1), and presenillin 2 (PS2), have been associated with familial forms of AD. These genes have been sequenced in lemurs. APP and PS1 distributions in mouse lemur brain are similar to that observed in humans and, similar to humans, are localized in the same brain structures (Calenda *et al*., 1996; Silhol *et al*., 1996). In contrast, few neurons can be marqued with combined PS2 protein and APP immunoreactivity (Calenda *et al*., 1998). The presence of genes related to AD in lemurs suggests that they are genetically susceptible to display mutations in genes involved in familial forms of AD. However, so far, neither

the presence of mutations involved in familial cases of AD nor the presence of mutational founder effects has been described in animals with amyloid deposits.

Variations in the age of onset and risk of AD are associated with the apolipoprotein E locus in humans (Strittmatter and Roses, 1996). Three isoforms (E2, E3, and E4) exist and are distinguished by cysteine-to-arginine substitutions at residues 158 and 112 (Mahley, 1988). A strong association has been found between the presence of the E4 isoform and the occurrence of AD (Schmechel *et al*., 1993; Gómez-Isla *et al*., 1996). The human apoE allele system has unique characteristics when compared to other primates (Finch and Sapolsky, 1999), and only the E4 isoform of apolipoprotein E was evidenced in the mouse lemur. ApoE genotyping revealed that nine amino acids differ between human and mouse lemur ApoE isoforms. However, the two amino acids characteristic of the human ApoE4 isoform are conserved, suggesting that the ApoE in mouse lemurs is phenotypically analogous to human ApoE4 (Calenda *et al*., 1995). This result is similar to results in macaque monkey, baboon, cow, pig, mouse, and rat, but not rabbit (Poduri *et al*., 1994).

V. Neurochemical Alterations

Immunocytochemical studies reveal that the structure of the cholinergic basal forebrain, essentially the nucleus basalis of Meynert, is very similar in both strepsirhine and haplorhine primates, including humans. Cholinergic neurons have been observed in the mouse lemur brain with choline acetyltransferase (ChAT) immunocytochemistry in the septum, the diagonal band of Broca, the nucleus accumbens, the nucleus basalis of Meynert, the caudate, the putamen, the globus pallidus, and the olfactory tubercle. In the aged mouse lemur, cytological changes and neuronal loss have been observed in these structures, suggesting an alteration of the cholinergic function during aging (Mestre and Bons, 1993). However, the ChAT activity increases during aging in lemurs (Dournaud *et al*., 1994). The apparent contradiction between immunocytochemical and biochemical studies might be related to different age ranges of the animals included in both studies. Studies of other neurotransmitter systems revealed a loss of serotonergic and catecholaminergic neurons during aging (Jallageas *et al*., 1998). Biochemical studies revealed that cortical somatostatin levels do not change with aging (Dournaud *et al*., 1994).

VI. Iron Accumulation

A. Captive Lemurs and Iron Overload

In various mammals, including human and nonhuman primates, a progressive increase of iron deposits in the brain, especially the basal ganglia, is characteristic of normal aging (see Koeppen, 1995, for a review). Strepsirhine primates are of particular interest in the study of iron absorption and deposition because hemosiderosis has been observed consistently in captive aged animals. Spelman *et al*. (1989) observed that all 49 lemurs necropsied since 1968 at the San Diego Zoo were hemosiderotic. Their high protein chow diet may have contributed to excessive iron absorption. The absorbed iron is first apparent in the macrophages of the intestinal lamina propria, in Kuppfer cells, in splenic cells, and in other nonparenchymal cells. With increasing iron overload, liver parenchymal cells are also affected, and hepatic cell necrosis, as well as periportal fibrosis, is observed. Iron overload in lemurs to some extent resembles human transfusional siderosis (Iancu and Shiloh, 1994). As in humans, the severity of the disorder is correlated to age. Indeed, iron homeostasis is disrupted during the aging process (Gelman, 1995).

Histochemistry of brain iron deposits, using Perl's staining, in old animals (8–15 years old) revealed that iron pigments are localized mainly in the globus pallidus, the substantia nigra, the neocortical and cerebellar white matter, the thalamus, and in anterior forebrain structures, including the nucleus basalis of Meynert (Fig. 27.6, see color insert) (Dhenain *et al*., 1998a; Gilissen *et al*., 1998, 1999b). This distribution agrees with previous findings in monkeys and humans, but among primates, only cheirogaleids and humans show iron deposits in the thalamus. Nonheme iron in the brain is encapsulated by ferritin, the primary tissue iron storage protein.

B. I*n* V*ivo* Detection of Iron with MRI during Brain Aging

MRI can detect *in vivo* naturally occurring brain depositions. Because of iron's paramagnetic characteristic, brain regions with a high iron content have short T2 relaxation times, yielding a hypointense (dark) signal in T2 or T2*-weighted images as compared to regions with a low iron content (Drayer, 1989). This effect is more prominent at a high magnetic field because susceptibility effects, such as iron-related T2 relaxation time decrease, are at least linearly dependent on magnetic field strength (Fisel *et al*., 1991; Lee, 1991; Bartzokis *et al*., 1993; Lee *et al*., 1995; Schenck, 1995; Vymazal *et al*., 1995; Gati *et al*., 1997). In addition, an iron-dependent contrast is enhanced with increasing field strength, whereas the other T2 effects are not changed. The effects of other processes that can affect T2 relaxation times (tissue characteristics) (Bartzokis *et al*., 1993) would therefore be proportionately less important at a high magnetic field.

Brain iron content was examined in young and aged cheirogaleid primates by Dhenain *et al*. (1997a,b, 1998a) with 4.7 T magnetic resonance (Bruker Biospec 47/30 system) and by Gilissen *et al*. (1998, 1999b) with high field magnetic resonance microscopy (Bruker AMX500 11.7 T MRI system) (Narasimhan and Jacobs, 1996). Results obtained from MR images were corroborated to histochemistry studies. The age-dependent MRI signal decrease in dwarf and mouse lemur brains appeared to be related to iron accumulation.

1. Iron Content of the Globus Pallidus: An I*n* V*ivo* Indice of Brain Aging

Dhenain *et al*. (1997a,b) observed very high correlations between the T2-weighted MRI signal decrease (T2ws) and the natural logarithm of mouse lemur age in the globus pallidus ($r = 0.95$), in the substantia nigra ($r = 0.81$), and in the thalamus ($r = 0.80$). The T2ws decreased rapidly until the age of 4 years. After this age, in middle-aged and older animals the signal decrease became less important. A rapid

T2ws decrease in young individuals, less rapid in middle-aged and slower in aged individuals, has also been observed in humans (Pujol *et al*., 1992; Schenker *et al*., 1993; Bartzokis *et al*., 1997). Further studies are necessary to assess if the kinetics of signal intensity decrease during aging is similar in mouse lemurs and humans.

The high correlation coefficient for the pallidum ($p < 0.0001$) suggests that it should be an excellent structure to study the T2 signal decrease as a marker of age. The T2 signal measure might be useful in assessing the efficiency of pharmaceutical agents aimed at reducing iron deposition in the aging process as well as standardizing the brain iron content during pharmaceutical tests.

2. Iron in the Basal Forebrain

The nucleus basalis of Meynert, the largest source of cholinergic fibers in the brain, is affected during pathological aging in humans (Samuel *et al*., 1991; Cullen *et al*., 1997 see Chapter 19). Bartzokis *et al*. (1994, 1997) suggested that future directions of *in vivo* evaluation of tissue iron with MR should focus on structures that are highly relevant for pathological aging, such as the basal forebrain. In particular, the nucleus basalis of Meynert is structurally and functionally closely associated with the ventral extension of the globus pallidus. Iron levels in the ventral globus pallidus are among the highest in the brain and are well visualized in humans with standard clinical magnets (1.5 T). Increased iron levels in the globus pallidus may be a marker of increased iron in the basal forebrain (Bartzokis, 1997). In humans, iron deposition in basal forebrain cholinergic structures is difficult to visualize *in vivo* because of their small size. High resolution and high magnetic field (11.7 T) MRI images of mouse lemurs allowed the detection of iron in the basal forebrain (Gilissen *et al*., 1999b). The areas of iron accumulation largely overlap the distribution of ChAT-immunoreactive neurons. Moreover, the MR contrast resulting from the iron content is comparable in the basal forebrain structures and in the globus pallidus (Figs. 27.5 and 27.6). This suggests that iron deposits affect basal forebrain cholinergic structures because of their close relationship with the ventral globus pallidus. Further longitudinal studies are necessary to assess how correlated are the iron depositions in the basal forebrain and the globus pallidus.

VII. Lipofuscin: Another Marker of Aging Unrelated to Iron Deposits

Accumulation of lipofuscin, an age pigment derived by lipid peroxidation, constitutes another reliable but invasive marker of aging. Iron promotes *in vivo* lipofuscin formation and is

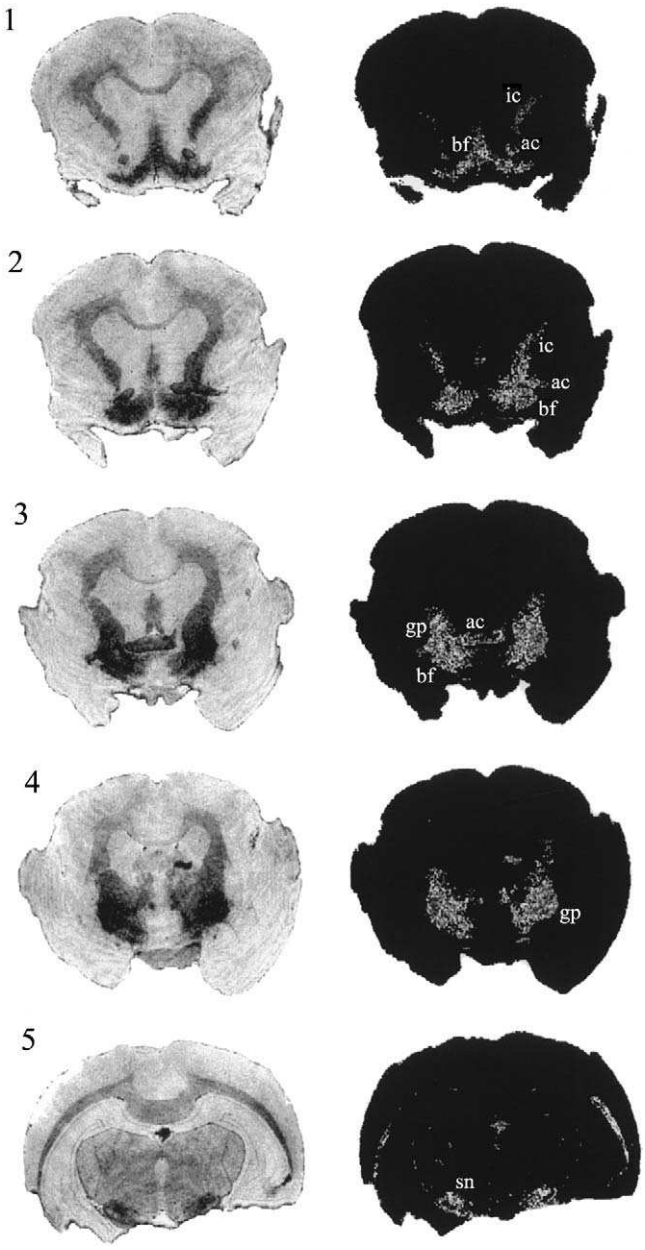

FIG. 27.5. Coronal MR scan T2*-weighted images of a 12-year-old mouse lemur brain. The hypointense (dark) signal on the left images corresponds to iron accumulation in basal forebrain structures (bf) such as the septum, the diagonal band of Broca, and the substantia innominata (1–3), in globus pallidus (gp) (3 and 4), and in substantia nigra (sn) (5). Iron is also present in the anterior commissure (ac) and internal capsule (ic). Signal intensity was measured using a gray scale ranging from 0 (black) to 255 (white). Gilissen *et al*. (1999b) assessed the maximum intensity value of the globus pallidus, substantia nigra, and basal forebrain structures. Upper thresholds were finally applied in order to set defined ranges of voxel values to be displayed. These thresholds correspond to the maximum intensity values of globus pallidus, basal forebrain structures, and substantia nigra. The lower voxel threshold was set at 0. In the right images, the voxel values beyond the upper thresholds are eliminated. In the case of the specimen illustrated here, upper thresholds were set at 35.2 (1–4) and 41.0 (5). To permit the remaining voxels to be visualized, the intensity histogram was adjusted to enhance contrast. In the right images, the resulting views display the basal forebrain structures (1–3), the globus pallidus (3 and 4), and the substantia nigra (5), as well as some adjacent structures with similar maximum intensity values. On a series of four aged animals (8–15 years old), the mean value of the upper threshold is 34.7 for the globus pallidus, 35.1 for the basal forebrain structures, and 40.8 for the substantia nigra (Gilissen *et al*., 1999b). The right images indicate that the contrast resulting from the iron content is similar in the basal forebrain structures and in the globus pallidus. (From Gilissen *et al*., *J. Neurol. Sci.*, vol. 168, 1999. Reprinted with the permission of Elsevier Publishers.)

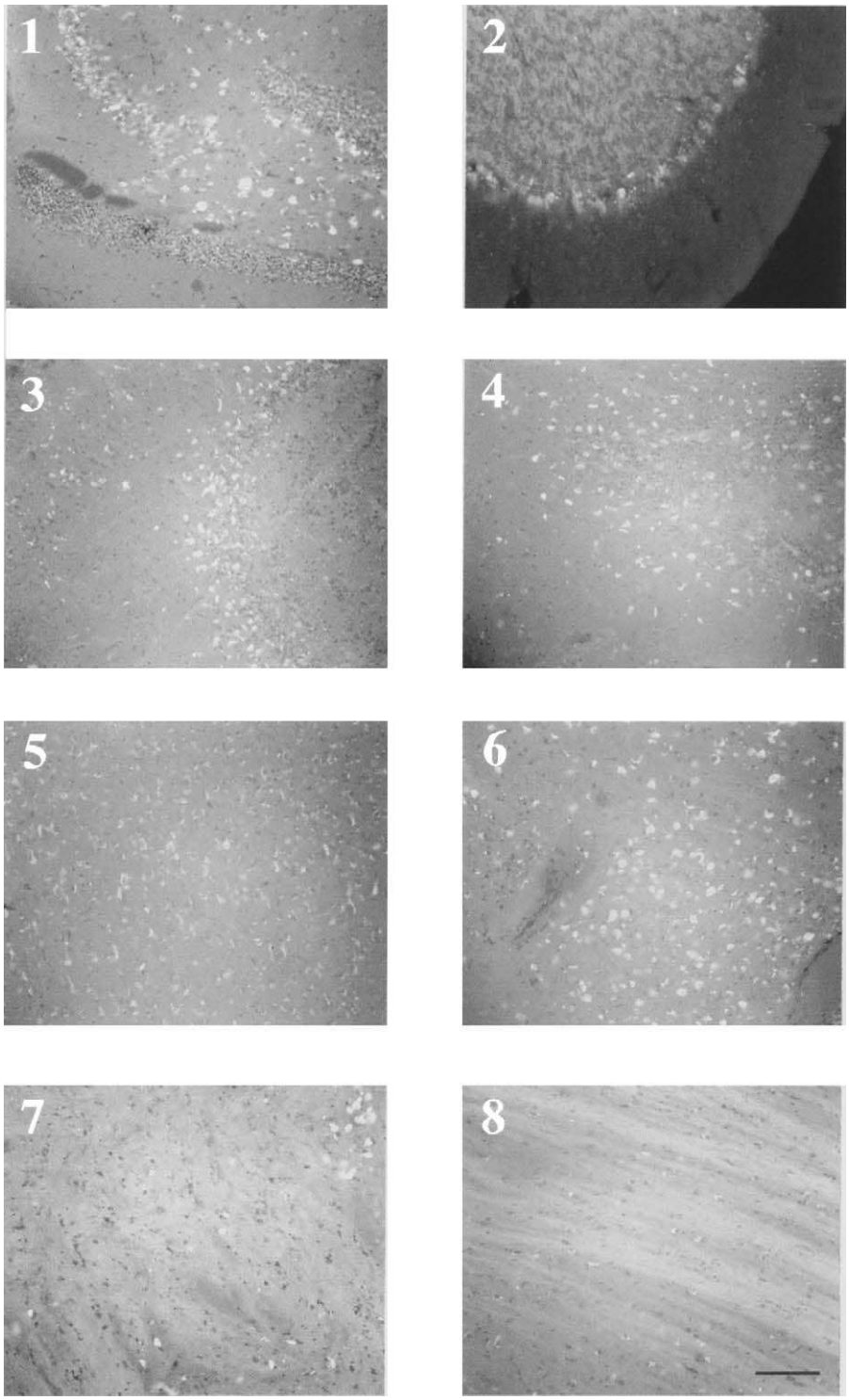

FIG. 27.7. Different fluorescence microscopy views of parasagittal cryostat sections of a 15-year-old dwarf lemur brain. The age pigments appear bright on the gray background. 1, hippocampus; 2, cerebellar folium; the age pigments are localized in the Purkinje cell layer; 3, olfactory bulb; the age pigments are mainly localized in the mitral cell layer; 4, olfactory nucleus; 5, neocortex; 6, basal forebrain; 7, globus pallidus; and 8, substantia nigra. The age pigments are less visible in the globus pallidus and the substantia nigra, where iron deposits are abundant. Scale bar: 0.1 mm. (From Gilissen *et al.*, *Am. J. Primatol.*, vol. 49, 1999. Reprinted with the permission of Wiley-Liss Publishers.)

usually detectable in high concentrations in lipofuscin granules. Nevertheless, even though iron is known to catalyze lipid oxidation (see Gilissen *et al*., 1999a, for a review), Gilissen *et al*., (1999a) observed that iron deposits and lipofuscin accumulation are not always coincident. Brain sections of aged (8–15 years old) and young (2–3 years old) dwarf and mouse lemurs were examined by autofluorescence and processed for iron histochemistry. Similar to iron, lipofuscin accumulation was observed in the aged animals but not in the young ones. Affected regions include the hippocampus (granular and pyramidal cells), where no iron accumulation was observed, the olfactory nucleus and the olfactory bulb (mitral cells), the basal forebrain, the hypothalamus, the cerebellum (Purkinje cells), the neocortex (essentially in the pyramidal cells), and the brain stem. Nevertheless, only a few scattered pigments were present in the thalamus, the globus pallidus, the substantia nigra, the striatal fundus, and the striatum, where dense iron deposits were observed (Fig. 27.7). Different biochemical and morphological cellular compartments might be involved in iron and lipofuscin deposition. Finally, the nonuniform distribution of lipofuscin indicates that brain structures are not equally sensitive to the factors causing their accumulation.

VIII. Manipulation of Aging: Changes in Photoperiodic Cycle

The mouse lemur is a good model system for the study of aging chronobiology. This primate exhibits behavioral and physiological seasonal cycles that are strictly controlled by variations in day length (photoperiodic variation). Perret (1997) revealed that, in the mouse lemur, seasonal rhythms can be accelerated by exposing captive animals to accelerated photoperiodic conditions consisting of 5 months of a long photoperiod followed by 3 months of a short photoperiod instead of 6 months of a long period followed by 6 months of a short period (as is the case in the natural environment). Long-term acceleration of seasonal rhythms obtained with accelerating photoperiodic cycles reduced the mean life span from 63.2 ± 2.5 to 45.5 ± 2.1 months and the maximal life span from 98 ± 3.9 to 79.3 ± 3.3 months. This corresponds to about a 30% reduction of life span and is neither accompanied by a desynchronization of biological rhythms nor related to an increase in reproduction or in duration of time spent in active conditions (Perret, 1997). A striking conclusion emerges from this study. When the number of seasonal cycles experienced by one individual is considered rather than chronological age, the mean life span was 5 seasonal cycles and maximum survival reached 9–10 cycles, independent of sex or of photoperiodic conditions. It is therefore possible that in mouse lemurs, and probably in other seasonal mammals, longevity may depend on the expression of a fixed number of seasonal cycles rather than on a fixed biological age (Perret, 1997).

IX. Summary and Conclusions

When compared to other primates, the life history parameters of the mouse and dwarf lemurs make them an excellent model system for the study of normal and pathological cerebral aging. Age-related behavioral alterations, as well as cerebral atrophy, have been observed in some aged animals. Amyloid deposits and neuritic degeneration associated with the presence of abnormally phosphorylated tau proteins (probably different than those observed in AD) have also been observed in the cerebral cortex of mouse lemurs. Other markers of aging, such as lipofuscin and iron deposits, have also been described in the brain of these primates. Iron distribution constitutes a reliable marker of cerebral aging. Brain regions with a high iron content have short relaxation times, yielding a hypointense (dark) signal in T2- and T2*-weighted MR images. T2 measures in the pallidum might be a useful *in vivo* marker for assessing the efficiency of pharmaceutical agents aimed at reducing iron deposition during the aging process. Because iron homeostasis is disturbed in pathological aging, the estimation of iron content in basal forebrain is of importance because of the implication of this structure in aging or AD. In humans, the basal forebrain is, however, very small when compared to the rest of the brain, and its iron content is difficult to study *in vivo*. Because of their specific brain proportions, mouse and dwarf lemurs appear to be an ideal model system for studying *in vivo* iron changes in the basal forebrain in relation to aging and neurodegeneration. Finally, this primate offers a good model system for aging chronobiology because its life span can be changed by manipulating photoperiodic cycles.

Aspects of age-related neurodegeneration differs between primate species or even between strepsirhine primates and related species (Schmechel *et al*., 1996). Different species-specific mechanisms can induce species-specific hallmarks of normal and pathological aging. If the combination of factors contributing to the onset of AD is specific to the human species, comparative studies are certainly of prime interest for dissecting the relationships between these factors and for elaborating therapeutic strategies.

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References

Aujard, F., and Perret, M. (1998). Age-related effects on reproductive function and sexual competition in the male prosimian primate, *Microcebus murinus. Physiol. Behav.* **64**, 513–519.

Baddeley, A. (1998). "Human Memory: Theory and Practice." Allyn & Bacon, New York.

Baddeley, A. D., Della Sala, S., and Spinnler, H. (1991). The two-component hypothesis of memory deficit in Alzheimer's disease. *J. Clin. Exp. Neuropsychol.* **13**, 372–380.

Ball, M., Braak, H., Coleman, P., Dickson, D., Duyckaerts, C., Gambetti, P., Hansen, L., Hyman, B., Jellinger, K., Markesbery, W., Perl, D.,

Powers, J., Price, J., Trojanowski, J. Q., Wisniewski, H., Phelps, C., and Khachaturian, Z. (1997). Consensus recommendations for the post-mortem diagnosis of Alzheimer's disease. *Neurobiol. Aging* **18**, S1–S2.

Bartus, R. T. (1979). Effects of aging on visual memory, sensory processing and discrimination learning in the non human primate. *In* "Aging" (J. M. Ordy and K. Brizzee, eds.), Vol. 10, pp. 85–114. Raven Press, New York.

Bartzokis, G. (1997). Magnetic resonance imaging of brain iron. *In* "Metals and Oxidative Damage in Neurological Disorders" (J. R. Connor, ed.), pp. 41–56. Plenum, New York.

Bartzokis, G., Aravagiri, M., Oldendorf, W. H., Mintz, J., and Marder, S. R. (1993). Field dependent transverse relaxation rate increase may be a specific measure of tissue iron stores. *Magn. Reson. Med.* **29**, 459–464.

Bartzokis, G., Sultzer, D., Mintz, J., Holt, L. E., Marx, P., Phelan, C. K., and Marder, S. R. (1994). In vivo evaluation of brain iron in Alzheimer's disease and normal subjects using MRI. *Biol. Psychiatry* **35**, 480–487.

Bartzokis, G., Beckson, M., Hance, D. B., Marx, P., Foster, J. A., and Marder, S. R. (1997). MR evaluation of age-related increase of brain iron in young adult and older normal males. *Magn. Reson. Imaging* **15**, 29–35.

Bons, N., and Mestre, N. (1993). Similitudes entre les plaques amyloïdes cérébrales d'un lémurien âgé et de l'homme atteint de maladie d'Alzheimer. *C. R. Seances Soc. Biol.* **187**, 516–525.

Bons, N., Mestre, N., and Petter, A. (1991a). Occurrence of neuritic plaques and neurofibrillary changes in the cerebral cortex of aged lemurian primate. *Paris, Life Sci.* **313**, 213–219.

Bons, N., Mestre, N., and Petter, A. (1991b). Senile plaques and neurofibrillary changes in the brain of an aged lemurian primate, *Microcebus murinus. Neurobiol. Aging* **13**, 99–105.

Bons, N., Mestre, N., Ritchie, K., Petter, A., Polisny, M., and Selkoe, D. (1994). Identification of amyloid beta protein in the brain of the small, short-lived lemurian primate *Microcebus murinus. Neurobiol. Aging* **15**, 215–220.

Bons, N., Jallageas, V., Silhol, S., Mestre-Frances, N., Petter, A., and Delacourte, A. (1995a). Immunocytochemical characterization of tau proteins during cerebral aging of the lemurian primate *Microcebus murinus. Paris, Life Sci.* **318**, 77–83.

Bons, N., Jallageas, V., Mestre-Francés, N., Silhol, S., Petter, A., and Delacourte, A. (1995b). *Microcebus murinus*, a convenient laboratory animal model for the study of Alzheimer's disease. *Alzheimer Res.* **1**, 83–87.

Braak, H., and Braak, E. (1991). Neuropathological stageing of Alzheimer related changes. *Acta Neuropathol.* **82**, 239–259.

Buerger, A. A., Gross, C. G., and Rocha-Miranda, C. E. (1974). Effects of ventral putamen lesions on discrimination learning by monkeys. *J. Comp. Physiol. Psychol.* **86**, 440–446.

Calenda, A., Jallageas, V., Silhol, S., Bellis, M., and Bons, N. (1995). Identification of a unique apolipoprotein E allele in *Microcebus murinus*; ApoE brain distribution and co-localization with beta-amyloid and tau proteins. *Neurobiol. Dis.* **2**, 169–176.

Calenda, A., Mestre-Francés, N., Czech, C., Pradier, L., Petter, A., Bons, N., and Bellis, M. (1996). Molecular cloning, sequencing, and brain expression of the presenilin 1 gene in *Microcebus murinus. Biochem. Biophys. Res. Commun.* **228**, 430–439.

Calenda, A., Mestre-Francés, N., Czech, C., Pradier, L., Petter, A., Perret, M., Bons, N., and Bellis, M. (1998). Cloning of the presenilin 2 cDNA and its distribution in brain of the primate, *Microcebus murinus:* Co-expression with beta APP and tau proteins. *Neurobiol. Dis.* **5**, 323–333.

Craik, F. I. M. (1977). Age differences in human memory. *In* "Handbook of the Psychology of Aging" (J. E. Birren and K. W. Schaie, eds.). Van Nostrand-Reinhold, New York.

Cullen, K. M., Halliday, G. M., Double, K. L., Brooks, W. S., Creasey, H., and Broe, G. A. (1997). Cell loss in the nucleus basalis is related to regional cortical atrophy in Alzheimer's disease. *Neuroscience* **78**, 641–652.

Davis, R. T. (1978). Old monkey behavior. *Exp. Gerontol.* **13**, 237–250.

Delacourte, A., Flament, S., Dibe, E. M., Hublau, P., Sablonnière, B., Hémont, B., Scherrer, V., and Defossez, A. (1990). Pathological proteins tau 64 and 69 are specifically expressed in the somatodendritic domain of the neurodegenerating cortical neurons during Alzheimer's disease. Demonstration with a panel of antibodies against tau proteins. *Acta Neuropathol.* **80**, 111–117.

Delacourte, A., Sautière, P.-E., Wattez, A., Mourton-Gilles, C., Petter, A., and Bons, N. (1995). Biochemical characterization of tau proteins during cerebral aging of the lemurian primate *Microcebus murinus. Paris, Life Sci.* **318**, 85–89.

Desgranges, B., Baron, J., de la Sayette, V., Petit-Taboue, M., Benali, K., Landeau, B., Lechevalier, B., and Eustache, F. (1998). The neural substrates of memory systems impairment in Alzheimer's disease—A PET study of resting brain glucose utilization. *Brain* **121**, 611–631.

Dhenain, M. (1998). Vieillissement cérébral de *Microcebus murinus:* Comportement, IRM, neuropathologie (Cerebral aging in *Microcebus murinus*: Behavior, MRI, neuropathology). Doctoral Thesis, University of Paris.

Dhenain, M., Volk, A., Picq, J. L., Perret, M., Boller, F., and Michot, J. L. (1997a). Age-dependence of the T2-weighted MRI signal in brain structures of a prosimian primate *(Microcebus murinus). Neurosci. Lett.* **237**, 85–88.

Dhenain, M., Michot, J. L., Volk, A., Picq, J. L., and Boller, F. (1997b). T2-weighted MRI studies of mouse lemurs: A primate model of brain aging. *Neurobiol. Aging* **18**, 517–521.

Dhenain, M., Volk, A., Michot, J.-L., Boller, F., and Picq, J.-L. (1997c). Réalisation d'un indice de vieillissement cognitif de microcèbes agés basé sur leurs comportements (Study of a cognitive aging rate for aged mouse lemurs based on their behavior). *Année Gérontol.* **11**, 218–226.

Dhenain, M., Duyckaerts, C., Michot, J. L., Volk, A., Picq, J. L., and Boller, F. (1998a). Cerebral T2-weighted signal decrease during aging in the mouse lemur primate reflects iron accumulation. *Neurobiol. Aging* **19**, 65–69.

Dhenain, M., Michot, J.-L., Volk, A., Gauthier, C.-A., Boller, F., and Picq, J.-L. (1998b). Etude préliminaire du vieillissement cognitif des microcèbes murins dans une version spatiale du test de non appariement retardé (Preliminary study of cerebral aging in mouse lemurs by a spatial version of the delayed non-matching to sample task). *Primatologie* **1**, 309–331.

Dhenain, M., Michot, J. L., Privat, N., Picq, J. L., Boller, F., Duyckaerts, C., and Volk, A. (2000). MRI description of cerebral atrophy in mouse lemurs primates *(Microcebus murinus). Neurobiol. Aging* (in press).

Dournaud, P., Gautron, J. P., Pattou, E., Bons, N., Mestre, N., Petter, A., Kordon, C., and Epelbaum, J. (1994). Age-related changes in choline acetyltransferase activity and somatostatin content in lemurian primate *Microcebus murinus* brain. *Neurobiol. Aging* **15**, 727–731.

Drayer, B. R. (1989). Basal ganglia: Significance of signal hypointensity on T2-weighted images. *Radiology* **173**, 311–312.

Ehrlich, A., Fobes, J. L., and King, J. E. (1976). Prosimian learning capacities. *J. Hum. Evol.* **5**, 599–617.

Eichenbaum, H., Otto, T., and Cohen, N. J. (1992). The hippocampus-What does it do? *Behav. Neural Biol.* **57**, 2–36.

Eisenberg, J. F. (1981). "The Mammalian Radiations. An Analysis of Trends in Evolution, Adaptation, and Behavior." University of Chicago Press, Chicago and London.

Finch, C. E., and Sapolsky, R. M. (1999). The evolution of Alzheimer disease, the reproductive schedule, and apoE isoforms. *Neurobiol. Aging* **20**, 407–428.

Fisel, C. R., Ackerman, J. L., Buxton, R. B., Garrido, L., Belliveau, J. W., Rosen, B. R., and Brady, T. J. (1991). MR contrast due to microscopically heterogeneous magnetic susceptibility: Numerical simulations and applications to cerebral physiology. *Magn. Reson. Med.* **17**, 336–347.

Flament, S., Delacourte, A., Hemon, A., and Defossez, A. (1989). Characterization of 2 pathological tau-proteins variants in Alzheimer brain cortices. *J. Neurol. Sci.* **92**, 133–141.

Fletcher, H. J. (1965). The delayed response problem. *In* "Behavior of Non-Human Primates" (A. M. Schrier, H. F. Harlow, and F. Stollnitz, eds.), pp. 129–166. Academic Press, New York.

Gati, J. S., Menon, R. S., Ugurbil, K., and Rutt, B. K. (1997). Experimental determination of the BOLD field strength dependence in vessels and tissue. *Magn. Reson. Med.* **38**, 296–302.

Gelman, B. B. (1995). Iron in CNS disease. *J. Neuropathol. Exp. Neurol.* **54**, 477–486.

Giannakopoulos, P., Silhol, S., Jallageas, V., Mallet, J., Bons, N., Bouras, C., and Delaère, P. (1997). Quantitative analysis of tau protein-immunoreactive accumulations and beta amyloid protein deposits in the cerebral cortex of the mouse lemur, *Microcebus murinus. Acta Neuropathol.* **94**, 131–139.

Gilissen, E., Ghosh, P., Jacobs, R. E., and Allman, J. M. (1998). Topographical localization of iron in brains of the aged fat-tailed dwarf lemur *(Cheirogaleus medius)* and grey lesser mouse lemur *(Microcebus murinus). Am. J. Primatol.* **45**, 291–299.

Gilissen, E., Jacobs, R. E., McGuinness, E. R., and Allman, J. M. (1999a). Topographical localization of lipofuscin pigment in the brain of the aged fat-tailed dwarf lemur *(Cheirogaleus medius)* and grey lesser mouse lemur *(Microcebus murinus):* Comparison with iron localization. *Am. J. Primatol.* **49**, 183–193.

Gilissen, E., Jacobs, R. E., and Allman, J. M. (1999b). Magnetic resonance microscopy of iron in the basal forebrain cholinergic structures of the aged mouse lemur. *J. Neurol. Sci.* **15**, 21–27.

Goldman, P. S., and Rosvold, H. E. (1970). Localization of function within the dorsolateral prefrontal cortex of the rhesus. *Exp. Neurol.* **27**, 291–304.

Gómez-Isla, T., West, H. L., Rebeck, G. W., Harr, S. D., Growdon, J. H., Locascio, J. J., Perls, T. T., Lispitz, L. A., and Hyman, B. T. (1996). Clinical and pathological correlates of apolipoprotein E epsilon 4 in Alzheimer's disease. *Ann. Neurol.* **39**, 62–70.

Hakeem, A., Sandoval, G. R., Jones, M., and Allman, J. (1996). Brain and life span in primates. *In* "Handbook of the Psychology of Aging" (J. E. Birren and K. W. Schaie, eds.), pp. 78–104. Academic Press, San Diego, CA.

Halliday, M. S. (1966). Exploration and fear in the rat. *In* "Play, Exploration and Territory in Mammals" (P. A. Jewel and C. Loizos, eds.), pp. 45–59. Acadamic Press, London.

Hauser, M. D., and Tyrrel, G. (1984). Old age and its behavioral manifestations: A study on two species of macaque. *Folia Primatol.* **43**, 24–35.

Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F., and Cole, G. (1996). Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. *Science* **274**, 99–102.

Hyman, B. T., Marzloff, K., and Arriagada, P. V. (1993). The lack of accumulation of senile plaques or amyloid burden in Alzheimer's disease suggests a dynamic balance between amyloid deposition and resolution. *J. Neuropathol. Exp. Neurol.* **52**, 594–600.

Iancu, T. C., and Shiloh, H. (1994). Morphologic observations in iron overload: An update. *In* "Progress in Iron Research" (C. Hershko, A. M. Konijn, and P. Aisen, eds.), pp. 255–265. Plenum, New York.

Jallageas, V., Privat, N., Mestre-Francés, N., and Bons, N. (1998). Age-related changes in serotonergic and catecholaminergic brain systems in the lemurian primate *Microcebus murinus. Ann. N.Y. Acad. Sci.* **839**, 628–630.

Kang, J., Lemaire, H. G., Unterbeck, A., Salbaum, J. M., Masters, C. L., Grzeschik, K. H., Multhaup, G., Beyreuther, K., and Müller-Hill, B. (1987). The precursor of Alzheimer's disease amyloid-A4 protein resembles a cell-surface receptor. *Nature (London)* **325**, 733–736.

Koeppen, A. H. (1995). The history of iron in the brain. *J. Neurol. Sci.* **134** (Suppl.), 1–9.

Lee, D. H. (1991). Mechanisms of contrast enhancement in magnetic resonance imaging. *Can. Assoc. Radiol. J.* **42**, 6–12.

Lee, J.-H., Garwood, M., Menon, R., Adriany, G., Andersen, P., Truwit, C. L., and Ugurbil, K. (1995). High contrast and fast three-dimensional magnetic resonance imaging at high fields. *Magn. Reson. Med.* **34**, 308–312.

Mahley, R. W. (1988). Apolipoprotein E: Cholesterol transport protein with expanding role in cell biology. *Science* **240**, 622–630.

Martin, R. (1972). A preliminary field study of the Lesser Mouse Lemur *(Microcebus murinus,* Miller 1777). *Z. Tierpsychol.* **9** (Suppl.), 42–90.

Mestre, N., and Bons, N. (1993). Age-related cytological changes and neuronal loss in basal forebrain cholinergic neurons in *Microcebus murinus* (lemurian, primate). *Neurodegeneration* **2**, 25–32.

Mestre-Francés, N., Silhol, S., and Bons, N. (1996). Evolution of β-amyloid deposits in the cerebral cortex of *Microcebus murinus* lemurian primate. *Alzheimer Res.* **2**, 19–28.

Mishkin, M., and Appenzeller, T. (1987) . The anatomy of memory. *Sci. Am.* **256**, 62–71.

Mishkin, M., and Pribram, K. H. (1955). Analysis of the effects of frontal lesions in monkey. I. Variations of delayed alternation. *J. Comp. Physiol. Psychol.* **48**, 492–495.

Narasimhan, P. T., and Jacobs, R. E. (1996). Neuroanatomical micro-magnetic resonance imaging. *In* "Brain Mapping: The Methods" (A. W. Toga and J. C. Mazziotta, eds.), pp. 147–167. Academic Press, San Diego, CA.

Nebes, R. D. (1989). Semantic memory in Alzheimer's disease. *Psychol. Bull.* **106**, 377–394.

Nebes, R. D. (1992). Cognitive dysfunctions in Alzheimer's disease. *In* "The Handbook of Aging and Cognition" (F. I. M. Craik and T. A. Salthouse, eds.), pp. 373–446. Erlbaum, Hillsdale,.

Olton, D. S. (1979). Mazes, maps, and memory. *Am. Psychol.* **34**, 583–596.

Olton, D. S., and Papas, B. C. (1979). Spatial memory and hippocampal function. *Neuropsychologia* **17**, 669–682.

Otto, T., and Eichenbaum, H. (1992). Complementary roles of the orbital prefrontal cortex and the perirhinal-entorhinal cortices in an odor-guided delayed-non-matching-to-sample task. *Behav. Neurosci.* **106**, 762–775.

Pages-Feuillade, E. (1988). Modalités de l'occupation de l'espace et relations interindividuelles chez un prosimien nocturne malgache, *(Microcebus murinus)* (Spatial distribution and interindividual relationships in a nocturnal Malagasy lemur *Microcebus murinus). Folia Primatol.* **50**, 204–220.

Perret, M. (1982). Stress-effects in *Microcebus murinus. Folia Primatol.* **39**, 63–114.

Perret, M. (1992). Environmental and social determinants of sexual function in the male lesser mouse lemur *(Microcebus murinus). Folia Primatol.* **50**, 1–25.

Perret, M. (1997). Change in photoperiodic cycle affects life span in a prosimian primate *(Microcebus murinus). J. Biol. Rhythms* **12**, 136–145.

Petit-Taboue, M., Landeau, B., Desson, J., Desgranges, B., and Baron, J. (1998). Effects of healthy aging on the regional cerebral metabolic rate of glucose assessed with statistical parametric mapping. *NeuroImage* **7**, 176–184.

Petrides, M. (1994). Frontal lobes and working memory: Evidence from investigations of the effects of cortical excisions in non human primates. *In* "Handbook of Neuropsychology" (F. Boller and J. Grafman, eds.), Vol. 9, pp. 59–82. Elsevier, Amsterdam.

Petter, J. J., Albignac, R., and Rumpler, Y. (1977). "Mammifères Lémuriens (Primates, Prosimiens). Faune de Madagascar [Lemurian mammals (prosimian, primates). Madagascar's wildlife]." ORSTOM-CNRS, Paris.

Picq, J.-L. (1992). Aging and social behaviour in captivity in *Microcebus murinus. Folia Primatol.* **59**, 217–220.

Picq, J.-L. (1993a). Etude des aspects comportementaux du vieillissement chez un prosimien, *Microcebus murinus* (Study of age-related behavioral features in the prosimian. *Microcebus murinus*). Doctoral Thesis, University of Paris.

Picq, J.-L. (1993b). Radial maze performance in young and aged grey mouse lemurs *(Microcebus murinus). Primates* **24**, 223–226.

Picq, J.-L. (1995). Effects of aging upon recent memory in *Microcebus murinus. Aging Clin. Exp. Res.* **7**, 17–22.

Picq, J.-L., and Dhenain, M. (1998). Reaction to new objects in young and aged grey mouse lemurs *(Microcebus murinus). J. Exp. Psychol., Sec. B* **51**, 337–348.

Poduri, A., Gearing, M., Rebeck, G. W., Mirra, S. S., Tigges, J., and Hyman, B. T. (1994). Apolipoprotein E4 and beta amyloid in senile plaques and cerebral blood vessels of aged rhesus monkeys. *Am. J. Pathol.* **144**, 1183–1187.

Pujol, J., Junqué, C., Vandrell, P. M., Grau, J., Marti-Vilalta, J. L., Olivé, C., and Gili, J. (1992). Biological significance of iron-related magnetic resonance imaging changes in the brain. *Arch. Neurol. (Chicago)* **49**, 711–717.

Rapp, P. (1993). Neuropsychological analysis of learning and memory in the aged nonhuman primate. *Neurobiol. Aging* **14**, 627–629.

Rapp, P. R., and Amaral, D. G. (1992). Individual differences in cognitive and neurobiological consequences of normal aging. *Trends Neurosci.* **15**, 340–345.

Ridley, R. M. (1991). A critical evaluation of monkeys models of amnesia and dementia. *Brain Res. Rev.* **16**, 15–37.

Samuel, W. A., Henderson, V. W., and Miller, C. A. (1991). Severity of dementia in Alzheimer disease and neurofibrillary tangles in multiple brain regions. *Alzheimer Dis. Assoc. Disord.* **5**, 1–11.

Schacter, D., Savage, C., Alpert, N., Rauch, S., and Albert, M. (1996). The role of hippocampus and frontal cortex in age-related memory changes: A PET study. *NeuroReport* **7**, 1165–1169.

Schenck, J. F. (1995). Imaging of brain iron by magnetic resonance: T2 relaxation at different field strengths. *J. Neurol. Sci.* **134** (Suppl.), 10–18.

Schenker, C., Meier, D., Wichmann, W., Boesiger, P., and Valavanis, A. (1993). Age distribution and iron dependency of the T2 relaxation time in the globus pallidus and putamen. *Neuroradiology* **35**, 119–124.

Schmechel, D. E., Saunders, A. M., Strittmatter, B. J., Crain, B. J., Hulette, C. M., Joo, S. H., Pericak-Vance, M. A., Goldgaber, D., and Roses, A. D. (1993). Increased amyloid beta peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late onset Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 9649–9653.

Schmechel, D. E., Burkhart, D. S., Ange, R., and Izard, M. K. (1996). Cholinergic axonal dystrophy and mitochondrial pathology in prosimian primates. *Exp. Neurol.* **142**, 111–127.

Sherman, P. W., Jarvis, J. U., and Alexander, R. D. (1991). ''The Biology of the Naked Mole-Rat.'' Princeton University Press, Princeton, NJ.

Silhol, S., Calenda, A., Jallageas, V., Mestre-Frances, N., Bellis, M., and Bons, N. (1996). Beta-amyloid protein precursor in *Microcebus murinus:* Genotyping and brain localization. *Neurobiol. Dis.* **3**, 169–182.

Spelman, L. H., Osborn, K. G., and Anderson, M. P. (1989). Pathogenesis of hemosiderosis in lemurs: Role of dietary iron, tannin, and ascorbic acid. *Zoo Biol.* **8**, 239–251.

Squire, L. R., Knowlton, B., and Musen, G. (1993). The structure and organization of memory. *Annu. Rev. Psychol.* **44**, 453–495.

Strittmatter, W. J., and Roses, A. D. (1996). Apolipoprotein E and Alzheimer's disease. *Annu. Rev. Neurosci.* **19**, 53–77.

Tulving, E. (1972). Episodic and semantic memory. *In* ''Organization of Memory'' (E. Tulving and W. Donaldson, eds.), pp. 381–403. Academic Press, New York.

Vauclair, J. (1987). A comparative approach to cognitive mapping. *In* ''Cognitive Processes and Spatial Orientation in Animal and Man'' (P. Ellen and C. Thinus-Blanc, eds.), Experimental Psychology and Ethology, Vol. 40, pp. 86–89. Martinus Nijhoff, Dordrecht, The Netherlands.

Vymazal, J., Brooks, R. A., Patronas, N., Hajek, M., Bulte, J. W. M., and Di Chiro, G. (1995). Magnetic resonance imaging of brain iron in health and disease. *J. Neurol. Sci.* **134** (Suppl.), 19–26.

Winocur, G. (1991). Functional dissociation of the hippocampus and prefrontal cortex in learning and memory. *Psychobiology* **19**, 11–20.

Wisniewski, H. M., Wegiel, J., Wang, K. C., Kujawa, M., and Lach, B. (1989). Ultrastructural studies of the cells forming amyloid fibers in classical plaques. *Can. J. Neurol. Sci.* **16**, 535–542.

Wright, A. A., Santiago, H. C., Sands, S. F., Kendrick, D. F., and Cook, R. G. (1985). Memory processing of serial lists by pigeons, monkeys, people. *Science* **229**, 287–289.

Zola-Morgan, S., and Squire, L. R. (1984). Preserved learning in monkeys with medial temporal lesions: Sparing of motor and cognitive skills. *J. Neurosci.* **4**, 1072–1085.

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Patrick R. Hof and Huiling Duan

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Age-Related Morphologic Alterations in the Brain of Old World and New World Anthropoid Monkeys

Anthropoid monkeys are subdivided into two large groups, the New World platyrrhine monkeys (callithricids and cebids) and the Old World catarrhine monkeys (macaques, baboons, guenons, and leaf-eating monkeys). Most taxa are poorly known from a neurobiological point of view, but many species are used for laboratory studies (in particular, some macaques and baboons [*Macaca* and *Papio*], the Patas monkey [*Erythrocebus*], and the Central and South American marmoset [*Callithrix*], owl [*Aotus*], squirrel [*Saimiri*], and capuchin [*Cebus*] monkeys). Aging is particularly well documented among these taxa from the long-tailed and rhesus macaques and from the squirrel monkey. The neurobiological basis of declining cortical function in primate aging remains to be defined. One possibility is that the structural integrity of the neocortex is compromised by frank neuronal degeneration, synaptic loss, or other morphologic alterations. The consensus emerging from recent studies, however, is that many cortical areas, including subdivisions of the hippocampal, prefrontal, motor, and sensory cortices known to participate critically in sensory integration and memory-related processes, are relatively resistant to cell death during normal aging in monkeys. In contrast, subcortical structures are more consistently affected in a manner that correlates with the severity of cognitive deficits. Importantly, recent ultrastructural and cellular analyses have demonstrated that subtle alterations involving the neuropil as well as restricted domains of the dendritic trees are likely to contribute massively, together with molecular changes in specific neurotransmitter receptor proteins, to the cognitive and memory deficits observed in aged anthropoid monkeys.

I. Introduction

Primates exhibit a considerable number of pathological alterations in the brain as well as a decline in certain cognitive functions during aging. Many anthropoid primate species including taxa commonly used in laboratory research, such as squirrel monkeys (*Saimiri*), macaque monkeys (*Macaca*), and baboons (*Papio*) have a potential life span in captivity of over 25 years, with some individuals surviving beyond 30 years, and a maximal longevity for macaques of about 35 years (Tigges *et al*., 1988). At such an advanced age, these animals present all of the characteristic senescent changes that are typically observed in aging human populations. In New World and Old World monkeys, these changes include amyloid deposition in several brain structures and impairment of delayed nonmatching-to-sample task, delayed recognition span task, and spatial and object reversal learning (Bartus *et al*., 1978; Moss *et al*., 1988, 1997; Rapp and Amaral, 1989, 1992; Heilbronner and Kemper, 1990; Walker, 1991, 1997; Baxter and Voytko, 1996; Peters *et al*., 1996; Rapp *et al*., 1996a,b, 1997; Herndon *et al*., 1997; Sloane *et al*., 1997; Voytko, 1998; see also Chapter 26). Monkeys offer a remarkable opportunity to collect a wide range of data on the process of primate senescence (Rapp *et al*., 1996b; Peters *et al*., 1996, 1998b; Lacreuse *et al*., 1998; Voytko, 1998), because they are the only practical laboratory animal model to be so closely related to humans. In addition, macaques have a large repertoire of behaviors that can be extensively tested using neuropsychological testing batteries derived from tasks developed originally for humans (see Chapter 26). Most importantly, monkeys (as well as great apes; see Chapter 29), do not present during aging with brain pathologies, such as Alzheimer's disease, which occur only in human, and therefore provide a model in which the effects of aging can be studied independently from confounding concomitant aging-related dementing illnesses (Lacreuse *et al*., 1998). Whereas age-related morphological alterations have been well described, the neural substrate of such changes is still poorly understood, and a wide range in behavioral deficits

indicates that a widespread cortical and subcortical dysfunction is likely to occur in aged monkeys (Bachevalier *et al*., 1991; Lai *et al*., 1995). In addition, other factors, such as hormonal status and reproductive senescence, dominance history and social rank, and degree of parenting are likely to influence the severity of cognitive decline and longevity potential in anthropoids (Roberts *et al*., 1997; Veenema *et al*., 1997; Allman *et al*., 1998).

Neurochemical investigations of the brain composition in aged monkeys have shown changes in the levels of certain metabolites such as *myo*-inositol and creatine in old animals, but failed to reveal clear relationships between these alterations and the cognitive status of the animals (Herndon *et al*., 1998a). Increased activation of microglial cells has been shown in the brain of old macaque monkeys using antibodies against HLA-DR, inducible nitric oxide synthase, and the indicator of protein nitration 3-nitrotyrosine (Sloane *et al*., 1999). This study demonstrated the occurrence of widespread microglial activation in the subcortical white matter as well as increased levels of protein nitration. Also, the density of activated microglial cells is correlated with cognitive impairment in old animals, suggesting a role for microglia in the pathologic events associated to normal brain aging (Sloane *et al*., 1999). Concomitantly, the subcortical white matter displays astrocytic hypertrophy and increased content of glial fibrillary acidic protein, indicating the presence of altered protein degradation and turnover in old macaque monkeys (Sloane *et al*., 2000). Magnetic resonance imaging demonstrated that the total volume of brain parenchyma decreases linearly with age at a rate of approximately 0.3% per year and affects mostly the gray matter (Andersen *et al*., 1999). Positron emission tomography studies of regional cerebral metabolic rates for glucose revealed that old macaque monkeys had lower metabolic rates in all cortical regions examined and particularly in the temporal neocortex (Eberling *et al*., 1995). Similarly, age-related changes in delayed response performance are correlated with lower metabolic rates in the hippocampus, and age is a predictor of the metabolic rate in the orbitofrontal cortex, linking deficits in delayed response task in aged monkeys to alterations of limbic and neocortical systems involved in learning and memory (Eberling *et al*., 1997).

However, most anatomopathologic studies of brains of aged monkeys point to the fact that few, if any, changes can be observed in total neuron numbers in the cerebral cortex and in subcortical structures, although a breakdown of myelin integrity and thinning of layer I in the cerebral cortex have been reported (Vincent *et al*., 1989; Tigges *et al*., 1990, 1995, 1996; Peters *et al*., 1994, 1996, 1997, 1998a,b; Peters, 1996, 1999; Gazzaley *et al*., 1997; Kim *et al*., 1997; O'Donnell *et al*., 1999; Hof *et al*., 2000). Moreover, brain weight does not decrease in aged macaque monkeys, unlike the situation in chimpanzee and humans (Herndon *et al*., 1998b, 1999). In this chapter we review anatomic and pathologic alterations that occur during aging in the brain of old monkeys, including amyloid deposition, possible occurrence of Alzheimer's disease-type neurofibrillary degeneration, variability in estimates of volumetric and region- and cell type-specific changes in neuronal numbers and morphology, ultrastructural changes, and subcellular pathology affecting neurotransmitter systems and their receptors in the context of identified neuronal circuits.

II. Age-Associated Deposition of Amyloid in the Monkey Brain

Amyloid deposition in the form of senile plaques and diffuse deposits is thought to play a pivotal role in the development of Alzheimer's disease and occurs consistently in the brain of elderly humans, even in cases displaying only very mild cognitive impairment or presenting with a normal cognitive status (Hof *et al*., 1999; see Chapter 7). The amyloid protein and its precursor are highly conserved in vertebrates (Selkoe *et al*., 1987; Podlisny *et al*., 1991). Amyloid deposits have been shown to occur in the brain of old salmonids and in birds (Nakayama *et al*., 1999; Maldonado *et al*., 2000), and their deposition has been studied in many mammalian species as well as in a variety of transgenic mouse models (see Chapters 25 and 30; Walker, 1991, 1997). In monkeys the amyloid precursor protein occurs in similar cellular localization as in human, as well as in large diffuse deposits and in neurites surrounding senile plaques in aged animals (Martin *et al*., 1991). Furthermore, immunoblots of macaque monkey cerebral cortex using antibodies against various regions of the amyloid precursor protein revealed a high similarity with human, and the predicted sequence of the 695-residue peptide is entirely congruent with that of human, whereas rare substitutions were found in peptides including the Kunitz protease domain (Podlisny *et al*., 1991). The frequent occurrence of amyloid protein in the brain of aged nonhuman primates (including prosimians, monkeys, and great apes; Bons *et al*., 1991, 1994; Walker, 1991, 1997; Gearing *et al*., 1994, 1996, 1997; Silhol *et al*., 1996; Giannakopoulos *et al*., 1997; Mestre-Francés *et al*., 2000; see Chapters 27 and 29) makes them a reliable model of β-amyloidosis in Alzheimer's disease (Podlisny *et al*., 1991) and permits to assess the potential toxicity of the β-amyloid protein (Aβ) in a nonhuman primate (Geula *et al*., 1998; McKee *et al*., 1998). In fact, a recent study has shown that following injections of the Aβ1-40 peptide into the frontal cortex of long-tailed macaques (*Macaca fascicularis*) and rhesus macaques (*Macaca mulatta*), dose-dependent lesions can be elicited, which in old animals include the presence of tau- and ubiquitin-immunoreactive neuronal perikarya as well as thioflavine S fluorescent materials in the vicinity of the Aβ depositions (McKee *et al*., 1998). Furthermore, injections of nonsoluble fibrillar Aβ at concentrations equivalent to those found in senile plaques in several neocortical regions in young and old monkeys have demonstrated an age-dependence of amyloid toxicity (Geula *et al*., 1998). In old animals, injections resulted in severe neuronal loss, gliosis, and formation of hyperphosphorylated tau-containing lesions, whereas no toxicity was observed in young animals. These effects were not observed in rats, and were present to a less severe degree in aged New World marmoset monkeys (*Callithrix*) than in the macaques, demonstrating that toxicity to Aβ may be species-specific (Geula *et al*., 1998).

Electron microscopic analyses of senile plaques in old macaque monkeys have shown that they are nearly identical to those seen in Alzheimer's disease, with a central dense amyloid core (Fig. 28.1), differing only by the absence in monkeys of twisted tubules (Wisniewski *et al*., 1973). In macaques, senile plaques are found predominantly in the prefrontal and primary somatosensory cortices, whereas lower densities of

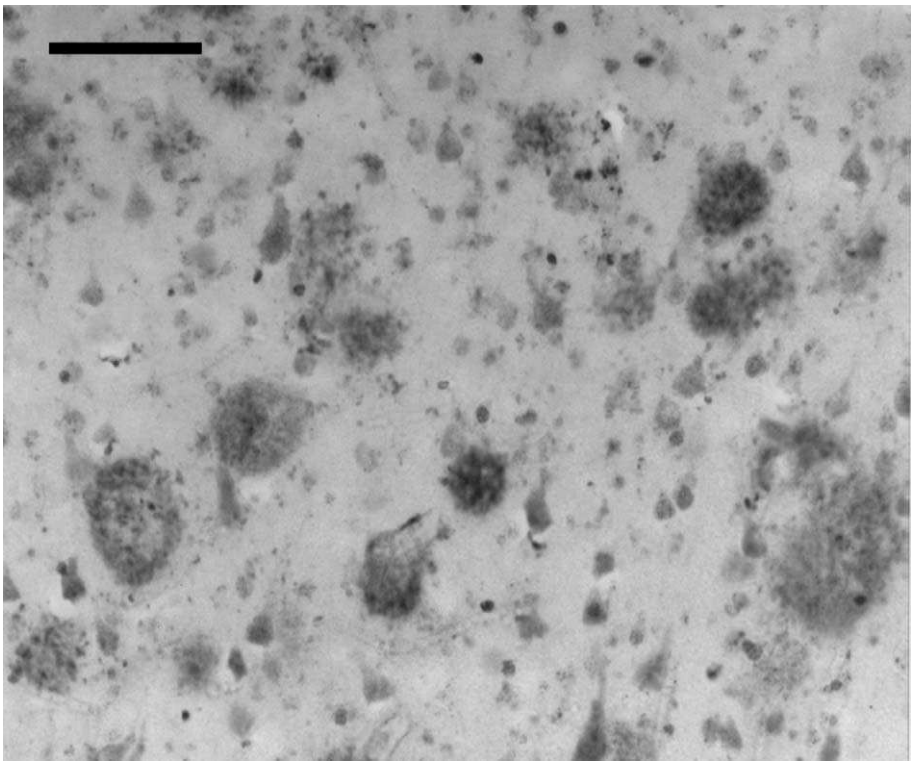

FIG. 28.1. Example of amyloid deposits in the temporal neocortex of a very old rhesus macaque (32 years old). Note the presence of classic senile plaques with well visible amyloid cores, as well as smaller deposits. Materials were stained with an antibody against Aβ1-40. Scale bar, 100 µm.

deposits have been reported in the hippocampus, the insula, and the cingulate, motor, auditory, visual, temporal and parietal cortices, and amygdala (Heilbronner and Kemper, 1990). This distribution differs considerably from that observed in Alzheimer's disease patients in whom these lesions predominate in the temporal limbic and parietal regions (Arnold *et al*., 1991). In the context of cytoarchitecture, it is interesting to note that the distribution of plaques in macaque monkeys does not appear to outline well-defined elements of the cortical circuitry as has been proposed in humans (Pearson *et al*., 1985; Rogers and Morrison, 1985; Hof *et al*., 1999) and that their densities are not correlated to cognitive deficits in old animals (Sloane *et al*., 1997). In fact, a consistent burden of amyloid is present only in some animals older than 25 years, but that number of individuals showing senile plaques increases with age (Sloane *et al*., 1997). It is important to note that in humans no clear correlation exist between the degree of amyloid deposition and the severity of cognitive deficits (Arriagada *et al*., 1992; Hof *et al*., 1999).

Interestingly, in the brain of the squirrel monkey (*Saimiri sciureus*), another species of New World anthropoid, the predominant type of amyloid-containing lesion is a widespread cortical angiopathy. In this species, vascular amyloid has been observed throughout the neocortex, the amygdala and the septal nuclei and is notably sparse or absent from subcortical structures. Moreover, the density of affected vessels is also correlated to the amyloid burden of the surrounding parenchyma (Walker *et al*., 1990; Walker, 1991). This species difference indicates that whereas the macaque represents an interesting model of cerebral amyloidosis, the squirrel monkey may be a more suitable model of vascular amyloid angiopathy. It has also been shown that the vascular Aβ deposits in macaques are stained strongly by antibodies differentiating the carboxy-terminal of the peptide. Thus, the vascular lesions contain preferentially Aβ42(43), whereas both Aβ1-40 and Aβ42(43) are observed in senile plaques, unlike the situation in humans. Furthermore, it appears that in monkeys the ratio of Aβ1-40 to Aβ42(43) is higher than in humans (Nakamura *et al*., 1995; Kanemaru *et al*., 1996), indicating that certain differences exist in amyloid deposition during the aging process between monkeys and humans.

A certain degree of glial response (Sloane *et al*., 1999, 2000) appears to occur prior to the development of senile plaques in macaque monkeys. In a study of the temporospatial relationships between neuronal injury and the deposition of the composants of senile plaques and parenchymal amyloid deposits, the earliest change associated to formation of senile plaques was recognized as the development in presynaptic terminals in dendrites of electron dense bodies and the accumulation of the amyloid precursor protein in astrocytes and microglial cells (Martin *et al*., 1994). In old monkeys, amyloid deposits containing nonfibrillar Aβ were observed in neurites and dendrites as well as glial elements, and the Aβ peptide was found in extracellular fibrils around mature plaques, suggesting that a synaptic pathology precedes the deposition of amyloid precursor protein in neurites and activated glia, and of extracellular fibrillar Aβ within senile plaques. In addition, it appears that the expression of the mRNAs for the 695- and 751-residue isoforms of the amyloid precursor protein is increased in adult macaques prior to reaching senescence, suggesting a role of

the amyloid precursor protein in early molecular events related to brain aging (Sirinathsinghji *et al*., 1995). Finally, in macaque monkeys, both senile plaques and amyloid angiopathy contain several proteins associated with these same lesions in humans. In particular, apolipoprotein E, α1-antichymotrypsin and complement factors C1q and C3c are encountered in these lesions in monkeys (Abraham *et al*., 1989; Podlisny *et al*., 1991; Poduri *et al*., 1994; Härtig *et al*., 1997), demonstrating a close similarity in the processes leading to senile plaque formation between monkeys and human.

III. Neurofibrillary Changes in Old Monkeys

Filamentous neuronal or neuritic changes have been reported in old monkeys albeit in far fewer number than in elderly human brains or in Alzheimer's disease cases. Early reports have described rare neuronal fibrillar materials in the form of 10 nm helical filaments exhibiting a periodicity of 50 nm as well as 13 nm granular, parallel filaments (Wisniewski *et al*., 1973). Other investigators described neurofibrillary tangles in the brains of cognitively impaired monkeys (Cork *et al*., 1989). More recently filamentous tau pathology, morphologically and biochemically comparable to the fibrillary lesions that are characteristics of a variety of human neurodegenerative disorders, have been described in a few aged baboons (*Papio hamadryas cynocephalus*) (Schultz *et al*., 2000), rhesus macaques, and Campbell's guenons (*Cercopithecus mona campbelli*) (Härtig *et al*., 2000). These lesions, that could be labeled by a panel of antibodies to hyperphosphorylated tau proteins, were observed in neurons, astrocytes, and oligodendrocytes of two old baboons, and occurred preferentially in the hippocampal formation. The fact that these lesions were found not only in nerve cells, but in glial cells as well, extend the nonhuman primate spectrum of age-related pathologies to a larger group of degenerative diseases that differ from Alzheimer's disease by their profile of tau proteins and characteristic lesion distributions. Why cellular lesions typical of Alzheimer's disease and other dementias, such as true tau-positive neurofibrillary tangles or glial coiled bodies, develop in only some individuals of a very restricted number of primate species (Cork *et al*., 1989; Härtig *et al*., 2000; Schultz *et al*., 2000; and see Chapter 29) remains to be elucidated. Also, the nature of the lesions observed in these animals differ morphologically and biochemically from the tangle-like lesions found in certain nonprimate species. In this context it is worth noting that fibrillary lesions have been described in the brain of old lesser gray mouse lemur (*Microcebus murinus*) a prosimian primate (Bons *et al*., 1991; Giannakopoulos *et al*., 1997; see Chapter 27). Tau proteins from this species have been shown to undergo age-related modifications in respect to their isoform composition (Delacourte *et al*., 1995), making it another interesting model for the study of biochemical dysfunctions during brain aging and Alzheimer's disease.

IV. Age-Related Ultrastructural Alterations in the Macaque Monkey Cerebral Cortex

There are few, if any, obvious changes to neuron morphology and numbers in the cerebral cortex of aged monkeys. In fact, several studies using traditional as well as stereologic methods have shown that the frequency of profiles displaying a nucleus or the total number of neurons remain unchanged in the neocortex and that the morphology of these nuclei is not altered in old animals (Peters *et al*., 1994, 1998b; Gazzaley *et al*., 1997; Hof *et al*., 2000). The neurons in area 46 of the prefrontal cortex have been shown to accumulate moderate amounts of lipofuscin during aging in macaques (Peters *et al*., 1994). However, there are notable changes in the morphology of the terminal dendrites of these neurons, which show evidence of degeneration particularly in layer I, where large numbers of dendritic branches exhibit changes in their cytoplasm including loss of organelles and accumulation of membranous materials (Peters *et al*., 1994). Peters and colleagues also reported a local thinning of layer I in this region and stereologic counts of the numbers of synapses revealed a consistent reduction in the old monkeys of 30 to 60% of the numerical densities of synapses per unit volume (Fig. 28.2A; Peters *et al*., 1998a; but see below for additional details). Such loss of synapses occurs in parallel to a decrease in the number of postsynaptic dendrites and spines in layer I, implying a considerable degree of involution of the apical tuft of dendrites of neocortical neurons takes place that during aging (Peters *et al*., 1998a). Recent preliminary data from our population of old macaque and Patas monkeys confirm these observations and reveal a significant degree of alterations in the apical dendritic tree of neurons in the prefrontal cortex as well as lower spine densities (see below and Fig. 28.5; Duan *et al*., 1999). Moreover, these dendritic and synaptic changes are accompanied by a degeneration of myelinated axons in the deep layers of the cortex and in the white matter, which correlates in old animals with deficits in visual and spatial recognition tasks (Peters *et al*., 1994). Together, these ultrastructural morphologic changes suggest that age-related cognitive deficits in old monkeys are not due to a loss of neurons, but rather evolve from cellular changes that lead to a disruption of the connectivity between the cerebral cortex and other brain regions.

The axonal pathology in aged monkeys appears to be consistently severe. Myelinated fibers have been shown to undergo several types of alterations. In some fibers there is accumulation of dense oligodendrocytic materials that leads to a splitting of the major dense line of the myelin sheaths (Peters *et al*., 2000). Some sheaths display severe ballooning, due to the opening of the intraperiod lines which becomes filled by fluid. These balloons in the myelin lamellae can be exceptionally large but usually have a diameter of about 10 μm (Feldman and Peters, 1998). Certain fibers display doubling of their myelin sheaths that present as layers of compact myelin surrounding each other, and other sheaths contain too much myelin so that their axons are enclosed by sheaths that are proportionally too large (Peters *et al*., 2000). Such changes of myelin sheaths occur under other conditions independently of aging (Feldman and Peters, 1998). However, their consistent presence in aged macaque monkeys indicate that during aging the oligodendrocytes are losing the capacity to maintain adequate myelin sheaths. It should also be noted that the changes have been observed not only in several regions of the cerebral cortex but in subcortical structures such as the inferior colliculus, cochlear and olivary nuclei, substantia nigra, and the cerebellum (Feldman and Peters, 1998).

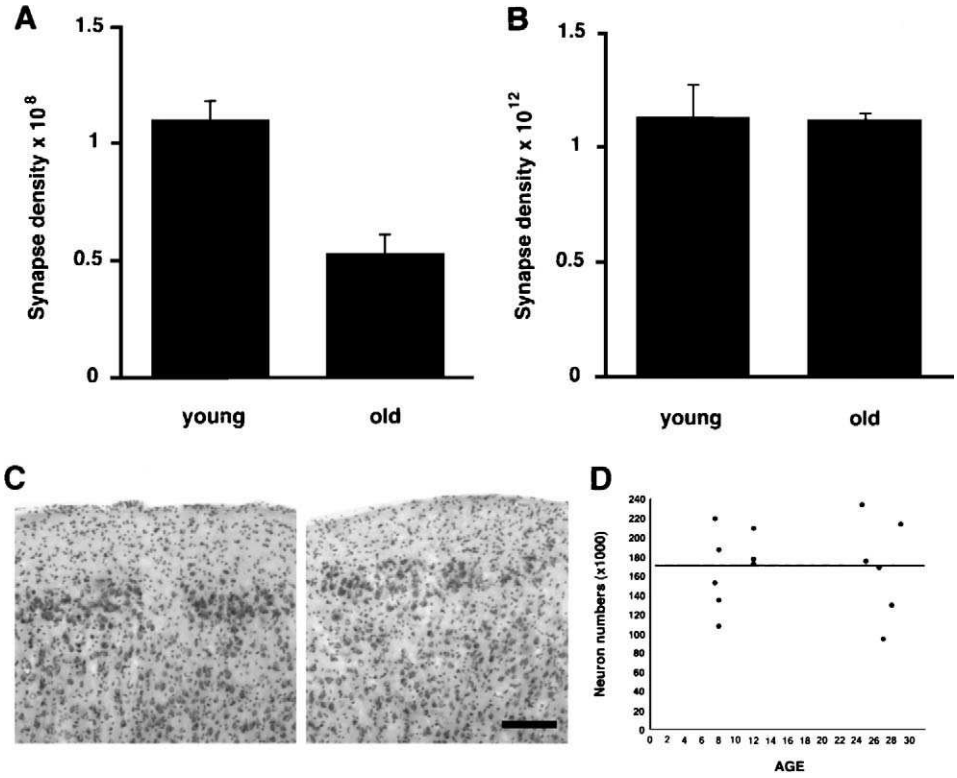

FIG. 28.2. Synapse densities in layer I of prefrontal cortex area 46 (A) and the dentate gyrus outer molecular layer (B) of old macaque monkeys compared to young animals. There is a decrease in the density of synaptic profiles counted under 1 mm^2 of cortical surface in the prefrontal cortex. In contrast, the densities of synapses per cm^3 are unchanged in the dentate gyrus of aged animals (data adapted from Peters *et al*., 1998b, and Tigges *et al*., 1996). (C, left) Layer II of the entorhinal cortex in a young adult rhesus monkey and (right) in an old animal. No cell loss is observed in the neurons of origin of the projection to the dentate gyrus in old monkeys as revealed by stereologic analyses (D; data adapted from Gazzaley *et al*., 1997). Quantitative data are shown as means ± SD. Scale bar on C, 100 μm.

These observations indicate that glial cells may be the principal target of age-related alterations in the brain of aged macaque monkeys, even though their numbers do not seem to change during aging (Peters *et al*., 1994). Further analyses of oligodendrocytes have demonstrated that these cells occur frequently in groups or arranged in rows in old animals, whereas in young adult they are commonly seen singly (Peters, 1996). In aged monkeys they are also observed in the vicinity of brain capillaries, at times with their cell bodies in contact with the basal membrane of the blood vessels, where they disrupt the normal limiting glial membrane formed by the astrocytes. In addition, these clumps of oligodendrocytes may form a network as tight junctions among their cell bodies have been observed (Peters, 1996). Finally, oligodendrocytes and other neuroglial cells accumulate abnormal inclusions or debris in their cytoplasm as do the pericytes (Peters *et al*., 1994).

V. Neuron and Synapse Numbers in the Central Nervous System of Old Macaque Monkeys

A considerable loss of synapses has been estimated in layer I of the prefrontal cortex (Fig. 28.2A; see above), in absence of obvious changes in the morphology of synapses (i.e., symmetric or asymmetric) and the distribution of the various types of synapses (i.e., axodendritic, abutting spines or dendritic shafts, and axosomatic). However, other regions of the cerebral cortex show milder changes. For instance, in the outer portion of the dentate gyrus no changes have been observed in the total counts of axonal terminals synapsing with dendritic spines and shafts, as well as in cross-sectional area of the terminals and length of postsynaptic densities (Fig. 28.2B; Tigges *et al*., 1995, 1996), although a minor decrease in the density of synapses on shafts was recorded when these synapses were considered alone. These data point to the fact that a major hippocampal network involved in memory is likely to be morphologically preserved during aging in these animals. These data agree with the fact that no neuronal loss has been observed in layer II of the entorhinal cortex where the neurons of origin of the projection to the outer molecular layer of the dentate gyrus reside, further supporting the morphological integrity of this pathway during aging (Figs. 28.2C and 28.2D; Gazzaley *et al*., 1997). It must be mentioned, however, that earlier studies of the hippocampal formation in old macaques have shown an approximately 50% decrease in the number of neurons in the CA1 field of the hippocampus proper and lesser differences in the prefrontal cortex, as well as a reduction in the thickness of these regions (Brizzee *et al*., 1980). These data were unfortunately not obtained using rigorous stereologic methods and await further confirmation, as they appear to dif-

fer radically from a host of more recent quantitative analyses (in this context it is worth mentioning that stereologic assessments of the numbers of neurons in the CA1 field and entorhinal cortex of great apes did not reveal any age-related differences; Erwin *et al*., 1999, and see Chapter 29). Similar observations have been made in the primary motor cortex of old macaque monkeys, where no loss in the numbers of the giant Betz cells and of the number of axosomatic appositions on their surface could be detected in spite of a slight age-related shrinkage in the volume of their perikarya (Tigges *et al*., 1990, 1992). It is in fact interesting to note that during adult development in macaque monkeys, the number of neurons in the primary motor cortex decreases significantly in the maturation period, but that the number of Betz cells increases at this time and remains stable for the entire life span of these animals (Tigges *et al*., 1990). It is therefore likely that the age-associated decrease in motility observed in aged macaques is not linked to a central loss of motoneurons and does not involve other components of the primary motor circuits, but is rather directly related to distal causes such as bone involution and joint disorders (DeRousseau, 1985).

The visual system of old monkeys has been extensively studied due to the remarkable similarities that exist between its functional organization and that of humans. Visual abilities decline during normal aging and involve deficits in visual acuity, spatial contrast and temporal-frequency contrast sensitivity and resolution, motion detection, and binocular processing (Spear, 1993; Spear *et al*., 1994). However, the anatomical and physiological study of aged monkeys has revealed very few significant alterations in the retino-geniculo-striate pathways, at least at the level of the retina and lateral geniculate nucleus, suggesting that visual deficits in aging may depend on more central dysfunctions (Spear, 1993; Spear *et al*., 1994). In fact, recent physiological data from old macaques clearly demonstrate the existence of degradation in orientation and direction selectivity in the primary visual cortex, accompanied by increased spontaneous activity and responsiveness, possibly due to an age-related deficit in intracortical inhibition (Schmolesky *et al*., 2000). Previous studies of retinal ganglion neurons and lateral geniculate nucleus neurons have generally failed to reveal any age-related decline in their numbers or size, suggesting that neither the parvocellular nor the magnocellular neurons are affected (Ahmad and Spear, 1993; Kim *et al*., 1996). In addition, aging has no clear effect on the number of neurons in histochemically identified compartments of the primary visual cortex, and the density of these functional domains (i.e., the cytochrome oxidase-rich blobs) remains unaltered in old animals (Kim *et al*., 1997). Furthermore, the area, volume, and thickness of the primary visual cortex is comparable in young and old macaque monkeys (Peters *et al*., 1997; Hof *et al*., 2000; Fig. 28.3A). However, as in other regions of the cerebral cortex, the neuropil in the primary visual cortex demonstrates vacuolar changes and degenerating myelin sheaths, in absence of obvious morphologic alterations to the neuronal perikarya (Vincent *et al*., 1989; Peters, 1996; Feldman and Peters, 1998; Peters *et al*., 2000).

It is therefore likely that the age-related deficits in visual function affect cortical structure at a finer grained level and may involve only certain neuronal subpopulations. One such identifiable cellular class comprises the large layer IVB neurons and the Meynert cells that form direct projections to cortical and subcortical regions involved in motion detection, ocular tracking, smooth pursuit movements, and saccade production (Hof *et al*., 2000). It is reasonable to argue that in view of their particular connectivity patterns these neurons may be affected by aging as the functions they are likely to subserve are not fully intact in old individuals. Used an unbiased stereologic method the optical fractionator estimates of the total numbers of neurofilament protein-containing layer IVB cells and Meynert cells have been obtained in the calcarine cortex and in the opercular cortex, separately (Hof *et al*., 2000). Not surprinsingly, a considerable degree of interindividual variability in neuron numbers and cortical volume was observed among both young and old animals. However, there were no differences in either Meynert cell or layer IVB cell numbers between the aged group and the young group in either parts of the primary visual cortex, confirming earlier data relying on density measurements (Fig. 28.3B; Peters and Sethares, 1993). These investigators had also determined that Meynert cells do not shrink during aging and our study revealed no morphological alterations to these cell populations (Fig. 28.3C). Interestingly, the oldest animal in our sample had the

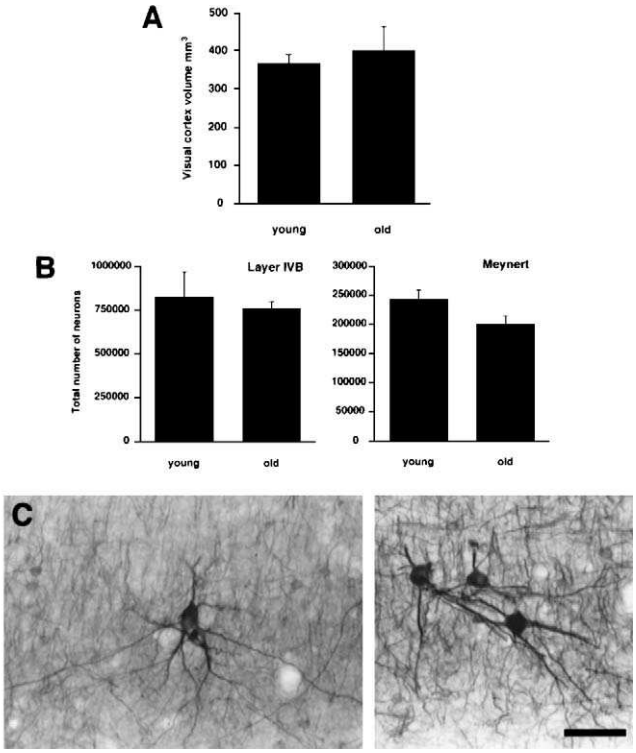

FIG. 28.3. Integrity of the primary visual cortex during aging in macaque monkeys. No changes in the total volume of the primary visual cortex occur in old animals (A). Specific populations of projection neurons, the large layer IVB and the Meynert cells, are not affected by aging as demonstrated by stereologic analyses (B). Furthermore, these particular neurons exhibit no age-related morphologic alterations (C). C shows clusters of Meynert cells in layer VI of young (left) and old (right) macaque monkeys. Note the distinct morphology of these neurons. Materials were stained with an antibody against nonphosphorylated neurofilament protein. Quantitative data are shown as means $\pm$ SD and were adapted from Hof *et al*. (2000). Scale bar on C, 100 μm.

lowest number of Meynert cells, suggesting that some animals have a certain degree of neuronal loss in the primary visual cortex during aging. These data suggest that the deficits occurring during aging of the visual system are not due to the loss of highly specific neocortical neuronal populations such as those analyzed in this particular study. It is, however, probable that more subtle alterations in the neurochemical characteristics or synaptic organization of the functional pathways subserving the different visual modalities are more directly responsible for these deficits. It should be kept in mind that these neuronal populations may undergo dendritic alterations that could not be detected with the methods used in the available studies. In view of the documented changes to the dendritic tree in other cortical regions (Peters *et al*., 1998b; Duan *et al*., 1999), it would be important to investigate the possible involution of dendritic arborizations as well as spine changes in Meynert cells in old monkeys.

VI. Neuronal Alterations and Loss in Subcortical Systems in Aged Macaque Monkeys

Whereas the cerebral cortex appears remarkably resistant to the aging process in old monkeys, significant alterations have been observed in subcortical nuclei that contain the neurons of origin of the cholinergic and aminergic systems. Cholinergic deficits have long been implicated in the memory deficits of aging and Alzheimer's disease. Although no definitive stereologic analyses have been performed in the nucleus basalis of Meynert and associated cholinergic cell groups in old monkeys, a study has documented an approximately 40% loss of cholinergic neurons in the caudalmost part of the nucleus of Meynert (Rapp and Amaral, 1992; Stroessner-Johnson *et al*., 1992). Age-related decreases in packing densities and numbers of neurons in the nucleus raphe centralis superior have been demonstrated in a group of old rhesus monkeys and concern a supopulation of small neurons and a group of serotoninergic neurons, with a cell loss of about 50% (Kemper *et al*., 1997). Finally, a recent study has shown that a substantial loss of dopaminergic neurons in the substantia nigra pars compacta and the ventral tegmental area occur in the macaque monkey during aging (Siddiqi and Peters, 1999; Siddiqi *et al*., 1999). The volume of these nuclei was not reported to change with aging in these studies, and the most severe neuronal loss in the substantia nigra involved the small, presumably GABAergic neurons (Siddiqi *et al*., 1999). Overall, about 25% of the substantia nigra pars compacta and 34% of the ventral tegmental area neurons are lost during aging. Furthermore, only slight changes in the size of individual nigral and tegmental neurons were observed by these investigators, although many of these cells accumulated lipofuscin and Marinesco bodies and underwent severe dendritic alterations during aging (Siddiqi and Peters, 1999). This study also revealed a considerable pathology of the neuropil of the substantia nigra pars compacta in aged animals, with increases in the number of astrocytic processes, and the presence of astrocyte-derived spheroids, oligodendrocytic inclusions, and breakdown of myelin sheaths.

Importantly, changes in all of these subcortical systems furnishing divergent projections to the cerebral cortex are correlated with the severity of cognitive deficits in aged animals (Rapp and Amaral, 1992; Stroessner-Johnson *et al*., 1992; Kemper *et al*., 1997; Siddiqi *et al*., 1999). These cellular alterations may exert a significant influence on the integrity of neurochemically specific afferent projections on neocortical function. In fact, age-related morphologic and pharmacological alterations of catecholaminergic systems are known to play a central role in the development of cognitive and memory impairment in aged monkeys (Arnsten, 1998, 1999). In this context, it is important to note that the cortical dopaminergic appears to be particularly affected (Goldman-Rakic and Brown, 1981; Wenk *et al*., 1989; Arnsten, 1998, 1999), which supports the morphological evidence (Siddiqi and Peters, 1999; Siddiqi *et al*., 1999). Interestingly, a recent study demonstrated that the age-related atrophy and loss of cholinergic neurons can be reversed by delivery of human nerve growth factor in old macaque monkeys (Smith *et al*., 1999). These data suggest that in spite of the fact that subcortical regions may exhibit greater vulnerability than other brain areas in aging macaques, cellular atrophy is an important contributing factor to the cognitive decline observed in these animals that can be potentially improved by gene transfer of neurotrophic molecules.

VII. Age-Related Cognitive Deficits in Monkeys Involve Subtle Morphological and Molecular Changes

The fact that only minor morphologic modifications in neuronal circuits are seen in old monkeys and that major changes appear to be restricted to a small number of specific systems, the cognitive deficits observed in aged animals may be more strongly related to rather minute alterations affecting certain populations of cortical neurons. Such alterations are additionally unlikely to be brought to evidence even by refined morphometric and stereologic methods, or during general neuropathologic evaluation of the brain, but require quantitative investigations at the subcellular level. The major age-related changes in catecholaminergic and cholinergic systems seen in old animlas (Arnsten, 1999) indicate a pathology involving specific receptor molecules and specific sites on the dendritic and somatic compartments of the target neurons. For example, a recent study of cortical volume of prefrontal cortex area 46 revealed no gross change in the volume of layer I and of the total cortex in macaque monkeys cognitively impaired on a delayed-response task (Fig. 28.4; O'Donnell *et al*., 1999). These data, together with those of other studies of neuropil changes in area 46 (Peters *et al*., 1996, 1998b), indicate that subtle subcellular alterations are likely to have substantial repercussions on the functional connectivity of affected cortical circuitry in aging. In addition, specific shifts in the expression of certain glutamate receptor subunit proteins, which may lead to functional decline without neurodegeneration, have been shown to occur in aged monkeys, but not in juvenile and adult monkeys (Gazzaley *et al*., 1996; Rosene and Nicholson, 1999). Recent data have demonstrated that NMDAR1 receptor levels detected by quantitative immunohistochemistry decrease specifically and consistently in the outer molecular layer of the dentate gyrus where part of the perforant path terminates, but there are no such changes in AMPA or kainate

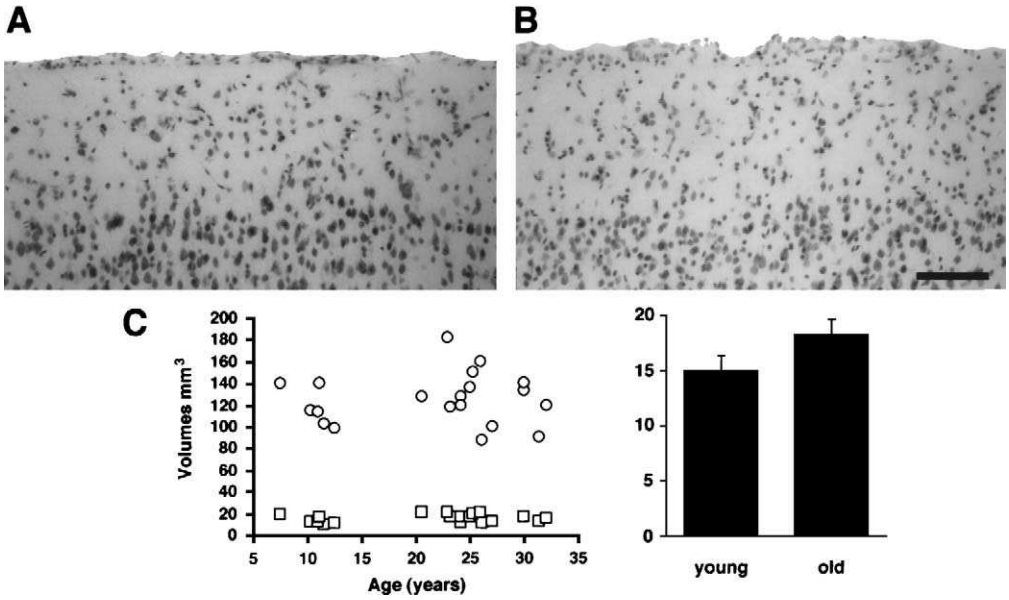

FIG. 28.4. Morphology and stereologic analysis of prefrontal cortex area 46 in macaque monkey aging. No gross alterations can be observed between young (A) and old (B) animals in the thickness and morphology of layer I. Volumetric analyses show no correlation between volumes of layer I or total volume of area 46 and age (C, left; total volumes are shown by open circles, and layer I volumes by open squares) or between the two groups of animals (C, right). Quantitative data are shown as means ± SD and were adapted from O'Donnell *et al*. (1999). Scale bar on B, 100 μm.

receptor subunits, and no morphologic reflection of degeneration of the perforant path (Gazzaley *et al*., 1996). In particular, there is no neuronal loss in layer II of the entorhinal cortex in these aged animals (Gazzaley *et al*., 1997). These data are consistent with the minor changes in synapses in the dentate gyrus of aged monkeys (Tigges *et al*., 1995), and with reports of receptor binding assays in old animals (Wenk *et al*., 1991; Rosene and Nicholson, 1999). Recent analyses of identified

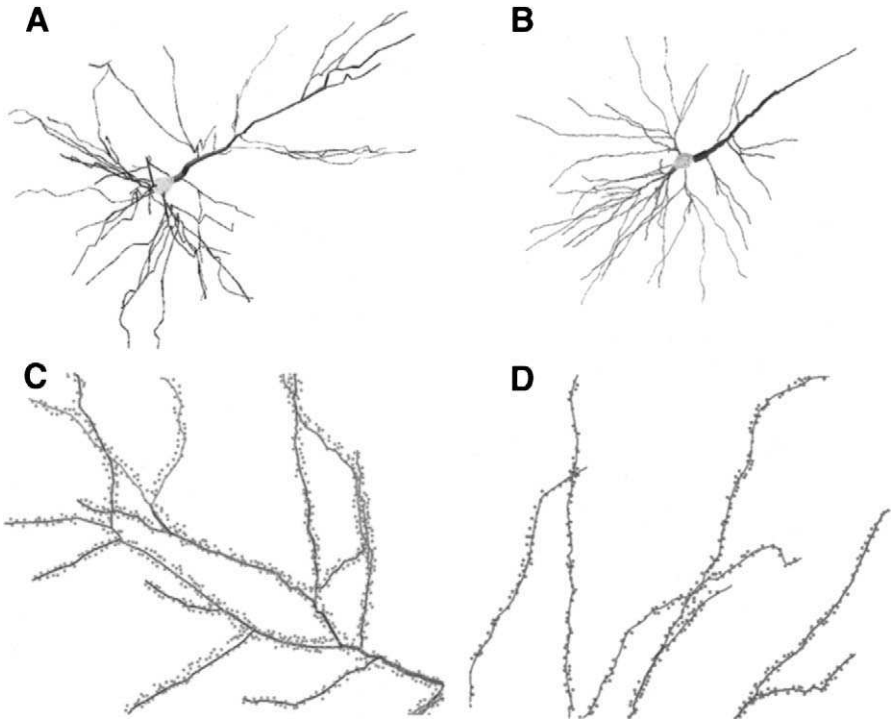

FIG. 28.5. Three-dimensional reconstruction of dye-loaded, retrogradely labeled neurons furnishing identified projections from the superior temporal cortex to prefrontal area 46 in a young (A) and an old (B) macaque monkey. Note the less complex and reduced dendritic arborization patterns in the old animal. Spines densities are also decreased in the old animal (D) compared to that seen in the young one (C). Spines were mapped in three dimensions at the same time that the neurons were reconstructed (Duan *et al*., 1999).

neurons furnishing corticocortical projections have demonstrated that a considerable downregulation in the expression of the glutamate receptor subunits NMDAR1 and G1uR2 occurs in old rhesus and Patas monkeys, so that in aged animals there are 20–40% fewer neurons expressing these subunit proteins depending on the type of corticocortical projections (Duan *et al*., 1999). Furthermore, the three-dimensional reconstruction and modeling of these cortical projection neurons showed that in both species they undergo a consistent impoverishment of the complexity of their distal dendritic arborizations and exhibit a severe decrease (20–30%) in spine densities (Fig. 28.5).

These findings suggest that the intradendritic parcellation of a neurotransmitter receptor is modifiable in an age-related and circuit-specific manner and that this change is anatomically positioned to influence the information-processing capacities of systems critical for normal learning and memory. Defining the functional significance of these alterations, however, will require a multidisciplinary approach, combining morphological and biochemical analysis in relation to the cognitive outcome of normal aging.

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References

Abraham, C. R., Selkoe, D. J., Potter, H., Price, D. L., and Cork, L. C. (1989). α1-Antichymotrypsin is present together with the β-protein in monkey brain amyloid deposits. *Neuroscience* **32**, 715–720.

Ahmad, A., and Spear, P. D. (1993). Effects of aging on the size, density, and number of rhesus monkey lateral geniculate neurons. *J. Comp. Neurol.* **334**, 631–643.

Allman, J., Rosin, A., Kumar, R., and Hasenstaub, A. (1998). Parenting and survival in anthropoid primates: Caretakers live longer. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 6866–6869.

Andersen, A. H., Zhang, Z., Zhang, M., Gash, D. M., and Avison, M. J. (1999). Age-associated changes in rhesus CNS composition identified by MRI. *Brain Res.* **829**, 90–98.

Arnold, S. E., Hyman, B. T., Flory, J., Damasio, A. R., and Van Hoesen, G. W. (1991). The topographical and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer's disease. *Cereb. Cortex* **1**, 103–116.

Arnsten, A. F. T. (1998). Catecholamine modulation of prefrontal cortical cognitive function. *Trends Cogn. Sci.* **2**, 436–447.

Arnsten, A. F. T. (1999). Age-related cognitive deficits and neurotransmitters—The role of catecholamine mechanisms in prefrontal cortical cognitive decline. *In* "Neurodegenerative and Age-Related Changes in Cerebral Cortex" (A. Peters and J. H. Morrison, eds.), Cerebral Cortex, Vol. 14, pp. 89–110. Kluwer Academic-Plenum, New York.

Arriagada, P. V., Growdon, J. H., Hedley-Whyte, E. T., and Hyman, B. T. (1992). Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology* **42**, 631–639.

Bachevalier, J., Landis, L. S., Walker, L. C., Erickson, M., Mishkin, M., Price, D. L., and Cork, L. C. (1991). Aged monkeys exhibit behavioral deficits indicative of widespread cerebral dysfunction. *Neurobiol. Aging* **12**, 99–111.

Bartus, R. T., Fleming, D., and Johnson, H. R. (1978). Aging in the rhesus monkey: Debilitating effects on short-term memory. *J. Gerontol.* **33**, 858–871.

Baxter, M. G., and Voytko, M. L. (1996). Spatial orienting of attention in adult and aged rhesus monkeys. *Behav. Neurosci.* **110**, 898–904.

Bons, N., Mestre, N., and Petter, A. (1991). Senile plaques and neurofibrillary changes in the brain of an aged Lemurian primate, *Microcebus murinus. Neurobiol. Aging* **13**, 99–105.

Bons, N., Mestre, N., Ritchie, K., Petter, A., Podlisny, M., and Selkoe, D. (1994). Identification of amyloid beta protein in the brain of the small, short-lived Lemurian primate *Microcebus murinus. Neurobiol. Aging* **15**, 215–220.

Brizzee, K. R., Ordy, J. M., and Bartus, R. T. (1980). Localization of cellular changes within multimodal sensory regions in aged monkey brain: Possible implications for age-related cognitive loss. *Neurobiol. Aging* **1**, 45–52.

Cork, L. C., Walker, L. C., and Price, D. L. (1989). Neurofibrillary tangles and senile plaques in cognitively impaired, aged nonhuman primates. *J. Neuropathol. Exp. Neurol.* **48**, 378.

Delacourte, A., Sautière, P. E., Wattez, A., Mourton-Gilles, C., Petter, A., and Bons, N. (1995). Biochemical characterization of Tau proteins during cerebral aging of the lemurian primate *Microcebus murinus. C. R. Acad. Sci. Paris [Life Sci.]* **318**, 85–89.

DeRousseau, C. J. (1985). Aging in the musculoskeletal system of rhesus monkeys: II. Degenerative joint disease. *Am. J. Phys. Anthropol.* **67**, 177–184.

Duan, H., He, Y., Wicinski, B., Yeung, G., Page, T. L., Janssen, W. G. M., Morrison, J. H., and Hof, P. R. (1999). Age-related changes in cortical projection neurons in macaque monkey: Dendrite morphology, spine density, and neurochemical features. *Soc. Neurosci. Abstr.* **25**, 362.

Eberling, J. L., Roberts, J. A., De Manincor, D. J., Brennan, K. M., Hanrahan, S. M., Vanbrocklin, H. F., Roos, M. S., and Jagust, W. J. (1995). PET studies of cerebral glucose metabolism in conscious rhesus macaques. *Neurobiol. Aging* **16**, 825–832.

Eberling, J. L., Roberts, J. A., Rapp, P. R., Tuszynski, M. H., and Jagust, W. J. (1997). Cerebral glucose metabolism and memory in aged rhesus macaques. *Neurobiol. Aging* **18**, 437–443.

Erwin, J. M., Perl, D. P., Nimchinsky, E. A., and Hof, P. R. (1999). Stereologic analyses of neuron number and volume in the entorhinal cortex of aged chimpanzees. *Soc. Neurosci. Abstr.* **25**, 105.

Feldman, M. L., and Peters, A. (1998). Ballooning of myelin sheaths in normally aged macaques. *J. Neurocytol.* **27**, 605–614.

Gazzaley, A. H., Siegel, S. J., Kordower, J. H., Mufson, E. J., and Morrison, J. H. (1996). Circuit-specific alterations of *N*-methyl-D-aspartate receptor subunit 1 in the dentate gyrus of aged monkeys. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 3121–3125.

Gazzaley, A. H., Thakker, M. M., Hof, P. R., and Morrison, J. H. (1997). Preserved number of entorhinal cortex layer II neurons in aged macaque monkeys. *Neurobiol. Aging* **18**, 549–553.

Gearing, M., Rebeck, G., Hyman, B. T., Tigges, J., and Mirra, S. S. (1994). Neuropathology and apolipoprotein E profile of aged chimpanzees: Implications for Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 9382–9386.

Gearing, M., Tigges, J., Mori, H., and Mirra, S. S. (1996). Aβ40 is a major form of β-amyloid in nonhuman primates. *Neurobiol. Aging* **17**, 903–906.

Gearing, M., Tigges, J., Mori, H., and Mirra, S. S. (1997). β-amyloid (Aβ) deposition in the brains of aged orangutans. *Neurobiol. Aging* **18**, 139–146.

Geula, C., Wu, C. K., Saroff, D., Lorenzo, A., Yuan, M., and Yankner, B. A. (1998). Aging renders the brain vulnerable to amyloid β-protein neurotoxicity. *Nat. Med.* **4**, 827–883.

Giannakopoulos, P., Silhol, S., Jallageas, V., Mallet, J., Bons, N., Bouras, C., and Delaère, P. (1997). Quantitative analysis of tau protein-im-

munoreactive accumulations and β-amyloid protein deposits in the cerebral cortex of the mouse lemur, *Microcebus murinus. Acta Neuropathol.* **94**, 131–139.

Goldman-Rakic, P. S., and Brown, R. M. (1981). Regional changes of monoamines in cerebral cortex and subcortical structures of aging rhesus monkeys. *Neuroscience* **6**, 177–187.

Härtig, W., Brückner, G., Schmidt, C., Brauer, K., Bodewitz, G., Turner, J. D., and Bigl, V. (1997). Co-localization of β-amyloid peptides, apolipoprotein E and glial markers in senile plaques in the prefrontal cortex of old rhesus monkeys. *Brain Res.* **751**, 315–322.

Härtig, W., Klein, C., Brauer K., Schüppel, C. F., Asendt, T., Brückner, G., and Bigl, V. (2000). Abnormally phosphorylated protein tau in the cortex of aged individuals of various mammalian orders. *Acta Neuropathol.* **100**, 305–312.

Heilbronner, P. L., and Kemper, T. L. (1990). The cytoarchitectonic distribution of senile plaques in three aged monkeys. *Acta Neuropathol.* **81**, 60–65.

Herndon, J. G., Constantinidis, I., and Moss, M. B. (1998a). Age-related brain changes in rhesus monkeys: A magnetic resonance spectroscopic study. *NeuroReport* **9**, 2127–2130.

Herndon, J. G., Tigges, J., Klumpp, S. A., and Anderson, D. C. (1998b). Brain weight does not decrease with age in adult rhesus monkeys. *Neurobiol. Aging* **19**, 267–272.

Herndon, J. G., Moss, M. B., Rosene, D. L., and Killiany, R. J. (1997). Patterns of cognitive decline in aged rhesus monkeys. *Behav. Brain Res.* **87**, 25–34.

Herndon, J. G., Tigges, J., Anderson, D. C., Klumpp, S. A., and McClure, H. M. (1999). Brain weight throughout the life span of the chimpanzee. *J. Comp. Neurol.* **409**, 567–572.

Hof, P. R., Bouras, C., and Morrison, J. H. (1999). Cortical neuropathology in aging and dementing disorders: Neuronal typology, connectivity, and selective vulnerability. *In* "Neurodegenerative and Age-Related Changes in Cerebral Cortex" (A. Peters and J. H. Morrison, eds.), Cerebral Cortex, Vol. 14, pp. 175–312. Kluwer Academic-Plenum, New York.

Hof, P. R., Nimchinsky, E. A., Young, W. G., and Morrison, J. H. (2000). Numbers of Meynert and layer IVB cells in area V1: A stereologic analysis in young and aged macaque monkeys. *J. Comp. Neurol.* **420**, 113–126.

Kanemaru, K., Iwatsubo, T., and Ihara, Y. (1996). Comparable amyloid β-protein (Aβ) 42 (43) and Aβ40 deposition in the aged monkey brain. *Neurosci. Lett.* **214**, 196–198.

Kemper, T. L., Moss, M. B., Rosene, D. L., and Killiany, R. J. (1997). Age-related neuronal loss in the nucleus centralis superior of the rhesus monkey. *Acta Neuropathol.* **94**, 124–130.

Kim, C. B. Y., Tom, B. W., and Spear, P. D. (1996). Effects of aging on the densities, numbers, and sizes of retinal ganglion cells in rhesus monkeys. *Neurobiol. Aging* **17**, 431–438.

Kim, C. B. Y., Pier, L. P., and Spear, P. D. (1997). Effects of aging on numbers and sizes of neurons in histochemically defined subregions of monkey striate cortex. *Anat. Rec.* **247**, 119–128.

Lacreuse, A., Herndon, J. G., and Moss, M. B. (1998). Vieillissement des fonctions cognitives chez l'homme et le macaque rhésus *(Macaca mulatta). Primatologie* **1**, 333–377.

Lai, Z. C., Moss, M. B., Killiany, R. J., Rosene, D. L., and Herndon, J. G. (1995). Executive system dysfunction in the aged monkey: Spatial and object reversal learning. *Neurobiol. Aging* **16**, 947–954.

Maldonado, T. A., Jones, R. E., and Norris, D. O. (2000). Distribution of β-amyloid and amyloid precursor protein in the brain of spawning (senescent) salmon: A natural, brain-aging model. *Brain Res.* **858**, 237–251.

Martin, L. J., Sisodia, S. S., Koo, E. H., Cork, L. C., Dellovade, T. L., Weidemann, A., Beyreuther, K., Masters, C., and Price, D. L. (1991). Amyloid precusrsor protein in aged nonhuman primates. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 1461–1465.

Martin, L. J., Pardo, C. A., Cork, L. C., and Price, D. L. (1994). Synaptic pathology and glial responses to neuronal injury precede the formation of senile plaques and amyloid deposits in the aging cerebral cortex. *Am. J. Pathol.* **145**, 1358–1381.

McKee, A. C., Kowall, N. W., Schumacher, J. S., and Beal, M. F. (1998). The neurotoxicity of amyloid beta protein in aged primates. *Amyloid Int. J. Exp. Clin. Invest.* **5**, 1–9.

Mestre-Francés, N., Keller, E., Calenda, A., Barelli, H., Checler, F., and Bons, N. (2000). Immunohistochemical analysis of cerebral cortical and vascular lesions in the primate *Microcebus murinus* reveal amyloid β1-42 and β1-40 immunoreactivity profiles. *Neurobiol. Dis.* **7**, 1–8.

Moss, M. B., Rosene, D. L., and Peters, A. (1988). Effects of aging on visual recognition memory in the rhesus monkey. *Neurobiol. Aging* **9**, 495–502.

Moss, M. B., Killiany, R. J., Lai, Z. C., Rosene, D. L., and Herndon, J. G. (1997). Recognition memory span in rhesus monkeys of advanced age. *Neurobiol. Aging* **18**, 13–19.

Nakamura, S., Tamaoka, A., Sawamura, N., Shoji, S., Nakayama, H., Ono, F., Sakakibara, I.,Yoshikawa, Y., Mori, H., Goto, N., and Doi, K. (1995). Carboxyl end-specific monoclonal antibodies to amyloid β protein (Aβ) subtypes (Aβ40 and Aβ42(43)) differentiate aβ in senile plaques and amuloid angiopathy in brains of aged cynomolgus monkeys. *Neurosci. Lett.* **201**, 151–154.

Nakayama, H., Katayama, K. I., Ikawa, A., Miyawaki, K., Shinozuka, J., Uetsuka, K., Nakamura, S. I., Kimura, N., Yoshikawa, Y., and Doi, K. (1999). Cerebral amyloid angiopathy in an aged great spotted woodpecker *(Picoides major). Neurobiol. Aging* **20**, 53–56.

O'Donnell, K. A., Rapp, P. R., and Hof, P. R. (1999). The volume of prefrontal cortex area 46 is preserved during aging in macaque monkeys. *Exp. Neurol.* **160**, 300–310.

Pearson, R. C. A., Esiri, M. M., Hiorns, R. W., Wilcock, G. K., and Powell, T. P. S. (1985). Anatomical correlates of the distribution of the pathological changes in the neocortex in Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4531–4534.

Peters, A. (1996). Age-related changes in oligodendrocytes in monkey cerebral cortex. *J. Comp. Neurol.* **371**, 153–163.

Peters, A. (1999). Normal aging in the cerebral cortex of primates. *In* "Neurodegenerative and Age-Related Changes in Cerebral Cortex" (A. Peters and J. H. Morrison, eds.), Cerebral Cortex, Vol. 14, pp. 49–80. Kluwer Academic-Plenum, New York.

Peters, A., and Sethares, C. (1993). Aging and the Meynert cells in rhesus monkey primary visual cortex. *Anat. Rec.* **236**, 721–729.

Peters, A., Leahu, D., Moss, M. B., and McNally, K. J. (1994). The effects of aging on area 46 of the frontal cortex of the rhesus monkey. *Cereb. Cortex* **4**, 621–635.

Peters, A., Rosene, D., Moss, M., Kemper, T., Abraham, C., Tigges, J., and Albert, M. (1996). Neurobiological bases of age-related cognitive decline in the rhesus monkey. *J. Neuropathol. Exp. Neurol.* **55**, 861–874.

Peters, A., Nigro, N. J., and McNally, K. J. (1997). A further evaluation of the effect of aging on striate cortex of the rhesus monkey. *Neurobiol. Aging* **18**, 29–36.

Peters, A., Morrisson, J. H., Rosene, D., and Hyman, B. T. (1998a). Are neurons lost from the primate cerebral cortex during normal aging? *Cereb. Cortex* **8**, 295–300.

Peters, A., Sethares, C., and Moss, M. B. (1998b). The effects of aging on layer 1 in area 46 of prefrontal cortex in the rhesus monkey. *Cereb. Cortex* **8**, 671–684.

Peters, A., Moss, M. B., and Sethares, C. (2000). Effects of aging on myelinated nerve fibers in monkey primary visual cortex. *J. Comp. Neurol.* **419**, 364–376.

Podlisny, M., Tolan, D. R., and Selkoe, D. J. (1991). Homology of the amyloid beta protein precursor in monkey and human supports a primate model for beta amyloidosis in Alzheimer's disease. *Am. J. Pathol.* **138**, 1423–1435.

Poduri, A., Gearing, M., Rebeck, G. W., Mirra, S. S., Tigges, J., and Hyman, B. T. (1994). Apolipoprotein E4 and beta amyloid in senile plaques and cerebral blood vessels of aged rhesus monkeys. *Am. J. Pathol.* **144**, 1183–1187.

Rapp, P. R., and Amaral, D. G. (1989). Evidence for task-dependent memory dysfunction in the aged monkey. *J. Neurosci.* **9**, 3568–3576.

Rapp, P. R., and Amaral, D. G. (1992). Individual differences in the cognitive and neurobiological consequences of normal aging. *Trends Neurosci.* **15**, 340–345.

Rapp, P. R., Kansky, M. T., and Eichenbaum, H. (1996a). Learning and memory for hierarchical relationships in the monkey: Effects of aging. *Behav. Neurosci.* **110**, 887–897.

Rapp, P. R., Kansky, M. T., Roberts, J. A., and Eichenbaum, H. (1996b). New directions for studying cognitive decline in old monkeys. *Semin. Neurosci.* **6**, 369–377.

Rapp, P. R., Kansky, M. T., and Roberts, J. A. (1997). Impaired spatial information processing in aged monkeys with preserved recognition memory. *NeuroReport* **8**, 1923–1928.

Roberts, J. A., Gilardi, K. V. K., Lasley, B., and Rapp, P. R. (1997). Reproductive senescence predicts cognitive decline in aged female monkeys. *NeuroReport* **8**, 2047–2051.

Rogers, J., and Morrison, J. H. (1985). Quantitative morphology and regional and laminar distributions of senile plaques in Alzheimer's disease. *J. Neurosci.* **5**, 2801–2808.

Rosene, D. L., and Nicholson, T. J. (1999). Neurotransmitter receptor changes in the hippocampus and cerebral cortex in normal aging. *In* ''Neurodegenerative and Age-Related Changes in Cerebral Cortex'' (A. Peters and J. H. Morrison, eds.), Cerebral Cortex, Vol. 14, pp. 111–128. Kluwer Academic-Plenum, New York.

Schmolesky, M. T., Wang, Y., Pu, M., and Leventhal, A. G. (2000). Degradation of stimulus selectivity of visual cortical cells in senescent rhesus monkeys. *Nat. Neurosci.* **3**, 384–390.

Schultz, C., Dehghani, F., Hubbard, G., Thal, D., Struckhoff, G., Braak, E., and Braak, H. (2000). Filamentous tau pathology in nerve cells, astrocytes, and oligodendrocytes of aged baboons. *J. Neuropathol. Exp. Neurol.* **59**, 39–52.

Selkoe, D. J., Bell, D. S., Podlisny, M. B., Price, D. L.,and Cork, L. C. (1987). Conservation of brain amyloid proteins in aged mammals and humans with Alzheimer's disease. *Science* **235**, 873–877.

Siddiqi, Z. A., and Peters, A. (1999). The effect of aging on pars compacta of the substantia nigra in rhesus monkey. *J. Neuropathol. Exp. Neurol.* **58**, 903–920.

Siddiqi, Z. A., Kemper, T. L., and Killiany, R. (1999). Age-related neuronal loss from the substantia nigra pars compacta and ventral tegmental area of the rhesus monkey. *J. Neuropathol. Exp. Neurol.* **58**, 959–971.

Silhol, S., Calenda, A., Jallageas, V., Mestre-Francés, N., Bellis, M., and Bons, N. (1996). β-Amyloid protein precursor in *Microcebus murinus:* Genotyping and brain localization. *Neurobiol. Dis.* **3**, 169–182.

Sirinathsinghji, D. J. S., Rigby, M., Heavens, R. P., Smith, D., Fernandez, J. M., Schuligoi, R., and Hill, R. G. (1995). Increased messenger RNA expression of the 695 and 751 amino acid isoforms of the β-amyloid protein precursor in the thalamus of 17-year-old cynomolgus *(Macaca fascicularis)* monkeys. *Neuroscience* **65**, 51–57.

Sloane, J. A., Pietropaolo, M. F., Rosene, D. L., Moss, M. B., Peters, A., Kemper, T., and Abraham, C. R. (1997). Lack of correlation between plaque burden and cognition in the aged monkey. *Acta Neuropathol.* **94**, 471–478.

Sloane, J. A., Hollander, W., Moss, M. B., Rosene, D. G., and Abraham, C. R. (1999). Increased microglial activation and protein nitration in white matter of the aging monkey. *Neurobiol. Aging* **20**, 395–405.

Sloane, J. A., Hollander, W., Rosene, D. G., Moss, M. B., Kemper, T., and Abraham, C. R. (2000). Astrocytic hypertrophy and altered GFAP degradation with age in subcortical white matter of the rhesus monkey. *Brain Res.* **862**, 1–10.

Smith, D. E., Roberts, J., Gage, F. H., and Tuszynski, M. H. (1999). Age-associated neuronal atrophy occurs in the primate brain and is reversible by growth factor gene therapy. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 10893–10898.

Spear, P. D. (1993). Neural bases of visual deficits during aging. *Vision Res.* **33**, 2589–2609.

Spear, P. D., Moore, R. J., Kim, C. B. Y., Xue, J. T., and Tumosa, N. (1994). Effects of aging on the primate visual system: Spatial and temporal processing by lateral geniculate neurons in young adult and old rhesus monkeys. *J. Neurophysiol.* **72**, 402–420.

Stroessner-Johnson, H. M., Rapp, P. R., and Amaral, D. G. (1992). Cholinergic cell loss and hypertrophy in the medial septal nucleus of the behaviorally characterized aged rhesus monkey. *J. Neurosci.* **12**, 1936–1944.

Tigges, J., Gordon, T. P., McClure, H. M., Hall, E. C., and Peters, A. (1988). Survival rate and life span of rhesus monkeys at the Yerkes Regional Primate Research Center. *Am. J. Primatol.* **15**, 263–273.

Tigges, J., Herndon, J. G., and Peters, A. (1990). Neuronal population of area 4 during the life span of the rhesus monkey. *Neurobiol. Aging* **11**, 201–208.

Tigges, J., Herndon, J. G., and Peters, A. (1992). Axon terminals on Betz cell somata of area 4 in rhesus monkey throughout adulthood. *Anat. Rec.* **232**, 305–315.

Tigges, J., Herndon, J. G., and Rosene, D. L. (1995). Mild age-related changes in the dentate gyrus of adult rhesus monkeys. *Acta Anat.* **153**, 39–48.

Tigges, J., Herndon, J. G., and Rosene, D. L. (1996). Preservation into old age of the synaptic number and size in the supragranular layer of the dentate gyrus in rhesus monkeys. *Acta Anat.* **157**, 63–72.

Veenema, H. C., Spruijt, B. M., Gispen, W. H., and Van Hoff, J. A. R. A. M. (1997). Aging, dominance history, and social behavior in Java-monkeys *(Macaca fascicularis). Neurobiol. Aging* **18**, 509–515.

Vincent, S. L., Peters, A., and Tigges, J. (1989). Effects of aging on the neurons within area 17 of rhesus monkey cerebral corex. *Anat. Rec.* **223**, 329–341.

Voytko, M. L. (1998). Nonhuman primates as models for aging and Alzheimer's disease. *Lab. Anim. Sci.* **48**, 611–617.

Walker, L. C. (1991). Animal models of cerebral amyloidosis. *Bull. Clin. Neurosci.* **56**, 86–96.

Walker, L. C. (1997). Animal models of cerebral beta-amyloid angiopathy. *Brain Res. Rev.* **30**, 70–84.

Walker, L. C., Masters, C., Beyreuther, K., and Price, D. L. (1990). Amyloid in the brains of aged squirrel monkeys. *Acta Neuropathol.* **80**, 381–387.

Wenk, G. L., Pierce, D. J., Struble, R. G., Price, D. L., and Cork, L. C. (1989). Age-related changes in multiple neurotransmitter systems in the monkey brain. *Neurobiol. Aging* **10**, 11–19.

Wenk, G. L., Walker, L. C., Price, D. L., and Cork, L. C. (1991). Loss of NMDA, but not GABA-A, binding in the brains of aged rats and monkeys. *Neurobiol. Aging* **12**, 93–98.

Wisniewski, H. M., Ghetti, B., and Terry, R. D. (1973). Neuritic (senile) plaques and filamentous changes in aged rhesus monkeys. *J. Neuropathol. Exp. Neurol.* **32**, 566–584.

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The Study of Brain Aging in Great Apes

The great apes are the closest biological relatives of humans. The resemblence is close from genetics to brain structure and cognitive function. Endocasts from fossil hominoids and hominids reveal that the brains of *Australopithecus* were similar in size and shape to those of modern chimpanzees, and that a dramatic increase in brain size occurred as *Homo* evolved. Studies of extant great apes (bonobos, chimpanzees, gorillas, and orangutans) in the wild and in captivity have provided evidence on patterns of sociality, behavior, communication, cognition, and self-awareness. These data have identified many characteristics that are apparently homologous among apes and humans, suggesting that these were also present in a common ancestor. Cerebral lateral asymmetries recently reported in the great apes suggest that some of the neurological foundations of language have long been present in the ape–human lineage. Neuronal loss with aging is associated with neurodegenerative pathology in humans. Studies of great ape brains using quantitative stereology have not yet found substantial neuronal loss associated with aging in the entorhinal cortex and CA1 field of the hippocampus, regions that are especially vulnerable to age-related cell loss in humans. However, two neuronal types have been recently found in anterior cingulate cortex that are unique to humans and great apes. One of these cell types, a large spindle cell found in a region implicated in self-awareness and regulation of autonomic functions, is diminished by about 60% in human victims of Alzheimer's disease. The application to great apes of improved methods of assessing cognitive decline, genetic risk, and gene expression, along with functional imaging and quantitative stereological research, offers prospects of additional insights into normal and pathological brain aging.

I. The Great Apes

The great apes are more like humans than are any other living creatures. Their genetic, anatomic, physiologic, cognitive, behavioral, and social similarity to humans recommends them highly for inclusion in comparative studies of many kinds, including studies of normal aging and age-related disorders. One of the most important reasons for studying the aging and other processes in the great apes is the greater prospect than with any other taxa of studying homologous structure, function, and malfunction.

Who are these great apes and why are we so much like them? The human species (*Homo sapiens*) is but one of about 200 extant species within the mammalian order, Primates. The Primate order includes the prosimians, the New World monkeys, the Old World monkeys, and the apes and humans. The living prosimians resemble widespread fossils dated beyond 40 million years before the present. The New World monkeys include the callitrichids (marmosets, tamarins, and *Callimico*) and the cebids (e.g., capuchins, squirrel monkeys, night monkeys). The Old World monkeys all fit into a single family, Cercopithecidae, with two branches cercopithecinae and colobinae. The cercopithecines include baboons, macaques, mangabeys, and guenons, while the colobines include leaf monkeys and odd-nosed monkeys. Authorities disagree on some aspects of the classification of apes and humans. Most agree that the lesser apes, Hylobatidae (gibbons and siamangs) form one family, but classification of the great apes and humans is problematic in several ways. This species cluster includes four genera, *Pongo* (orangutan), *Gorilla* (gorilla), *Pan* (''common'' chimpanzee and bonobo), and *Homo* (humans). Some authorities place the African apes (bonobo, chimpanzee, and gorilla) with the Asian orangutan in family Pongidae, while others reserve the name Panidae for the African ape family (Tuttle, 1999). Modern humans and their fossil predecessors (including several species of *Australopithecus* and *Homo*) are reliably assigned to family Hominidae. Several authorities place all the great apes and humans in family Hominidae (Goodman, 1974; Groves, 1986). Orangutans are assigned to subfamily, Ponginae. Gorillas, chimpanzees, and bonobos are assigned to subfamily Paninae (Gantt, 1986) or Homininae (Groves, 1986). Humans are classed in subfamily Homininae (Goodman, 1974; Groves, 1986). The nomenclature can be confusing when references to ''hominids'' (members of family Hominidae) can mean all great apes and ''hominoids'' (members of superfamily Hominoidea) can mean all apes, including gibbons.

Regardless of nomenclature, it is clear that all great apes and humans have shared common ancestry within about the past 12–15 million years, that the common ancestor of African

apes and humans lived less than 10 million years ago, and that the common ancestor of humans and chimpanzees lived 5–8 million years ago (Sarich and Wilson, 1967; Ciochon, 1983; McHenry, 1984; Kelley and Pilbeam, 1986). By contrast, the common ancestor of humans and Old World monkeys (such as rhesus macaques) lived about 30–32 million years ago. Humans are separated from the ancestors they share with all nonprimate mammals by at least 60 million years of evolution. Considering that both divergent lineages were evolving from that common ancestor, the minimum evolutionary distance between extant forms should be twice the postdivergence interval (i.e., more than 120 million years separate humans from any nonprimate mammal and perhaps as little as 10 million years from the chimpanzee). Reference to this time scale can be useful as we discuss the brains of great apes in relation to those of humans and other primates. Clearly the greatest homology must be expected between humans and chimpanzees or bonobos, somewhat less for gorilla, and less yet for orangutan.

II. Brain Evolution

The most obvious difference between the human brain and the brains of chimpanzees and other great apes is size. Whether we consider brain weight, cranial volume, or an encephalization index that considers brain weight relative to body weight, human brains are extraordinarily large relative to those of our nearest relatives (Tobias, 1971; Stephan *et al*., 1981, 1988; Semendeferi *et al*., 1997; Rilling and Insel, 1998, 1999; Semendeferi and Damasio, 2000). Brain weights given by Stephan *et al*. (1981, 1988) are as follows: rhesus (93 g), chimpanzee (405 g), and human (1330 g). When adjusted for body weight to produce an encephalization index, the values were 8.0, 11.4, and 30.1, respectively. Of the 45 species examined, the encephalization index of the chimpanzee was second only to that of humans. It should be noted that the gorilla brain is larger than that of the chimpanzee, but due to the gorilla's huge body mass, its encephalization index is less than that of the chimpanzee. It should be obvious that brain size and function can vary independently of body mass, so modest differences in encephalization index should not be regarded as indicating differences in cognitive functioning or intelligence; however, the difference is enormous between humans and chimpanzees, both in encephalization index and absolute brain size (Fig. 29.1). Interestingly, recent data suggest that although the absolute brain volume varies considerably among hominoids, the relative volume and cellular composition of certain regions, such as the prefrontal cortex, remain quite constant among these taxa (Semendeferi *et al*., 1997; Semendeferi and Damasio, 2000).

Another indicator of relative brain size can be obtained by measuring endocranial volumes. Holloway (1974, 1996) has used endocranial casts of fossil hominid skulls to measure size constraints and organizational features apparent form shape. Tobias (1971, 1996) reviewed the cranial volumes that had been found in studies of a large number of great ape skulls and related these data to those obtained for fossil *Australopithecus* and *Homo*, as well as modern humans (see also Falk, 1986). The endocranial volumes for the great apes were the following (pooled male and female data sets): orangutans, 404.8 cm^3 (n = 402); gorillas, 504.6 cm^3 (n = 668); bonobo, 343.7 cm^3 (n = 11); and chimpanzee, 383.4 cm^3 (n =363). The corresponding values for *Australopithecus* range from 350 to 530 cm^3, well within the range of extant great ape species. Early *Homo* (*habilis*) endocranial volume ranged from about 450 cm^3 to nearly 900 cm^3, and *H. erectus* averaged more than 900 cm^3, ranging from about 650 cm^3 to nearly 1400 cm^3—within the range of modern humans (mean = 1370 cm^3; range, 1070–1670). Interestingly, classic Neanderthal brain size (mean = 1470 cm^3; range, 1145–1795) was greater than that of modern humans.

Endocasts of *Australopithecus* crania do not usually provide much detail regarding the topical features of the brains they contained (Holloway, 1974; Eccles,1989; but see also Kochetkova, 1978). The features shared by *Australopithecus* specimens from South Africa and East Africa but not by extant apes are sumarized by Tobias (1996). Some controversy exists over the degree to which sulcal patterns are evident from these endocasts. The most important point is that modest differences in the topography of sulci and gyri may or may not indicate functional changes underlying these landmarks. While it seems likely that some reorganization of the brain was already in progress in *Australopithecus*, we may never know what these changes were. The endocasts of early *Homo* (*habilis*), however, reveal a dozen, or so, clear departures from the patterns seen in extant apes (summarized by Tobias, 1996). The changes in size and shape suggest that a dramatic reorganization of the brain was in progress as *H. erectus* emerged and eventually gave rise to *H. sapiens*. The morphological changes evident within the past 2 million years may have increased human vulnerability to age-related changes, but many age-related phenomena encountered by present-day humans seldom, if ever, could have been experienced during the relatively brief life spans of our ancestors. Human life expectancy has increased dramatically in recent times (over 120 years in one documented case) by comparison with that which was typical throughout the evolutionary history of *Homo*. The life expectancy of great apes in the wild is certainly less than 30 years, but in captivity, with the benefits of hygiene, nutrition, and health care, they can live beyond 40, 50, and possibly 60 (Erwin *et al*., 1998) (Fig. 29.2).

III. History

Comparative brain anatomy studies of great apes were underway early in the 20th century, including studies of orangutan and chimpanzee (Campbell, 1905), orangutan (Mauss, 1911), chimpanzee (Brodmann, 1912), and gorilla (Bolk, 1910). These included examination not only of surface topography, but also detailed microscopic examination of underlying tissue and documentation of homologies between ape and human brain areas (Campbell, 1905). Research on the effects of ablations and lesions on learning, memory, and emotionality continued for many years (Blum, 1948; Rosvold *et al*., 1961; see Markowitsch, 1988, for a review). Especially significant was a series of studies by Bailey and colleagues comparing and mapping human and chimpanzee neocortex (Bailey, 1948; Bailey *et al*., 1950). While these studies did not focus

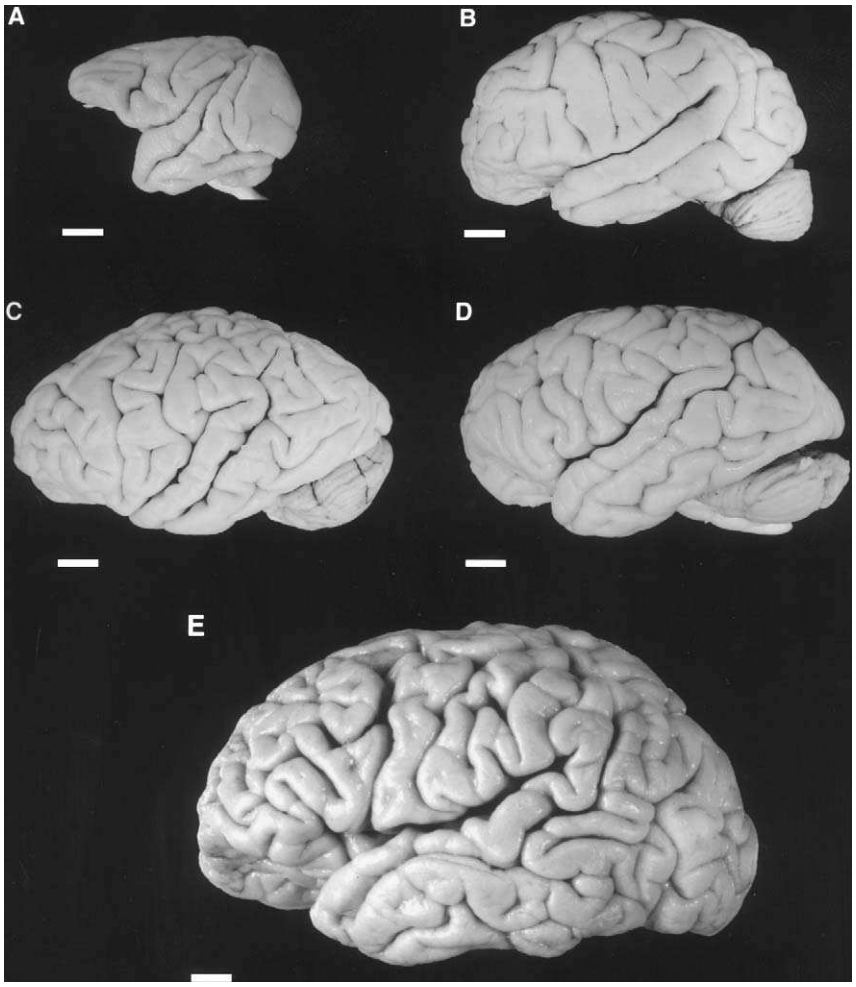

FIG. 29.1. Lateral views of the left hemisphere of the brains of (A) a 19-year-old patas monkey (*Erythrocebus patas*), (B) a 37-year-old Sumatran orangutan (*Pongo pygmaeus abelii*), (C) a 48-year-old Western Lowland gorilla (*Gorilla gorilla gorilla*), (D) a 45-year-old common chimpanzee (*Pan troglodytes*), and (E) a nondemented 82-year-old human. All specimens are shown at the same scale (scale bars, 1 cm). Note the similar size of the gorilla and chimpanzee specimens and the considerable increase in the volume of the frontal and temporal regions in hominids compared to the patas monkey. Although all of these specimens were obtained from relatively old individuals, no gross age-related alterations are visible.

on aging, the results are useful in understanding the functional neurobiology of great ape and human brains. It is unlikely that further ablation or lesion studies will be done on great apes. Most invasive research involving great apes has ceased, partly due to studies such as those cited below that indicate how similar the great apes are to humans.

IV. Communication

Many attempts have been made to establish language communication with great apes. Two notable (unsuccessful) attempts to teach chimpanzees to speak were those of Kellogg and Kellogg (1933) and Hayes and Hayes (1951). Another attempt at sophisticated communication was more successful. Premack used nonverbal symbols on a form board to successfully establish a system of communication with a chimpanzee named ''Sarah'' (Premack, 1971). Even though chimpanzees lack the ability to produce articulated speech (Lenneberg, 1975), they were found to be capable of a remarkable facility to use sign language and other artificial languages, and several successful demonstrations of this have been carried out with chimpanzees (Gardner and Gardner, 1969; Fouts, 1974; Rumbaugh, 1977; Matsuzawa, 1999), orangutans (Miles, 1980), a gorilla named ''Koko'' (Patterson and Cohn 1990), and a most remarkable bonobo named ''Kanzi'' (Savage-Rumbaugh and Lewin, 1994). The extent to which language production and comprehension of symbolic communication have been demonstrated is astonishing. While the communication systems established in these cases do not constitute actual human language, they certainly *approach* human language and challenge alternative explanations. A few groups have recently revisited the issue of functional asymmetry of language areas in the brain (Gannon *et al*., 1998a,b, 1999; Hopkins *et al*., 1998; Kheck *et al*., 1998). They have reported that the anatomic pattern and left hemisphere size predominance of the

FIG. 29.2. Photograph of an elderly female common chimpanzee. This individual is close to her 6th decade of life and is known, according to monitoring by the zoo keepers, to exhibit what may represent age-related cognitive and behavioral disturbances. More than 200 chimpanzees older than 35 years live in various facilities thoughout the world.

planum temporale, a language area of the human brain (Wernicke's posterior receptive language area), are also present in chimpanzees. This discovery suggests that human language functions evolved within an anatomic substrate that was already lateralized to the left hemisphere in the common ancestor of humans and chimpanzees 5–8 million years ago. Functional imaging studies of humans and some of the chimpanzees that have been involved in communication and language studies may reveal whether or not homologous brain regions are used for similar tasks. One such study compared two language-experienced chimpanzees, ''Panzee'' and ''Lana,'' with humans (Rilling *et al*., 1999). Humans, but not chimpanzees, exhibited lateralized activation of Heschl's gyrus, planum temporale, and frontal cortical areas. The two chimpanzees exhibited different patterns of activation from the humans and from each other, suggesting that different neural substrates are used for language-like processing in chimpanzees than are typically used for language in humans.

V. Tool Use and Culture

The invention, manufacture, and use of tools by the great apes exceeds that exhibited by any other nonhuman. The use of tools to solve problems was impressively demonstrated by Koehler (1925) with captive chimpanzees in the Canary Islands. Many other investigators replicated and extended this work (Yerkes, 1943; Beck, 1980). The most impressive reports of tool use have been those from field studies, in which great apes, especially chimpanzees, were reported to manufacture and use tools to crack nuts (Nishida, 1973; Boesch and Boesch, 1983) or retrieve termites from termite mounds (Goodall, 1964; Suzuki, 1966; McGrew, 1974; for a review, see Candland, 1987). Tool use in captivity and the wild have been directly compared (Matsuzawa, 1999). The case has recently been made that the differing tool-using traditions observed in various chimpanzee populations constitute distinct ''cultures'' (Whiten *et al*., 1999).

VI. Self-Awareness

The question of whether or not nonhumans are aware of themselves has been a matter of much speculation. Gallup (1970) devised a clever method of objectively addressing this question by studying responses of animals to their mirror images. Most of the animals tested responded to their mirror image as if the reflection were another animal. Gallup argued that this indicated a lack of self-concept. He observed that chimpanzees responded differently. They at first acted as if their reflection was another animal, but after a period of 2 or 3 days of mirror exposure they began to engage in self-directed behavior while observing the reflection of themselves doing so. Gallup devised a ''mark test'' in which chimpanzees were anesthetized, marked with red dye on an eyebrow ridge and ear, and allowed to regain consciousness. When they were again exposed to the mirror they immediately responded by touching and inspecting the marks while watching the reflection. ''Visual self-recognition'' in chimpanzees and orangutans was replicated by several investigators and shown to be a reliable phenomenon. Numerous other primate species were tested using this technique, but all failed to exhibit self-recognition (Gallup, 1987). Although gorillas that were specifically tested did not demonstrate self-awareness (Suarez and Gallup, 1981; Ledbetter and Basin, 1982), Patterson (1978) reported that ''Koko'' gorilla engaged in purposive self-directed behavior using mirrors. The demonstration that humans and great apes, but not lesser apes or other primates, exhibit visual self-awareness is an intriguing finding that suggests an exceptional cognitive capacity. Some of the great apes involved in language and cognition research are now elderly. It will be interesting to see how their cognition is affected by aging.

VII. Maps, Math, and Models

The evidence for the development of cognitive maps was reviewed by Meador *et al*. (1987). Among the most compelling studies reported were those of Menzel *et al*. (1978). Chimpanzees who were allowed to watch on a small black-and-white television monitor a caretaker hiding food in an outdoor enclosure were more efficient at finding the hidden food than were those who were not allowed to view the monitor. More recently, Boysen and Berntsen (1995) demonstrated that some chimpanzees (but not others) were able to use information from a scale model to solve a similar hidden reward problem. Matsuzawa (1985) and his colleagues (Biro and Matsuzawa, 1999) have been studying the use of numbers by a chimpanzee named ''Ai'' and have recently reported responses to introduction of the numerical concept ''zero.'' In other studies, Boysen demonstrated ordinality and transitivity in chimpanzees and found an interesting paradox in quantitative cognition (Boysen *et al*., 1993; Boysen and Berntson, 1995; see Deacon, 1997, for discussion). Given the choice between two piles of candy, chimpanzees could not inhibit choice of the larger pile, even if that meant that they were rewarded only with the smaller pile. This

response is similar to that observed in human children. When Arabic numerals were substituted for the piles of candy the chimpanzees could solve the problem. Boysen is currently developing noninvasive methods of assessing cognitive decline associated with aging and is currently working with Premack's "Sarah," who is over 40 years of age.

VIII. Nervous System and Aging

What do nervous systems do, and how are their functions vulnerable to the effects of aging? Willott (1999) sets forth seven fundamental functions of nervous systems, including the following: (a) maintain vital functions; (b) obtain information; (c) store information; (d) produce behavior; (e) modulate overall activity; (f) integrate information and behavior; (g) promote reproduction. Aging is likely to affect every aspect of the nervous system from control of vital functions and sensory input to memory and behavior. Few aspects of aging have been addressed in the great apes, partly because so few great apes in the past reached very advanced age in captivity, and partly due to the difficulty of obtaining a sufficient number of brains from great apes of different ages. This problem is currently being addressed by our Great Ape Aging Project, which includes a great ape central nervous system tissue bank component (Erwin *et al*., 1998, 1999, 2000).

One way of prioritizing neurobiology of aging studies of great apes is to focus first on points of vulnerability in humans. The sensory systems responsible for informational input almost inevitably decline with aging, including audition, vision, chemical senses (olfaction and taste), somatosensory modes (touch, pressure, heat, cold, pain, proprioception, etc.), and vestibular sensation, with some of the deficits occurring in peripheral sensory mechanisms and others resulting from central processing mechanisms. The processing of visual information, for example, is extremely complex for primates (Allman and McGuinness, 1988; Kaas and Huerta, 1988; Rodieck, 1988; Yin and Medjbeur, 1988), as are audition (Newman, 1988) and the somatosensory system (Kaas and Pons, 1988). Interestingly, a recent study has demonstrated unique patterns of expression of cytoskeletal proteins in the human primary visual cortex, as well as derived features in this cortical region in humans and great apes compared to that seen in Old and New World monkeys (Preuss *et al*., 1999). There is a need to understand not only how these systems are affected by aging but how they differ among the various primates including humans. Methods of testing for peripheral sensory deficits can include the use of visual evoked potentials (Boysen and Berntson, 1985), auditory evoked potentials (Kraus *et al*., 1985), and other innovative techniques. These clinical assessments can contribute to fundamental advances in knowledge relevant to aging, but they can also be of great value for clinical diagnosis of peripheral sensory deficits for individuals. Fundamental comparative data on motor systems are also important to an understanding of the processes of aging and to the treatment of age-related disorders, but few studies to date have focused on aging of motor systems in the great apes. Relevant reviews are available regarding primate motor cortex (Hepp-Reymond, 1988), neural control of vocalization (Sutton and Jürgens, 1988), and cerebellum (Haines, 1986).

The central processing mechanisms involved in learning and memory functions can change with age. Some of the structures involved are the hippocampus, neocortex, basal forebrain, amygdala, thalamus and hypothalamus, and the basal ganglia. The hippocampus and associated regions, such as entorhinal cortex, are especially implicated in some neurodegenerative processes associated with aging in humans, so comparative studies of cell populations in this area are important.

IX. Entorhinal Cortex

As a part of the Great Ape Aging Project we have initiated neuropathology screening of great ape brains using stereologic methods. We are focusing on regions that are severely affected in Alzheimer's disease (AD) and are involved early by age-related neuropathologic changes in humans. To analyze the cellular characteristics of brain aging in the great apes, we estimated the total number and cell volume of neurons in layer II of the entorhinal cortex using stereologic tools in a series of 18 postmortem brain specimens of common chimpanzees ages 1 to more than 45 years. There was no apparent neuron loss in the oldest case relative to the younger ones, and no significant shrinkage of mean neuronal volume related to age was detected (Erwin *et al*., 1999; Hof *et al*., 1999) (Figs. 29.3, see color insert, and 29.4). These data are similar to those from rhesus macaques (Peters *et al*., 1996, 1998; Gazzaley *et al*., 1997; O'Donnell *et al*., 1999; Hof *et al*., 2000). They also resemble the pattern seen in brains from healthy elderly humans, in which degenerative disorders are not present—but they do not resemble the results of studies of humans exhibiting neurodegenerative pathology. Additional studies are needed, including studies of the brains of older individuals with or without accompanying neuropathology. Perhaps chimpanzees are just not vulnerable to cell loss and loss of cell volume. If they are not, we must learn why, and find ways of using that knowledge to forestall the onset of neurodegenerative pathology in humans.

X. Senile Plaques and Neurofibrillary Tangles

Up to now, β-amyloid plaques have been found in the brains of elderly great apes (Gearing *et al*., 1994, 1997), but the neurofibrillary tangles typical of AD have not been reported for great apes. We know of only two reports of neurofibrillary tangles in nonhuman primates, one for an elderly rhesus macaque (Cork *et al*., 1989) and another, more recently, for elderly hamadryas baboons and guenons (Härtig *et al*., 2000; Schultz *et al*., 2000). These animals ages 26 and 30 years at the time of death, exhibited a combination of neuronal and glial cytoskeletal pathology that preferentially affected limbic areas such as the hippocampus and amygdala. In the hippocampus of the older animal, numerous tau-immunoreactive inclusions were seen in the granule cells of the dentate gyrus. The granule cells even displayed argyrophilic neurofibrillary tangles. The cytoskeletal changes of glial cells, previously unreported in nonhuman primates, affected both astrocytes and oligodendrocytes. The occurrence of neurofibrillary tangles in a nonhuman primate suggests that vulnerability extends beyond humans.

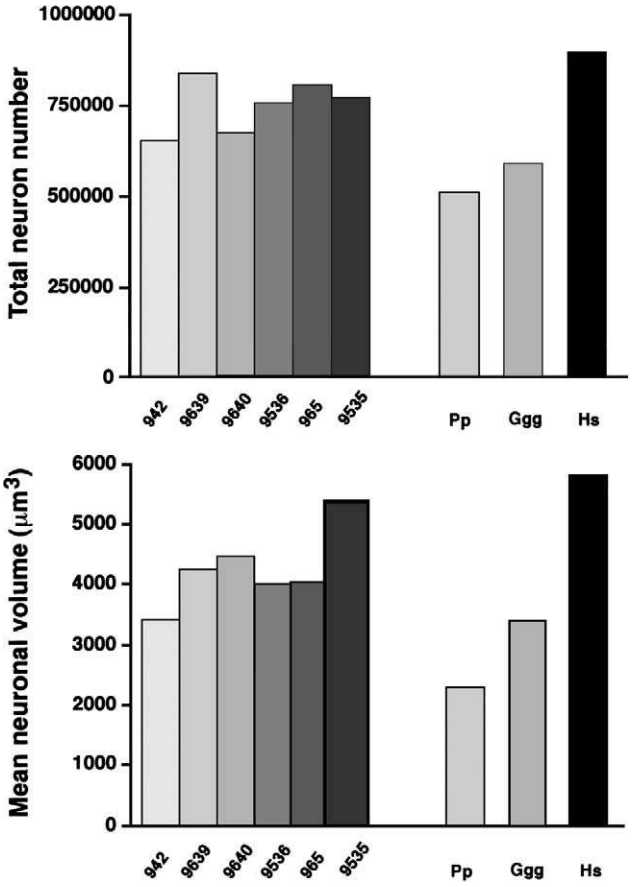

FIG. 29.4. Quantitative analysis of the total number of neurons (top) and mean neuronal volume (bottom) in layer II of the entorhinal cortex in a series of six chimpanzees (13–45 years old, see bars at left on each graph) and, for comparison, in a 13-year-old orangutan (Pp), a 34-year-old gorilla (Ggg), and an 81-year-old normal human (Hs). There are no age-related differences among the chimpanzees for these parameters. Overall, it appears that the orangutan and the gorilla have smaller and fewer layer II neurons than *Pan* and *Homo*.

XI. Unique Neurons in Anterior Cingulate Cortex

The cerebral cortex is reduced in volume in the brain of elderly victims of AD and this is associated with neural cell loss (for reviews, see Morrison and Hof, 1997; Willott, 1999). Large pyramidal cells in the frontal, parietal, and temporal lobes are among the most vulnerable neurons, and the death of these cells certainly disrupts cognitive function. Cells in various cortical layers are differentially affected. For example, layers I and V in the entorhinal cortex have the greatest concentrations of neurofibrillary tangles. The cingulate cortex is also vulnerable to cell loss in AD. Nimchinsky *et al*. (1995) found that the abundance of a large spindle neuron located in layer Vb of the anterior cingulate cortex (subareas 24a and 24b, following the nomenclature of Vogt *et al*., 1995) was reduced by about 60% in the brains of AD victims relative to that seen in normal controls. These neurons are characterized by a very elongate, gradually tapering, large-sized soma that is virtually symmetrical about its vertical and horizontal axes, as well as a light staining pattern with Nissl stain (Fig. 29.5A, see color insert). The first description of this type of neuron was by Betz (1881), and it had been documented a few times prior to the discovery of its vulnerability in AD. Unlike most other cell types in the neocortex, spindle neurons seemed to be uniquely human. They were not present, for example, in the anterior cingulate cortex of rhesus macaques. A literature review revealed a single report of this cell type in a chimpanzee (Rose, 1927). Nimchinsky *et al*. (1999) assembled anterior cingulate cortex specimens from 28 primate species, ranging from mouse lemurs to humans, including specimens from great apes. None of the prosimian, New World monkey, Old World monkey, or gibbon specimens yielded any spindle cells. The spindle cells were sparse, but were clearly present in the orangutan specimens. They were more abundant in gorilla, and even more so in common chimpanzee (Fig. 29.5B, see color insert). In the bonobo, spindle cells were not only abundant, but were clustered in a manner very similar to that seen in humans. Spindle cell volumes were estimated from measures of samples from each individual specimen. Those from humans were more than twice as large as those from chimpanzees and bonobos, while those for orangutans and gorillas were smaller yet. The functions of these spindle cells are not yet known for certain, so we do not know what the functional implications of differential cell volume might be. Spindle neurons are not the only cell type affected in AD, nor is AD neuron loss confined to the anterior cingulate cortex, but this region of the brain clearly is involved in attention, memory, emotionality, vocalization, and other functions, including visual self-recognition. A second neuron type, an intermediate size pyramidal cell that contains the calcium-binding protein calretinin, has now been discovered in the same region. The prospect that the presence of these cell types might be involved in phenomena such as self-recognition is intriguing. The anterior cingulate cortex is connected with the hippocampus and amygdala and the neocortex. Disruption of such circuitry would surely have dramatic consequences, possibly including some of the memory and visual recognition disorders, as well as autonomic and language deficits that accompany AD.

Age-related loss of spindle cells in great apes has not yet been documented—nor has any evidence of AD beyond deposition of β-amyloid been found in great apes. The brains thus far examined, however, have all been from individual great apes who had not been reported to show signs of dementia. The Great Ape Aging Project currently is obtaining behavioral profiles, cognitive assessments, and videographic documentation of motor behavior of elderly great apes in zoos and research institutes. These data in addition to clinical records and caretaker reports will be used to identify individual great apes who may exhibit some impairment. Following natural death, neuropathology assessments are conducted to identify neurodegeneration if it exists.

XII. The Future of Ape Research

The role of zoos is tremendous in caring for great apes and participating in cooperative projects such as the Great Ape Aging Project. All the orangutans and gorillas and most of

the bonobos involved in the project reside in zoological gardens. The vast majority of captive common chimpanzees live in research facilities—more than 1600 of them (Fig. 29.2). Many of these, especially the older ones, have been designated as "surplus to research needs." In the year 2000, there were more than 200 chimpanzees over 35 years of age. A small number of the oldest ones were provided with full or partial support from the Great Ape Aging Project with some funding from the National Institute on Aging. We are hopeful that more of these individuals will be supported in comfortable circumstances for noninvasive studies of aging. The maximum life span of chimpanzees is above 60 years, and other great apes have lived into their 50s. They are susceptible to many of the same age-related diseases and disorders as are humans, including arthritis, osteoporosis, diabetes, benign prostate hypertrophy, and cardiovascular disease. Further study, especially of elderly individuals may also reveal instances of cancer, Parkinson's disease, and AD. Genetic risk factors for humans and the great apes can be compared and basic studies of neurochemistry and neurogenetics may be compared between humans and our nearest biological kin. We should learn what we can from worms, fruit flies, zebrafish, and mice, and we should continue the precise and productive experimental neuroscience research with nonhuman primates such as rhesus macaques; but we also have an obligation and an opportunity to continue learning from the great apes in ways that contribute to their well being and our own.

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References

Allman, J., and McGuinness, E. (1988). Visual cortex in primates. *In* "Comparative Primate Biology" (D. Steklis and J. Erwin, eds.), Vol. 4, pp. 279–326. Alan R. Liss, New York.

Bailey, P. (1948). Concerning cytoarchitecture of the frontal lobe of chimpanzee (*Pan satyrus*) and man (*Homo sapiens*). *In* "The Frontal Lobes" (J. Fulton, C. Aring, and S. Wortis, eds.), pp. 84–94. Williams & Wilkins, Baltimore, MD.

Bailey, P., von Bonin, G., and McCulloch, W. (1950). "The Isocortex of the Chimpanzee." University of Illinois Press, Urbana.

Beck, B. (1980). "Animal Tool Behavior: The Use and Manufacture of Tools By Animals." Garland STPM Press, New York.

Betz, W. (1881). Über die feinere Struktur der Gehirnrinde des Menschen. *Z. Med. Wiss.* **19**, 193–195, 209–213, 231–234.

Biro, D., and Matsuzawa, T. (1999). Numerical ordering in chimpanzee (*Pan troglodytes*): Planning, executing, and monitoring. *J. Comp. Psychol*. **113**, 178–185.

Blum, R. (1948). The effect of bilateral removal of the prefrontal granular cortex on delayed response performance and emotionality in chimpanzee. *Am. Psychologist* **3**, 237–238.

Boesch, C., and Boesch, H. (1983). Optimization of nut-cracking with natural hammers by wild chimpanzees. *Behaviour* **83**, 265–285.

Bolk, L. (1910). Beiträge zur Affenanatomie: Das Gehirn von Gorilla. *Z. Morphol. Anthropol*. **12A**, 141–242.

Boysen, S., and Berntson, G. (1985). Visual evoked potentials in the great apes. *Electroencephalogr. Clin. Neurophysiol*. **62**, 150–153.

Boysen, S., and Berntson, G. (1995). Responses to quantity: Perceptual versus cognitive mechanisms in chimpanzees (*Pan troglodytes*). *J. Exp. Psychol.: Anim. Behav. Process.* **21**, 82–86.

Boysen, S., Berntson, G., Shreyer, T., and Quigley, K. (1993). Processing of ordinality and transitivity by chimpanzees (*Pan troglodytes*). *J. Comp. Psychol*. **107**, 208–215.

Brodmann, K. (1912). Ergebnisse über die vergleichende histologische Lokalisation der Grosshirnrinde mit besonderer Berücksichtigung des Stirnhirns. *Anat. Anz.* **41**(Suppl.), 157–216.

Campbell, A. (1905). "Histological Studies on the Localization of Cerebral Function." Cambridge University Press, Cambridge, UK.

Candland, D. (1987). Tool use. *In* "Comparative Primate Biology" (G. Mitchell and J. Erwin, eds.), Vol. 2B, pp. 85–103. Alan R. Liss, New York.

Ciochon, R. (1983). Hominoid cladistics and the ancestry of modern apes and humans—a summary statement. *In* "New Interpretations of Ape and Human Ancestry" (R. Ciochon and R. Corruccini, eds.), pp. 783–843. Plenum, New York.

Cork, L., Walker, L., and Price, D. (1989). Neurofibrilary tangles and senile plaques in cognitively impaired, aged nonhuman primates. *J. Neuropathol. Exp. Neurol*. **48**, 378.

Deacon, T. (1997). "The Symbolic Species: The Coevolution of Language and the Brain." Norton, New York.

Eccles, J. (1989). "Evolution of the Brain: Creation of the Self." Routledge, London.

Erwin, J. M., Perl, D. P., Nimchinsky, E. A., and Hof, P. R. (1999). Stereologic analyses of neuron number and volume in the entorhinal cortex of aged chimpanzees. *Soc. Neurosci. Abstr.* **25**, 105.

Erwin, J. M., Bloomsmith, M., Boysen, S., Hof, P. R., Holloway, R., Lowenstine, L., McManamon, R., Nimchinsky, E., Perl, D., Reite, M., Young, W., and Zihlman, A. (1998). Development of a comparative neurobiology of aging resource: Progress during the first year. *Am. J. Primatol*. **45**, 179.

Erwin, J. M., Hof, P. R., Gannon, P., Holloway, R., and Perl, D. (2000). A research resource for comparative and evolutionary primate biology. *Am. J. Phys. Anthropol*. **30** (Suppl.), 146–147.

Falk, D. (1986). Endocranial casts and their significance for primate brain evolution. *In* "Comparative Primate Biology" (D. Swindler and J. Erwin, eds.), Vol.1, pp. 477–490. Alan R. Liss, New York.

Fouts, R. (1974). Origins, language, and chimpanzees. *J. Hum. Evol*. **3**, 475–482.

Gallup, G. (1970). Chimpanzees: Self-recognition. *Science* **167**, 86–87.

Gallup, G. (1987). Self-Awareness. *In* "Comparative Primate Biology" (G. Mitchell and J. Erwin, eds.), Vol. 2B, pp. 3–16. Alan R.Liss, New York.

Gannon, P. J., Holloway, R. L., Broadfield, D. C., and Braun, A. R. (1998a). Asymmetry of chimpanzee planum temporale: Humanlike pattern of Wernicke's brain language area homologue. *Science* **279**, 220–222.

Gannon, P. J, Broadfield, D. C., Kheck, N. M., Hof, P. R., Braun, A. R., Erwin, J. M., and Holloway, R. L. (1998b). Brain language area evolution I: Anatomic expression of Heschl's gyrus and planum temporale asymmetry in great apes, lesser apes, and Old World monkeys. *Soc. Neurosci. Abstr.* **24**, 160.

Gannon, P. J., Kheck, N. M., and Hof, P. R. (1999). Brain language area evolution. III: Left hemisphere predominant asymmetry of cytoarchitectonic, but not gross anatomic, planum temporale homolog in Old World monkeys. *Soc. Neurosci. Abstr.* **25**, 105.

Gannt, D. (1986). Enamel thickness and ultrastructure in hominoids: With reference to form, function, and phylogeny. *In* "Comparative Primate Biology" (D. Swindler and J. Erwin, eds.), Vol. 1, pp. 453–475. Alan R. Liss, New York.

Gardner, R., and Gardner, B. (1969). Teaching sign language to a chimpanzee. *Science* **165**, 664–672.

Gazzaley, A. H., Thakker, M. M., Hof, P. R., and Morrison, J. H. (1997). Preserved number of entorhinal cortex layer II neurons in aged macaque monkeys. *Neurobiol. Aging* **18**, 549–553.

Gearing, M., Rebeck, G., Hyman, B. T, Tigges, J., and Mirra, S. S. (1994). Neuropathology and apolipoprotein E profile of aged chimpanzees: Implications for Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 9382–9386.

Gearing, M., Tigges, J., Mori, H., and Mirra, S. S. (1997). Beta-amyloid (Aβ) deposition in the brains of aged orangutans. *Neurobiol. Aging* **18**, 139–146.

Goodall, J. (1964). Tool-using and aimed throwing in a community of free-living Chimpanzees. *Symp. Zool. Soc. London*. **10**, 1264–1266.

Goodman, M. (1974). Biochemical evidence on hominid phylogeny. *Annu. Rev. Anthropol*. **3**, 203–228.

Groves, C. (1986). Systematics of the great apes. *In* "Comparative Primate Biology" (D. Swindler and J. Erwin, eds.), Vol. 1, pp. 187–217. Alan R. Liss, New York.

Haines, D. (1986). The primate cerebellum. *In* "Comparative Primate Biology" (D. Swindler and J. Erwin, eds.), Vol. 1, pp. 491–535. Alan R. Liss, New York.

Härtig, W., Klein, C., Brauer K., Schüppel, C. F., Asendt, T., Brückner, G., and Bigl, V. (2000). Abnormally phosphorylated protein tau in the cortex of aged individuals of various mammalian orders. *Acta Neuropathol.* **100**, 305–312.

Hayes, K., and Hayes, C. (1951). The intellectual development of a home-raised Chimpanzee. *Proc. Am. Psychol. Soc*. **95**, 105–109.

Hepp-Reymond, M. C. (1988). Functional organization of motor cortex and its participation in voluntary movements. *In* "Comparative Primate Biology" (H. Stelkis and J. Erwin, eds.), Vol. 4, pp. 601–624. Alan R. Liss, New York.

Hof, P. R., Perl, D. P., Nimchinsky, E. A., and Erwin, J. M. (1999). The entorhinal cortex in aged chimpanzees (*Pan troglodytes*): Stereologic analyses of neuron number and volume. *Am. J. Primatol*. **49**, 60–61.

Hof, P. R., Nimchinsky, E. A., Young, W. G., and Morrison, J. H. (2000). Numbers of Meynert and layer IVB cells in area V1: A stereologic analysis in young and aged macaque monkeys. *J. Comp. Neurol*. **420**, 113–126.

Holloway, R. (1974). The casts of fossil hominid brains. *Sci. Am*. **231**, 106–115.

Holloway, R. (1996). Toward a synthetic theory of human brain evolution. *In* "Origins of the Human Brain" (J. P. Changeux and J. Chavaillon, eds.), pp. 42–60. Clarendon Press, Oxford.

Hopkins, W. D., Marino, L., Rilling, J. K., and MacGregor, L. A. (1998). Planum temporale asymmetries in great apes as revealed by magnetic resonance imaging (MRI). *NeuroReport* **9**, 2913–2918.

Kaas, J., and Huerta, M. (1988). The subcortical visual system of primates. *In* "Comparative Primate Biology" (H. Stelkis and J. Erwin, eds.), Vol. 4, pp. 327–391. Alan R. Liss, New York.

Kaas, J., and Pons, T. (1988). The somatosensory system of primates. *In* "Comparative Primate Biology" (H. Stelkis and J. Erwin, eds.), Vol. 4, pp. 421–468. Alan R. Liss, New York.

Kelley, J., and Pilbeam, D. (1986). The Dryopithecines: Taxonomy, comparative anatomy, and phylogeny of Miocene large Hominoids. *In* "Comparative Primate Biology" (D. Swindler and J. Erwin, eds.), Vol. 1, pp. 361–411. Alan R. Liss, New York.

Kellogg, W., and Kellogg, L. (1933). "The Ape and the Child." McGraw-Hill, New York.

Kheck, N., Gannon, P., Hof, P., Braun, A., and Erwin, J. (1998). Brain language area evolution. II: Human-like pattern of hemispheric asymmetry in planum parietale of chimpanzees. *Soc. Neurosci. Abstr.* **24**, 160.

Kochetkova, V. (1978). "Paleoneurology." V. H. Winston, Washington, DC.

Koehler, W. (1925). "The Mentality of Apes." Harcourt Brace, New York.

Kraus, N., Smith, D., Reed, N., Willott, J., and Erwin, J. (1985). Auditory brainstem and middle latency responses in nonhuman primates. *Hear. Res.* **17**, 219–226.

Ledbetter, D., and Basen, J. (1982). Failure to demonstrate self-recognition in gorillas. *Am. J. Primatol*. **2**, 307–310.

Lenneberg, E. (1975). A neuropsychological comparison between man, chimpanzee, and monkey. *Neuropsychologica* **13**, 125.

Markowitsch, H. (1988). Anatomical and functional organization of the primate prefrontal cortical system. *In* "Comparative Primate Biology" (H. Stelkis and J. Erwin, eds.), Vol. 4, pp. 99–153. Alan R. Liss, New York.

Matsuzawa, T. (1985). Use of numbers by a chimpanzee. *Nature (London)* **315**, 57–59.

Matsuzawa, T. (1999). Intelligence and culture in chimpanzees. *In* "Evolution of the Apes and Origin of the Human Beings" (O. Takenaka, ed.), p. 43. Primate Research Institute of Kyoto University, Inuyama.

Mauss, T. (1911). Die Faserarchitektonische Gliederung des Cortex Cerebri der anthropomorphen Affen. *J. Psychol. Neurol*. **18**, 410–467.

McGrew, W. (1974). Tool use by wild chimpanzees in feeding upon driver ants. *J. Hum. Evol*. **3**, 501–508.

McHenry, H. (1984). The common ancestor. *In* "The Pygmy Chimpanzee" (R. Susman, ed.), pp. 201–230. Plenum, New York.

Meador, D., Rumbaugh, D., Pate, J., and Bard, K. (1987). Learning, problem solving, cognition, and intelligence. *In* "Comparative Primate Biology" (G. Mitchell and J. Erwin, eds.), Vol. 2B, pp. 17–83. Alan R. Liss, New York.

Menzel, E., Premack, D., and Woodruff, G. (1978). Map reading by chimpanzees. *Folia Primatol*. **12**, 241–249.

Miles, L. (1980). Acquisition of gestural signs by an infant orangutan (*Pongo pygmaeus*). *Am. J. Phys. Anthropol.* **52**, 256–257.

Morrison, J. H., and Hof, P. R. (1997). Life and death of neurons in the aging brain. *Science* **278**, 412–419.

Newman, J. (1988). Primate hearing mechanisms. *In* "Comparative Primate Biology" (H. Stelkis and J. Erwin, eds.), Vol. 4, pp. 469–499. Alan R. Liss, New York.

Nimchinsky, E. A., Vogt, B. A., Morrison, J. H., and Hof, P. R. (1995). Spindle neurons of the human anterior cingulate cortex. *J. Comp. Neurol*. **355**, 27–37.

Nimchinsky, E. A, Gilissen, E., Allman, J. M., Perl, D. P., Erwin, J. M., and Hof, P. R. (1999). A neuronal morphologic type unique to humans and great apes. *Proc. Natl. Acad. Sci. U.S.A*. **96**, 5268–5273.

Nishida, T. (1973). The ant-gathering behavior by the use of tools among wild chimpanzees of the Mahale Mountains. *J. Hum. Evol*. **2**, 357–370.

O'Donnell, K. A., Rapp, P. R., and Hof, P. R. (1999). The volume of prefrontal cortex area 46 is preserved during aging in macaque monkeys. *Exp. Neurol*. **160**, 300–310.

Patterson, F. (1978). Conversations with a gorilla. *Natl. Geogr.* **154**, 438–465.

Patterson, F., and Cohn, R. (1990). Language acquisition by a lowland gorilla: Koko's first ten years of vocabulary development. *Word* **41**, 97–143.

Peters, A., Rosene, D., Moss, M., Kemper, T., Abraham, C., Tigges, J., and Albert, M. (1996). Neurobiological bases of age-related cognitive decline in the rhesus monkey. *J. Neuropathol. Exp. Neurol*. **55**, 861–874.

Peters, A., Morrison, J. H., Rosene, D., and Hyman, B. T. (1998). Are neurons lost from the primate cerebral cortex during normal aging? *Cereb. Cortex* **8**, 295–300.

Premack, D. (1971). Language in a chimpanzee? *Science* **172**, 808–822.

Preuss, T. M., Qi, H., and Kaas, J. H. (1999). Duistinctive compartmental organization of human primary visual cortex. *Proc. Natl. Acad. Sci. U.S.A*. **96**, 11601–11606.

Rilling, J. K., and Insel, T. R. (1998). Evolution of the cerebellum in primates: Differences in relative volume among monkeys, apes and humans. *Brain Behav. Evol*. **52**, 308–314.

Rilling, J. K., and Insel, T. R. (1999). Differential expansion of neural projection systems in primate brain evolution. *NeuroReport* **10**, 1453–1459.

Rilling, J. K., Kilts, C., Williams, S., Kelley, J., Beran, M., Giroux, M., Hoffman, J., Savage-Rumbaugh, S., and Rumbaugh, D. (1999). Functional neuroimaging of linguistic processing in chimpanzees. *In* "Evolution of the Apes and the Origin of the Human Beings" (O. Takenaka, ed.), p. 51. Primate Research Institute of Kyoto University, Inuyama.

Rodieck, R. (1988). The primate retina. *In* "Comparative Primate Biology" (H. Stelkis and J. Erwin, eds.), Vol. 4, pp. 203–278. Alan R. Liss, New York.

Rose, M. (1927). Gyrus limbicus anterior und Regio retrosplenialis (Cortex holoprotoptychos quinquestratificatus)—Vergleichende Architektonik bei Tier und Menschen. *J. Psychol. Neurol*. **35**, 5–217.

Rosvold, H., Szarcbart, M., Mirsky, A., and Mishkin, M. (1961). The effect of frontal lobe damage on delayed response performance in chimpanzees. *J. Comp. Physiol. Psychol*. **54**, 368–374.

Rumbaugh, D. (1977). "Language Learning by a Chimpanzee: The Lana Project." Academic Press, New York.

Sarich, V., and Wilson, A. (1967). Immunological time scale for hominid evolution. *Science* **158**, 1200–1203.

Savage-Rumbaugh, S., and Lewin, R. (1994). "Kanzi: The Ape at the Brink of the Human Mind." Wiley, New York.

Schultz, C., Dehghani, F., Hubbard, G., Thal, D., Struckhoff, G., Braak, E., and Braak, H. (2000). Filamentous tau pathology in nerve cells, astrocytes, and oligodendrocytes of aged baboons. *J. Neuropathol. Exp. Neurol*. **59**, 39–52.

Semendeferi, K., and Damasio, H. (2000). The brain and its main anatomical subdivisions in living hominoids using magnetic resonance imaging. *J. Hum. Evol*. **38**, 317–332.

Semendeferi, K., Damasio, H., Frank, R., and Van Hoesen, G. W. (1997). The evolution of the frontal lobe: A volumetric analysis based on three-dimensional reconstruction scans of human and ape brains. *J. Hum. Evol*. **32**, 375–388.

Stephan, H., Frahm, H., and Baron, G. (1981). New and revised data on volumes of brain structures in insectivores and primates. *Folia Primatol*. **35**, 1–29.

Stephan, H., Baron, G., and Frahm, H. (1988). Comparative size of brains and brain components. *In* "Comparative Primate Biology" (H. Stelkis and J. Erwin, eds.), Vol. 4, pp. 1–38. Alan R. Liss, New York.

Suarez, S., and Gallup, G. (1981). Self-recognition in chimpanzees and orangutans, but not gorillas. *J. Hum. Evol*. **10**, 175–188.

Sutton, D., and Jürgens, U. (1988). Neural control of vocalizations. *In* "Comparative Primate Biology" (H. Stelkis and J. Erwin, eds.), Vol. 4, pp. 625-647. Alan R. Liss, New York.

Suzuki, A. (1966). On the insect-eating habits among wild chimpanzees living in the savanna woodland of western Tanzania. *Primates* **7**, 481–487.

Tobias, P. (1971). "The Brain in Hominid Evolution." Columbia University Press, New York.

Tobias, P. (1996). The brain of the first hominids. *In* "Origins of the Human Brain" (J. P. Changeux and J. Chavaillon, eds.), pp. 61–83. Clarendon Press, Oxford.

Tuttle, R. (1999). Are human beings apes, or are apes people too? *In* "Evolution of the Apes and the Origin of the Human Beings" (O. Takenaka, ed.), p. 72. Primate Research Institute of Kyoto University, Inuyama.

Vogt, B. A., Nimchinsky, E. A., Vogt, L. J., and Hof, P. R. (1995). Human cingulate cortex: Surface features, flat maps, and cytoarchitecture. *J. Comp. Neurol*. **359**, 490–506.

Whiten, A., Goodall, J., McGrew, W., Nishida, T., Reynolds, V., Sugiyama, Y., Tutin, C., Wrangham, R., and Boesch, C. (1999). Cultures in chimpanzees. *Nature (London)* **399**, 682–685.

Willott, J. (1999). "Neurogerontology: Aging and the Nervous System." Springer, New York.

Yerkes, R. (1943). "Chimpanzees: A Laboratory Colony." Yale University Press, New Haven, CT.

Yin, T., and Medjbeur, S. (1988). Cortical association areas and visual attention. *In* "Comparative Primate Biology" (H. Stelkis and J. Erwin, eds.), Vol. 4, pp. 393–419. Alan R. Liss, New York.

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Elizabeth Head, Norton William Milgram, and Carl W. Cotman

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Neurobiological Models of Aging in the Dog and Other Vertebrate Species

The rodent and nonhuman primate are not the only species to develop neuropathologic lesions similar to those seen in the aged human brain. Dogs and cats develop diffuse senile plaques with age, but do not show neurofibrillary tangle formation. The goat, sheep, bear, and wolverine, by contrast, are reported to develop neurofibrillary-like lesions but, in the first two cases, not senile plaques. The only one of these six species that has been extensively studied, however, is the dog, and the neurobiology of aging in canines is therefore the main focus of this chapter. Dogs exhibit extensive individual variability in the aging process on measures of learning and memory and in extent of β-amyloid (Aβ) deposition in the brain. Dogs, like humans, also show variability in cognitive aging. Some dogs develop severe cognitive impairment; these same dogs develop extensive Aβ deposition. Other dogs remain cognitively intact, and also show little or only moderate Aβ deposition. These findings suggest that dogs can be used to model the earliest phases of cognitive and neuroanatomical changes associated with pathological aging in humans. In addition, the existence of aged dogs that can learn and remember as well as younger dogs may also offer us insight into promoting successful aging in humans.

I. Introduction

Previous chapters in this volume have discussed the behavior and anatomy of the aging rat and the nonhuman primate. The present chapter will discuss the functional neurobiology of aging in other vertebrate species (excluding nonhuman primates, see Chapters 26–29). Our primary emphasis will be on the dog, but we will also include descriptions of the sheep, goat, bear, wolverine, and cat. Both dogs and cats are appropriate models for the study of aging in humans since they share many of the same age-related diseases such as arthritis, cardiovascular disease, cancer, bone loss, hypertension, and atherosclerosis (Committee on Animal Models for Research on Aging, 1981). As this chapter will also discuss, many of these other vertebrate species develop some of the hallmark lesions of both normal and pathological aging in the human brain.

Companion animals, such as the dog and the cat, provide two unique advantages as aging models. First, they inhabit environments similar to those of their owners, which may provide a unique opportunity to evaluate the effects of environmental variables on the aging process. Second, the possibility for analysis of genetic contributions to both brain and cognitive aging can be studied by taking advantage of the presence of different breeds of dogs and cats and studying littermates (Russell *et al*., 1992; Bobik *et al*., 1994).

Our underlying strategy for studying aging in the dog is to use a clinical-neuropathology approach in which we first give dogs a battery of tasks in an effort to test the function of several cognitive domains such as spatial attention or learning. In using this approach we have modeled our evaluation of the cognitive function of dogs after that with humans where both global and then more specific tests such as those for memory, learning, and visuospatial skills are administered. We combine cognitive evaluations of our dogs with extensive analyses of neuropathology in an effort to determine whether it is possible to detect various forms of pathology in the brain on the basis of behavior.

II. Cognitive Function and Aging in the Dog

Cognitive aging in humans is a complex process. Cognitive deterioration does not occur uniformly; some cognitive abilities show age-related decline, whereas others remain relatively intact. Striking individual differences are more likely the rule than the exception; some individuals show little decline (successful agers), some show mild decline (age-associated memory impairment), and yet others of the same age may develop dementia. As we will discuss in more detail later in the chapter, the same pattern of individual differences in aging occurs in dogs, with some showing evidence of successful aging, others showing relatively mild impairments, and still others showing severe impairment corresponding, perhaps, to a global dementia. Also, we have evidence that learning pro-

cesses and memory processes are dissociable in dogs, and we believe that age-associated learning impairments can occur independently of age-related memory impairments and vice versa.

A. Dogs Show Age-Related Learning Impairments That Are Task-Dependent

This section will describe age effects in dogs on tests of simple procedural learning tasks, associative learning problems, and more complex rule-learning problems.

1. Age and Previous Experience Play a Role in Procedural Learning

Procedural learning tasks are those that reflect a form of skill learning such as learning to ride a bike. In dogs, procedural learning is used to describe tasks given to teach the skills necessary to work in the testing apparatus. We have developed standardized procedures for the initial training of dogs in the Toronto General Test Apparatus, which is a modified form of the Wisconsin General Test Apparatus used to test cognitive function in nonhuman primates (Milgram *et al*., 1994). After training dogs to expect a food reward, the dogs are then tested on a procedure called reward approach learning. In this task, animals are rewarded if they respond to the sight of food. After learning this, the dogs are then trained on object approach learning, which is intended to teach dogs the skills to manipulate objects by pushing them away from a food well to reveal the reward. The first task appears trivial, but that does not seem to be the case for many of our dogs. Learning these types of skills appears to depend on both the age of the dog and potentially previous experience. For example, aged random-source dogs that were formerly companion animals learned these skills as quickly as younger random-source dogs (Milgram *et al*., 1994; Head *et al*., 1998). On the other hand, kennel-reared beagle dogs showed evidence of slower learning on these same tasks.

2. Associative Learning: Simple and Complex Discriminations Have Differential Age Sensitivities

Associative learning tasks involve the establishment of a link between two events as a consequence of repeated pairing of those events. Discrimination learning is one example of an associative learning task in which an animal is required to establish an association between a particular stimulus and delivery of a reward, while another stimulus is not associated with a reward. Dogs readily acquire simple discrimination learning problems, such as a visually based object discrimination task where the two stimuli are different objects (Milgram *et al*., 1994).

We have also looked at more complex types of discrimination tasks. These tasks include a size discrimination task (where the objects are identical in all respects except for size), a double discrimination task (two pairs of objects are used in alternating trials), concurrent discrimination learning (10 pairs of objects presented once per day), and olfactory discrimination learning (where the objects are associated with different odors) (Head *et al*., 1996, 1998).

Discrimination learning, in other animal models, is typically insensitive to age, except when the discrimination is difficult (Bartus *et al*., 1979; Arnsten and Goldman-Rakic, 1985; Moss *et al*., 1988; Markowska *et al*., 1989; Rapp, 1990; Baxter and Gallagher, 1996). We have obtained similar findings in dogs. We typically find no difference between old and young dogs on a simple object discrimination task. We do, however, find greater variability among old animals. This is probably because some aged dogs show a global impairment on all tests of cognitive function, and this can also include impairments on simple discrimination problems.

The individual variability in discrimination learning tasks, in general, may be a consequence of existing object preferences. We tested this hypothesis directly by determining object preferences prior to training on a simple object discrimination learning task. Assigning a preferred object as a positive stimulus to aged dogs actually conferred an advantage over that of young dogs, resulting in a trend toward lower error scores. On the other hand, aged dogs assigned a nonpreferred object performed more poorly than young dogs. Thus, existing object preferences lead to higher or lower error scores on a visual discrimination task, which is particularly true for older dogs (Head *et al*., 1998).

One possible explanation for this effect is increased perseverative responding in aged dogs, which is likely dependent upon frontal lobe function (Brush *et al*., 1961; Mishkin *et al*., 1964). In fact, as will be discussed later in the chapter, visual discrimination learning using a nonpreferred object is indeed sensitive to prefrontal cortex neuropathology (Head *et al*., 1998). This may also be the reason for olfactory discrimination impairments in aged dogs; although detection thresholds were similar in young and old dogs, an olfactory task is sensitive to orbitoventral frontal lobe dysfunction (Allen, 1939, 1943).

3. Complex Rule Learning Tasks Reveal Three Groups of Aged Dogs: Successful Agers, Impaired, and Severely Impaired

More complex learning tasks are also age-sensitive in dogs and other animals. These include both complex discrimination learning and tasks such as reversal learning, requiring both the inhibition of an existing response and the acquisition of a new response. In addition, the acquisition of a new association appears to be age-sensitive in dogs and other animals (Bartus *et al*., 1979; Milgram *et al*., 1994; Head *et al*., 1998) but see (Rapp, 1990; Rapp and Amaral, 1991). One hypothesis is that these more complex rule-learning tasks recruit several brain regions or a cortical circuit, leading to a greater chance of detecting brain dysfunction. For example, size discrimination learning requires the intact function of a ventral processing stream thought to underlie object identification (Mishkin, 1972; Felleman and van Essen, 1991; van Essen *et al*., 1992). This ventral processing stream consists of a circuit beginning with visual input from the primary visual cortex that is transfered to the striate cortex and then ventrally into the inferotemporal cortex. Reversal learning of an object discrimination task would also require this ventral processing stream in addition to the orbitoventral prefrontal cortex (Mishkin, 1964; Jones and Mishkin, 1972).

An object-recognition memory task, which is typically used in monkeys and has also been applied to dogs, tests the function of several components of the ventral processing stream

(Mishkin and Delacour, 1975; Milgram *et al*., 1994). The first component of this problem is teaching animals the nonmatching-to-sample rule necessary to perform the task. Dogs are first shown a single object covering a food reward in the center of a presentation tray. Following a brief delay period, dogs are presented with the previous object and a novel object each covering the left and right food wells. The correct response is for dogs to select the novel object. Our first study of object recognition memory acquisition in dogs suggested that old dogs were impaired relative to young dogs, which was consistent with impairments found in aged monkeys (Moss *et al*., 1988; Rapp and Amaral, 1989, 1991; Milgram *et al*., 1994). The most notable observation was that the task was extremely difficult, and only a small proportion of the subjects was able to meet a very weak learning criterion. We have subsequently discovered that dogs are capable of excellent performance on this task if we take into consideration that the dogs' visual near point is between 25 and 30 cm (Callahan *et al*., in press).

Whereas object recognition memory is thought to rely upon the ventral processing stream, another form of complex rule learning problem, spatial learning, is thought to depend on a dorsal processing stream (Mishkin, 1972; Felleman and van Essen, 1991; van Essen *et al*., 1992). The dorsal stream includes the visual cortex, the posterior parietal cortex and the dorsolateral prefrontal cortex. We can test dogs for their ability to learn a spatial nonmatching-to-sample rule using a similar procedure as that described for object recognition memory training. Dogs are first shown an object covering a food reward on either the left or right side of the presentation tray. After a brief delay interval, dogs are offered a choice between selecting two identical objects, one each covering both the left and right food wells. The correct response is to select the object on the side opposite to that seen previously. When testing dogs with a spatial memory task, delayed nonmatching to position, consistent age effects, has been found (Head *et al*., 1995; Adams *et al*., 2000). The results of these studies indicate that aged dogs are impaired when learning the nonmatching-to-position rule. More importantly, it is possible to differentiate three groups of aged dogs on the basis of spatial learning, that some old dogs obtain scores similar or better than young dogs (unimpaired), some obtain scores that are poorer (impaired), and others obtained scores that are impaired relative to other old dogs (severely impaired) (Fig. 30.1)

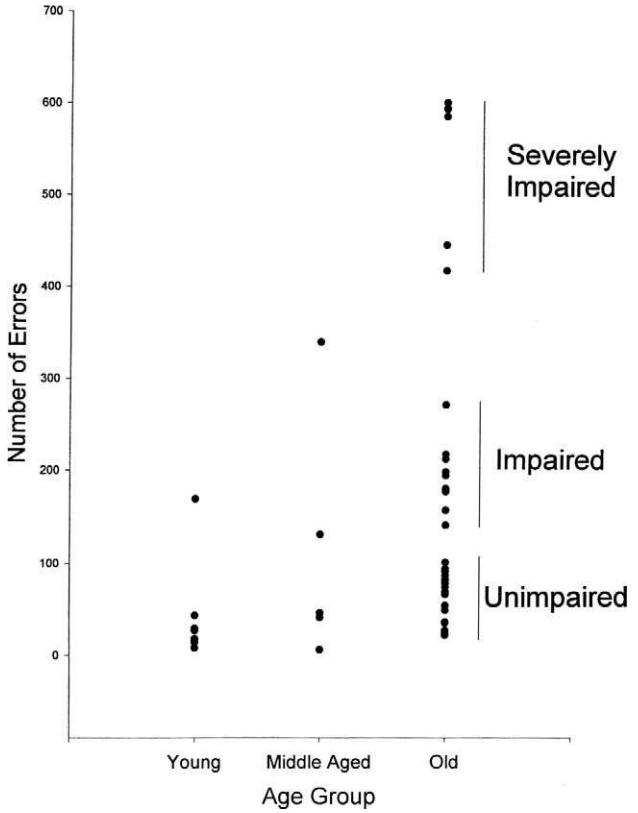

FIG. 30.1. Spatial learning as a function of age in dogs reveals that old dogs can be characterized as belonging to one of three groups: unimpaired (scores fall within the range of young dogs' scores), impaired (scores fall outside of those obtained by young dogs), and severely impaired (at least one standard deviation outside the average of the old dogs).

B. Memory Tasks Reveal Striking Differences in Young and Old Dogs

In humans, memory impairment is one of the most important criteria for diagnosing dementia associated with neurodegenerative disorders such as Alzheimer's disease. We have two protocols for studying memory in the dog. One involves measuring accuracy of responses as a function of increasing memory demands (working memory) and the second involves progressively increasing the memory demand until a dog fails a task (maximal memory). These are dissociable from deficits in learning ability, as will be shown shortly.

As discussed in the previous section, spatial learning ability is compromised in a subset of aged dogs. However, some old dogs can learn the rules of the task as adequately or as rapidly as younger dogs but then go on to have difficulty when the memory demands are increased. We have developed two different protocols for studying the effect of memory demands on spatial abilities. The first uses a variable delay paradigm in which the interval between the initial presentation of an object on the left or right side of the presentation tray and the choice presentation is set at either 20, 70, or 110 sec. Typically, all dogs show a decline in accuracy scores with increasing memory demands, which can be plotted as a "memory curve." We typically see much more variation in old dogs on this task than we do in young dogs. The large majority of young dogs show a very small performance decrement with increasing delays. This is also true of some old dogs. Others, by contrast, show a marked decrement (Head *et al*., 1995). This age-dependent memory impairment is not necessarily associated with an impairment in learning the rules of the task. Thus, dogs can show dissociable age effects in spatial learning and in memory; one old dog may be impaired in learning a task, but once acquired can remember information as long as younger dogs. The opposite can also occur, where an aged dog can learn the task as quickly as a younger dog but cannot attain the same accuracy as the memory demands are increased.

The second protocol involves progressively increasing the delay interval over which dogs must remember information in a stepwise procedure (Adams *et al*., 2000). Prior to advancing to a longer delay interval, and thus higher memory

demand, dogs are required to reach a criterion level of responding. Dogs are tested until they fail to reach criterion in a set number of test sessions. It is this delay interval where dogs can no longer reach a criterion level of accuracy that we call the maximal memory capacity. Young dogs can typically solve the spatial memory task with delays ranging up to 210 sec. Old dogs have a shorter spatial memory capacity and on average can reach delays of only 50 sec.

C. Individual Variability in Learning and Memory Is a Consistent Feature of Aging in Dogs

Aged dogs are not globally deficient across all cognitive tasks and not all old dogs are impaired. Some of the individual variability in aged dogs may be related to factors such as breed differences, previous experience, reproductive status, and selection biases. Our preliminary cognitive studies in dogs included two populations: random source pound dogs and kennel-reared beagle dogs. Although young dogs from both populations learned the cognitive tasks with a similar number of errors and trials, aged dogs showed some very interesting differences, suggesting a possible age by source interaction. In some tasks, aged random source dogs were deficient and in others they performed with fewer errors than both young and old beagle dogs. Some of the variability may be a result of differences in previous experience. For example, aged kennel-reared beagle dogs were poor at learning procedural-type tasks, whereas old random-source dogs learned as well as younger dogs; this may be due to the lack of experience of beagle dogs and thus these tasks are a form of new learning.

D. Clinical Indices of Cognitive Dysfunction in Pet Dogs

As in humans, tests of neuropsychological function can yield results that are quite different from tests of daily function. A similar phenomenon occurs in dogs. Pet dogs are evaluated in a clinic and global indices of behavioral dysfunction in dogs indicate a number of age-related deficits. In a survey of 26 pet dogs owners with dogs greater than or equal to 10 years of age the most common complaints were destructive behavior in the house, inappropriate urination or defecation, and excessive vocalization. In some cases, pet owners noticed an increase in separation anxiety characterized as an increase in vocalization when the owner is absent (Ruehl *et al*., 1995). These behavioral symptoms are observed in dogs that were normal when younger and the existence of other medical conditions to account for abnormal behavior have been eliminated (Chapman and Voith, 1990). In fact, one sign of canine cognitive dysfunction frequently used by veterinarians is the existence of urinary incontinence, which typically results in the pet owner's decision to euthanize their pet (Mosier, 1988).

III. Neuropathology in Aging Dogs

The pattern of intact and impaired learning and memory abilities in aging dogs suggests a selective age-associated vulnerability in specific brain circuits. Whereas the first goal of our work is to characterize cognitive changes as a function of age, the second major goal of our development of the canine model of human aging is to characterize the pattern of underlying brain pathology. This section will describe candidate pathologies including neuronal loss, the deposition of β-amyloid (Aβ) in the form of diffuse senile plaques, and pretangle formation in aged dogs.

A. Aged Dogs Show Evidence of Neuron Loss and Dysfunction

The issue of the occurrence of neuronal loss during normal aging has been controversial (Coleman and Flood, 1987; Flood and Coleman, 1988). Neuron loss in the human brain was thought to be a consistent feature of aging as measured by decreased brain weight at death, cortical atrophy, and increased ventricular volume both at autopsy and during magnetic resonance image (MRI) scans (Katzman and Terry, 1992). One reasonable explanation for the loss of brain tissue is the loss of neurons and recent evidence provided by unbiased stereological cell counting techniques seems to suggest significant cell number losses in the subiculum and hilus of the dentate gyrus of the hippocampus, but not area CA1, with age (West, 1993). In this respect, normal aging contrasts with a dramatic loss of area CA1 neurons in the hippocampus of Alzheimer's disease brain (West *et al*., 1994). Stereological studies to detect cell loss in the aged dog brain have not been conducted; however, there are reports of selective neuron loss in layers III and V of the prefrontal, temporal, and occipital cortex along with the claustrum (Wisniewski *et al*., 1970; Morys *et al*., 1994). More recent evidence of cortical atrophy was found in an MRI experiment examining changes in cortical volume with age in a group of beagle dogs ranging in age from 4.5 to 15.3 years (Su *et al*., 1998). After the age of 10 years, the size of the lateral ventricles increased exponentially until the age of 15 years (Fig. 30.2). These data suggest the need for controlled, unbiased sampling stereological techniques for neuron counts in aged dog brains.

A number of other morphological hallmarks of dysfunction in aging neurons can be seen in the dog brain. Neurons in the prefrontal cortex of aged dogs (13–18 years) show distorted soma, loss of dendritic spines and shrinkage of dendritic branches, tortuous apical dendrites (Mervis, 1978). Similar changes have also been reported in hippocampal neurons of the human brain (Scheibel *et al*., 1975, 1976, 1977; Mervis, 1978). These changes may also be reflected as a loss of synapses as has been demonstrated in the Alzheimer's disease brain (Terry *et al*., 1991), but a similar study has yet to be conducted in aged canine brain tissue. This is an area that requires further research since synaptic loss is more likely to correlate with cognitive dysfunction than gross structural changes *per se*.

Another potential indicator of neuron dysfunction is the presence of DNA damage. As with aging human and Alzheimer's disease brain, aged dogs show an increase in the extent of neurons with damaged DNA (Anderson *et al*., 1996; Cotman and Su, 1996; Kiatipattanasakul *et al*., 1996). The study by Kiatipattanasakul and colleagues (1996) did not find any significant correlation between DNA damage and the extent of Aβ deposition nor were apoptotic bodies observed. Anderson *et al*. (1997), on the other hand, did find a significant correlation between DNA and Aβ deposition. We have observed apoptotic bodies

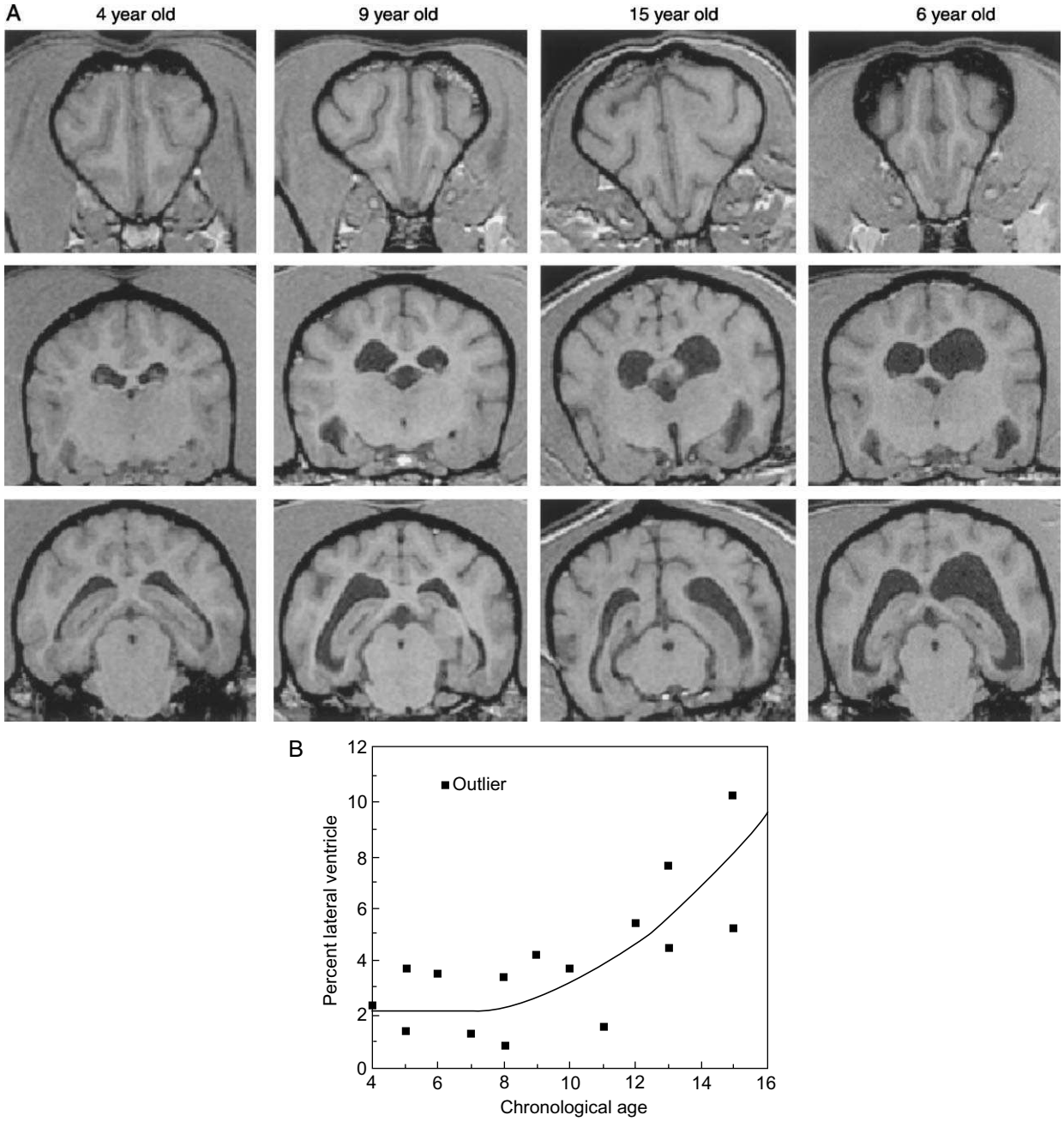

FIG. 30.2. (A) T2-weighted anatomical images obtained from a group of beagle dogs with representative samples from a 4-, a 9-, a 15-year-old dog. Note the age-associated increase in ventricular volume and cortical atrophy. The images on the far right are from a 6-year-old female beagle that showed premature Aβ deposition. (B) Graph illustrating that ventricular volume increases with age in an exponential function with volumes increasing dramatically after 11 years of age. Reprinted from Su *et al*. (1998), with permission from Elsevier Science.

in at least one aged beagle dog that had extensive Aβ deposition and was cognitively impaired (Fig. 30.3, see color insert).

B. β-Amyloid Deposition Is a Consistent Feature of Aging in Dogs

One of the hallmarks of pathology in the human brain is the formation of senile plaques in several cortical and subcortical brain regions. Aβ, the principal component of senile plaques, is a hydrophobic 39- to 43-amino-acid peptide that can adopt a β-pleated sheet configuration. Aβ is derived from the proteolytic processing of a longer amyloid precursor protein (APP), a member of a large family of 70 kDa transmembrane glycoproteins derived from alternative splicing of precursor mRNA (Kang *et al*., 1987; Price *et al*., 1991; Selkoe, 1994, 1996). The sequence of the APP is identical in dogs and humans (Johnstone *et al*., 1991). In addition, there are several cleavage products of APP but dogs deposit predominantly the longer

42/43-amino-acid-long peptide rather than the shorter 39/40 protein (Cummings *et al*., 1996b; Wegiel *et al*., 1996; Wisniewski *et al*., 1996; Nakamura *et al*., 1997). There are many features of Aβ deposits in the dog brain that parallel those seen in human brain, including similar morphology, extent of deposition with age, the pattern of deposition, the types of proteins associated with these deposits, and association with blood vessels.

The morphology of Aβ deposits in aged dog brain is that of a diffuse subtype (Fig. 30.4, see color insert). These plaques are not stained by thioflavin S and therefore probably lack β-pleated sheet formation (Cummings *et al*., 1993). These diffuse deposits appear to contain intact neurons, which are also seen in the brains of individuals with Down syndrome and in normal elderly human brain (Fig. 30.4). The extent of Aβ deposition in the dog brain does not appear to vary significantly with age in studies where a number of different breeds of dogs are included in the sample (Wegiel *et al*., 1996; Yoshino *et al*., 1996). However, in studies where a single breed of dog is used with a broad range of ages, the correlation between extent of Aβ and age at death is significant (Cummings *et al*., 1993; Cummings *et al*., 1996b; Russell *et al*., 1996; Head *et al*., 1998). The lack of an age association most likely reflects the fact that a heterogenous population of dogs was used and there is evidence of breed differences in the rate and age of onset of Aβ deposition in dogs (Bobik *et al*., 1994). Breed differences may also be a consequence of differential aging rates in large breeds of dogs as compared to smaller breeds where typically the former have a shorter life span. Thus, making the assumption that a 10-year-old larger breed of dog is the same ''biological age'' as a smaller breed may not be entirely valid and emphasizes the importance of controlling for variability due to breed.

Although Aβ deposition in the dog brain increases with age, as with human aging, not all old dogs are affected to the same extent. Another factor that contributes to the individual variability in Aβ deposition is dependent upon the brain region sampled. The pattern of deposition of Aβ as a function of age in dogs is similar to that of humans in terms of regional vulnerabilities (Braak *et al*., 1993; Head *et al*., 1998, 2000). The earliest and most consistent site of Aβ deposition is in the prefrontal cortex, with the development of senile plaques in the parietal and occipital lobe at a later age. In the entorhinal cortex, early deposition of Aβ is seen only in a subset of aged dogs. Within individual cortical layers, Aβ is first deposited in deep layers adjacent to white matter and these clouds appear to coalesce into more compact deposits, later appearing in more superficial layers with increasing age (Satou *et al*., 1997). Unlike humans with AD, diffuse plaques are rarely, if ever, seen in the molecular layer (layer I) of cortex in the dog (Fig. 30.4).

Aβ deposition in the canine also varies within brain structures. In the hippocampus, a cloud of diffuse deposition has been consistently found in the outer molecular layer (Cummings *et al*., 1993; Osmand and Switzer, 1992). This is the terminal field of the perforant pathway, which are the axons and terminals of layer II entorhinal neurons (Steward, 1976; Steward and Scoville, 1976). Diffuse deposition in this brain region strongly suggests a neuronal origin for Aβ; deposition can occur as a result of aberrant processing of APP within neurite terminals (Koo *et al*., 1990). In addition, more recent reports show that entorhinal cortex lesions given to PDAPP transgenic mice lead to a loss of Aβ deposition in the outer molecular layer (Chen *et al*., 1998).

Several proteins coexist within senile plaques in the dog brain that may play a role in the formation of these lesions. The sequence of APP, for example, contains domains that are homologous to Kunitz-type serine protease inhibitors (Kitaguchi *et al*., 1988; Ponte *et al*., 1988; Tanzi *et al*., 1988). APP is deposited in swollen neurites in senile plaques of both aged dog and human brain (Catteruccia *et al*., 1990; Younkin, 1991; Okuda *et al*., 1994). This is in addition to the presence of other factors that are associated with senile plaques in the aged dog brain, which are listed in Table 30.1.

Aged dogs also develop Aβ angiopathy, which has been reviewed in more detail elsewhere (Walker, 1997). Aβ angiopathy in the dog consists of both the longer and the shorter Aβ species as reported in Alzheimer's disease brain and in cats (Cummings *et al*., 1996b). We have also noticed that the occipital lobes of aged dogs are particularly vulnerable to this form of pathology, which is also the case in the human brain (Katzman and Terry, 1992). Aβ angiopathy in the dog brain is also associated with intense ApoE immunoreactivity (Uchida *et al*., 1997), which is interesting in light of the fact that ApoE is a risk factor associated with Alzheimer's disease in humans (Saunders *et al*., 1993).

C. Aged Dogs Do Not Develop Neurofibrillary Tangles

Neurofibrillary tangles consist of abnormally phosphorylated tau proteins, which, in their normal state, bind to microtubules and stabilize them to form a functioning cytoskeleton in neurons (Goedert, 1993; Trojanowski *et al*., 1993a,b;

TABLE 30.1 Constituents of Senile Plaques in the Aged Dog Brain

Marker	Present/absent	Reference
Cathepsin B	−	Uchida *et al*., 1997
Cathepsin D	+	Uchida *et al*., 1997
Cystatin C	+	Uchida *et al*., 1997
	+	Uchida *et al*., 1992a
Antichymotrypsin	+	Uchida *et al*., 1997
	−	Uchida *et al*., 1992b
HSP70	−	Uchida *et al*., 1997
Ubiquitin	+	Uchida *et al*., 1997
	+	Uchida *et al*., 1992a
	+	Okuda *et al*., 1994
ApoE	+	Uchida *et al*., 1997
Tau	Rare	Uchida *et al*., 1992a
	−	Okuda *et al*., 1994
	−	Cummings *et al*., 1993
HSGAG	−	Cummings *et al*., 1993
bFGF	−	Cummings *et al*., 1993
APP	+	Okuda *et al*., 1994
SOD	+	Kiatipattanasakul *et al*., 1997

Tucker, 1990). In the human literature, several studies demonstrate a relationship between cognitive status and the extent of neurofibrillary degeneration in aging human brain and in Alzheimer's disease (Dayan, 1970b; Alafuzoff *et al*., 1987; Braak and Braak, 1991, 1995; Dickson *et al*., 1995; Dournaud *et al*., 1995; Langui *et al*., 1995; Duyckaerts *et al*., 1997). One of the drawbacks to almost all animal models studied to date, except possibly the bear, wolverine, goat, and sheep (to be discussed at the end of the chapter) and in the mouse lemur (see Chapter 27), is the lack of spontaneously occurring neurofibrillary tangle formation with age. Dogs do not develop full-blown tangles (Ball *et al*., 1983; Selkoe *et al*., 1987; Giaccone *et al*., 1990; Wisniewski *et al*., 1990; Cummings *et al*., 1993). Although aged dogs do not develop full-blown neurofibrillary tangles, several reports suggest the development of early tangle formation (Ball *et al*., 1983; Selkoe *et al*., 1987; Giaccone *et al*., 1990; Wisniewski *et al*., 1990; Cummings *et al*., 1993). Another study reported neurofibrillary targles in dogs but the morphology of these were not identical to those seen in human brain at the ultrastructural level of analysis (Fisher *et al*., 1986). Our studies of aging dog brain have used the marker for early neurofibrillary tangles, AT8, and our preliminary data indicate that middle-aged dogs, but not old dogs, appear to have hyperphosphorylated tau in select populations of neurons (Fig. 30.5, see color insert). These never appear to progress to full neurofibrillary tangles.

One possible explanation for a lack of neurofibrillary tangle formation in aged dog brain is that the tau protein itself is different from the human tau protein. A preliminary RT-PCR analysis certainly suggests isoform differences from human tau with apparent excesses in four repeat sequences, which is similar to transgenic mice that also do not develop neurofibrillary tangles despite extensive Aβ deposition (M. Hutton, personal communication).

IV. Functional Neurobiology of Aging in the Dog

Can we account for the selectivity in age-associated cognitive dysfunction by selectivity in the development of neuropathology? As discussed previously, not all aged dogs are cognitively impaired, and chronological age is not the best predictor of cognitive status, which parallels human aging (Rowe and Kahn, 1987; Albert and Funkenstein, 1992). There must be some other factor rather than age *per se* that can account for the variability in the aging process in dogs as it does in humans. To approach this question, the fact that not all old dogs are impaired, becomes a useful tool for identifying markers of neuropathology that can differentiate the two groups.

Our first study of the relationship between cognition and neuropathology examined the extent of Aβ deposition in a group of cognitively characterized dogs. A group of 29 dogs ranging in age from 1.6 to 12.3 years had been tested for reward and object approach learning, visual discrimination and reversal, and spatial and object recognition memory. The prefrontal cortex, hippocampus, and entorhinal cortex was examined for the extent of Aβ deposition using image analysis techniques. A discriminant analysis of the cognitive test scores indicated that two major clusters were present in the behavioral data, one that we termed procedural-type tasks (reward and object approach learning) and the second termed declarative-type tasks (visual discrimination, reversal, and memory tasks). The declarative-type tasks, but not the procedural-type tasks were strongly correlated with Aβ deposition in both the prefrontal and entorhinal cortices. In fact, up to 68.9% of the variability in cognitive test scores could be accounted for by the amount of Aβ deposition.

In a second study, a more detailed examination was made of 20 beagle dogs ranging in age from 4.5 to 15.3 years of age. In these dogs, we tested object and reward approach learning (which we previously established to be independent of Aβ deposition), object discrimination learning using either a preferred or a nonpreferred object, and size discrimination learning along with long-term retention. Based on the nonhuman primate literature, we predicted that some tasks would be sensitive to prefrontal lobe pathology (reversal learning, object discrimination learning with a nonpreferred object) and that others would be sensitive to temporal lobe function (size discrimination task). Dogs were first classified as being impaired or unimpaired based upon individual error scores obtained from the cognitive tasks listed previously. An impaired dog was defined as an animal that obtained an error score falling outside the range of error scores obtained by the young dogs. If dogs are separated on this basis then those old dogs that were impaired had higher amounts of Aβ deposition. In addition, as illustrated in Fig. 30.6, dogs with impairments in reversal learning and in object discrimination learning with a nonpreferred object accumulated significantly more Aβ in the prefrontal cortex than unimpaired dogs. This was also true for size discrimination and reward approach learning; impaired dogs had higher amounts of Aβ in the entorhinal cortex. Thus, it may be possible to predict the presence of Aβ deposition in specific brain regions based upon cognitive test scores (Head *et al*., 1998).

Significant associations between extent of Aβ pathology and cognitive dysfunction in the dog parallels that reported in humans (Blessed *et al*., 1968; Dayan 1970a; Wisniewski, 1979; Alafuzoff *et al*., 1987; Duyckaerts *et al*., 1990; Price *et al*., 1991; Cummings and Cotman, 1995; Dickson *et al*., 1995; Langui *et al*., 1995; Cummings *et al*., 1996c). The fact that not all old dogs are impaired is consistent with taking a random sample of old individuals: we would expect a mix of successfully aging individuals in addition to those suffering from some type of neurodegenerative disorder resulting in increasing individual variability with increasing age.

V. Aging Cats: Behavior and Neuropathology

Behavioral information on aging cats is primarily anecdotal. The most common behavioral problems noted in geriatric pet cats are related to secondary age-related diseases such as arthritis, cardiovascular or renal disease, and diabetes. In addition, sensory decline in visual and auditory ability occurs with age in cats (Harrison and Buchwald, 1982). Aged cats show a heightened sensitivity to changes in the environment, which can cause changes in eating or patterns of elimination or possibly even aggression (Houpt and Beaver, 1981). Other age-linked behavioral deficits include reduced classical condi-

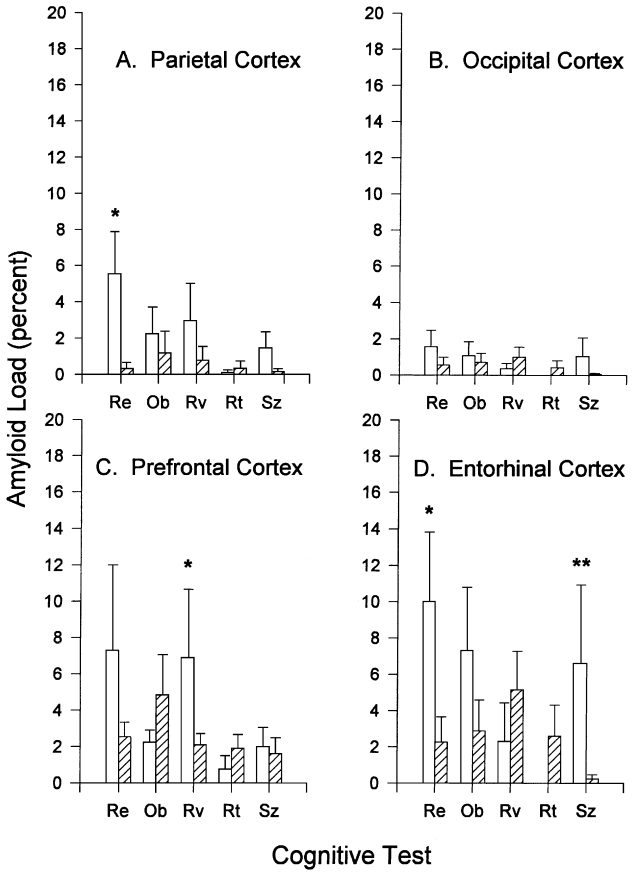

FIG. 30.6. The relationship between cognitive task, cognitive status, and Aβ accumulation is represented as a function of brain region: (A) parietal cortex, (B) occipital cortex, (C) prefrontal cortex, and (D) entorhinal cortex. Aged dogs were classified as being impaired or unimpaired for each behavioral task and the amount of Aβ present in each brain region was compared. Re, reward approach learning; Ob, object approach learning; Rv, reversal learning; Rt, retention testing; Sz, size discrimination learning. $^{*}p < 0.05$;$^{**}p < 0.075$. Reprinted from Head *et al.* (1998), with permission from Elsevier Science.

tioning and disturbances in patterns of habituation in tests of locomotor activity and auditory reactivity (Harrison and Buchwald 1983; Levine *et al.*, 1987). In the latter study, three groups of cats (1–3, 5–9, and 11–16 years of age) were tested for locomotor activity, fine motor coordination, reactivity to auditory stimuli, and spatial reversal learning. The rather surprising finding of improved spatial reversal learning in aged cats relative to younger cats may reflect previous experience variables—or poorer motivation in the young cats. The results of this study highlight a potential problem in working with cats, a difficulty in obtaining reliable behavior during the test situation. The authors suggest that these changes in behavior may be associated with caudate dysfunction since impairments in habituation are a symptom of caudate-lesioned cats (Villablanca *et al.*, 1978). On the other hand, the lack of spatial reversal impairments in aged cats suggest that caudate dysfunction does not contribute to the age-dependent cognitive dysfunction (Olmstead *et al.*, 1976).

Very few studies of the neuropathology of the aging cat exist. The presence of Aβ, the protein constituent of senile plaques has been observed in some studies and not others. Visualizing Aβ in the cat brain, as for the dog, is dependent upon the staining technique used. For example, in silver stains, no senile plaques have been found in cats up to the age of 17 years (Braak *et al.*, 1994). On the other hand, more sensitive immunohistochemical techniques consistently identify diffuse senile plaques in cats over 16 years (Cummings *et al.*, 1996b; Nakamura *et al.*, 1996; Kuroki *et al.*, 1997; E. Head, unpublished observations—Fig 30.4). In addition, the longer form of Aβ, 42 to 43 amino acids long, is found in diffuse senile plaques of aged cat brain, which is consistent with the dog and with human brain tissue (Cummings *et al.*, 1996b). No evidence for early tangle formation has been found in aged cat brain (Kuroki *et al.*, 1997).

The age range for the development of senile plaques (>16 years) suggests that very old cats would be the most appropriate for studies of age-dependent cognitive decline. The relationship between neuropathology and behavioral dysfunction has not been well explored in cats, however, in the Cummings *et al.* (1996b) study, the three aged cats with diffuse senile plaques were reported to be exhibiting abnormal behavior such as wandering, confusion, and inappropriate vocalization.

VI. Neuropathology of Aging Sheep, Goats, Bears, Wolverines, Camels, and Birds

In contrast with many other animal models of aging, goats, sheep, bears, and possibly wolverines are the few vertebrates where there are reports of naturally occurring neurofibrillary tangle-like formations but in the former two cases, a lack of senile plaque formation. Silver staining in aged goats and sheep up to 10 years of age was negative for Aβ (Braak *et al.*, 1994) and this was confirmed in a second study of sheep using immunohistochemical techniques (Nagata, 1997). On the other hand, there are several reports of neurofibrillary tangle-like formations in these two species (Braak *et al.*, 1994; Nelson *et al.*, 1994; Nagata, 1997). Antibodies against hyperphosphorylated tau (AT8, PHF-1, and Alz-50), a component of neurofibrillary tangles, reveal tangle-like structures in aged sheep (Braak *et al.*, 1994; Nelson *et al.*, 1994). A recent study has demonstrated the occurrence of tangle-like lesions in the brains of other large artiodactyls such as llamas and bison (Härtig *et al.*, 2000). However, another study, involving samples from sheep between the ages of 2 and 14 years was negative for neurofibrillary tangles (Kuroki *et al.*, 1997). In addition to sheep and goats, bears and wolverines may also exhibit neurofibrillary tangle-like structures (Cork *et al.*, 1988; Roertgen *et al.*, 1996; Härtig *et al.*, 2000). Reports of amyloid angiopathy in a 16-year-old woodpecker and senile plaques in a 20-year-old camel suggest that in fact, birds and many artiodactyls may also develop some of the characteristic lesions of human brain aging (Nakamura *et al.*, 1995; Nakayama *et al.*, 1999). To our knowledge no information is available regarding behavioral decline as a function of age in these animals.

VII. Summary

Both aged cats and dogs develop senile plaques in many brain regions that very likely have functional consequences.

In aged dogs, the relationship between the extent of Aβ deposition and the extent of cognitive dysfunction shows a strong association. However, we do not account for all behavioral dysfunction with Aβ accumulation alone, suggesting that other factors likely contribute. The potential role for environment, breed, previous experience, and genetics all highlight the utility of the dog as a model of human aging. In addition, particularly with respect to the dog, genetic contributions to both brain and cognitive aging can be studied by taking advantage of the presence of different breeds of dogs and studying littermates (Russell *et al*., 1992; Bobik *et al*., 1994). Our own studies have evaluated these factors to some degree but we are currently studying aging in a colony of beagle dogs that are extremely well-characterized clinically with health records maintained since birth. This colony of dogs is also unique in that all animals have been raised and maintained in the same environment, given the same diet and water, and housed with the same kennelmate for their entire lives. Thus, we are able to eliminate many potential variables that can confound experimental studies of aging.

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References

Adams, B., Chan, A., Callahan, H., Siwak, C., Tapp, D., Ikeda-Douglas, C., Head, E., Cotman, C. W., and Milgram, N. W. (2000). Spatial learning and memory in the dog as a model of cognitive aging. *Behav. Brain Res.* **108**, 47–56.

Alafuzoff, L., Iqbal, K., Friden, H., Adolfsson, R., and Winblad, B. (1987). Histopathological criteria for progressive dementia disorders: Clinical-pathological correlation and classification by multivariate analysis. *Acta Neuropathol.* **74**, 209–225.

Albert, M. S., and Funkenstein, H. H. (1992). The effects of age: Normal variation and its relation to disease. *In* "Disorders of the Nervous System: Clinical Neurology" (W. I. M. A. K. Asburg and G. M. McKhanney, eds.), 2nd ed., pp. 598–611. Saunders, Philadelphia.

Allen, W. F. (1939). Effect of ablating the frontal lobes, hippocampi, and occipito-parieto-temporal (excepting pyriform areas) lobes on positive and negative olfactory conditioned reflexes. *Am. J. Physiol*. **159**, 525–532.

Allen, W. F. (1943). Effect of prefrontal brain lesions on correct conditioned differential responses in dogs. *Am. J. Physiol.* **159**, 525–532.

Anderson, A. J., Su, J. H., and Cotman, C. W. (1996). DNA damage and apoptosis in Alzheimer's disease: colocalization with c-Jun immunoreactivity, relationship to brain area and effect of postmortem delay. *J. Neurosci*. **16**, 1710–1719.

Anderson, A. J., Ruehl, W. W., Fleischmann, L. K., Stenstrom, K., Entriken, T. L., and Cummings, B. J. (1997). DNA damage is correlated with Aβ deposition and unrelated to cytoskeletal neuropathology in the canine model of Alzheimer's disease. *Soc. Neurosci. Abstr.*. **23**, 539.

Arnsten, A. F. T., and Goldman-Rakic, P. S. (1985). Alpha 2-adrenergic mechanisms in prefrontal cortex associated with cognitive decline in aged nonhuman primates. *Science* **230**, 1273–1276.

Ball, M. J., MacGregor, J., Fyfe, I. M., Rapoport, S. I., and London, E. (1983). Paucity of morphological changes in the brains of ageing beagle dogs: Further evidence that Alzheimer lesions are unique for primate central nervous system. *Neurobiol. Aging* **4**, 127–131.

Bartus, R. T., Dean, R. L., and Fleming, D. L. (1979). Aging in the Rhesus monkey: Effects on visual discrimination learning and reversal learning. *J. Gerontol*. **34**, 209–219.

Baxter, M. G., and Gallagher, M. (1996). Neurobiological substrates of behavioral decline: Models and data analytic strategies for individual differences in aging. *Neurobiol. Aging* **17**, 491–495.

Blessed, G., Tomlinson, B. E., and Roth, M. (1968). The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. *Br. J. Psychiatry* **114**, 797–811.

Bobik, M., Thompson, T., and Russell, M. J. (1994). Amyloid deposition in various breeds of dogs. *Soci. Neurosci. Abstr.* **20**, 172.

Braak, H., and Braak, E. (1991). Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol*. **82**, 239–259.

Braak, H., and Braak, E. (1995). Staging of Alzheimer's disease-related neurofibrillary changes. *Neurobiol. Aging* **16**, 271–284.

Braak, H., Braak, E., and Bohl, J. (1993). Staging of Alzheimer-related cortical destruction. *Rev. Clin. Neurosci*. **33**, 403–408.

Braak, H., Braak, E., and Strothjohann, M. (1994). Abnormally phosphorylated tau protein related to the formation of neurofibrillary tangels and neuropil threads in the cerebral cortex of sheep and goat. *Neurosci. Lett*. **171**, 1–4.

Brush, E. S., Mishkin, M., and Rosvold, H. E. (1961). Effects of object preferences and aversions on discrimination learning in monkeys with frontal lesions. *J. Comp. Physiol. Psychol*. **54**, 319–325.

Callahan, H., Ikeda-Douglas, C., Head, E., Cotman, C. W., and Milgram, N. W. (in press). Development of a protocol for studying object recognition memory in the dog. *Prog. Neuro-Psychopharm. Biol. Psychiatry.*

Catteruccia, N., Willingale-Theune, J., Bunke, D., Prior, R., Masters, C. L., Crisanti, A., and Beyreuther, K. (1990). Ultrastructural localization of the putative precursors of the A4 amyloid protein associated with Alzheimer's disease. *Am. J. Pathol.* **137**, 19–26.

Chapman, B. L., and Voith, V. L. (1990). Behavioral problems in old dogs: 26 cases (1984–1987). *J. Am. Vet. Med. Assoc*. **196**, 944–946.

Chen, K. S., Soriano, F., Lyn, W., Grajeda, H., Masliah, E., and Games, D. (1998). Effects of entorhinal cortex lesions on hippocampal β-amyloid deposition in PDAPP transgenic mice. *Soc. Neurosci. Abstr.* **24**, 1502.

Coleman, P. D., and Flood, D. G. (1987). Neuron number and dendritic extent in normal aging and Alzheimer's disease. *Neurobiol. Aging* **8**, 521–545.

Committee on Animal Models for Research on Aging. (1981). "Carnivores," pp. 180–242. National Academy Press, Washington, DC.

Cork, L. C., Powers, R. E., Selkoe, D. J., Davies, P., Geyer, J. J., and Price, D. L. (1988). Neurofibrillary tangles and senile plaques in aged bears. *J. Neuropathol. Exp. Neurol*. **7**, 629–641.

Cotman, C. W., and Su, J. H. (1996). Mechanisms of neuronal death in Alzheimer's disease. *Brain Pathol.* **6**, 493–506.

Cummings, B. J., and Cotman, C. W. (1995). Image analysis of beta-amyloid "load" in Alzheimer's disease and relation to dementia severity. *Lancet* **346**, 1524–1528.

Cummings, B. J., Su, J. H., Cotman, C. W., White, R., and Russell, M. J. (1993). Beta-amyloid accumulation in aged canine brain: A model of plaque formation in Alzheimer's disease. *Neurobiol. Aging* **14**, 547–560.

Cummings, B. J., Head, E., Ruehl, W. W., Milgram, N. W., and Cotman, C. W. (1996a). Beta-amyloid accumulation correlates with cognitive dysfunction in the aged canine. *Neurobiol. Learn. Memory* **66**, 11–23.

Cummings, B. J., Satou, T., Head, E., Milgram, N. W., Cole, G. S., Savage, M. J., Podlisny, M. B., Selkoe, D. J., Siman, R., Greenberg, B. D., and Cotman, C. W. (1996b). Diffuse plaques contain C-terminal Aβ 1-42 and not Aβ 1-40: Evidence from cats and dogs. *Neurobiol. Aging* **17**, 653–659.

Cummings, B. J., Pike, C. J., Shankle, R., and Cotman, C. W. (1996c). Beta-amyloid deposition and other measures of neuropathology predict cognitive status in Alzheimer's disease. *Neurobiol. Aging* **17**, 921–933.

Dayan, A. D. (1970a). Quantitative histological studies on the aged human brain. I. Senile plaques and neurofibrillary tangles in ''normal'' patients. *Acta Neuropathol*. **16**, 85–94.

Dayan, A. D. (1970b). Quantitative histological studies on the aged human brain. II. Senile plaques and neurofibrillary tangles in senile dementia (with an Appendix on the occurrence in cases of carcinoma). *Acta Neuropathol*. **16**, 95–102.

Dickson, D. W., Crystal, H. A., Bevona, C., Honer, W., Vincent, I., and Davies, P. (1995). Correlations of synaptic and pathological markers with cognition of the elderly. *Neurobiol. Aging* **16**, 285–304.

Dournaud, P., Delaère, P., Hauw, J. J., and Epelbaum, J. (1995). Differential correlation between neurochemcial deficits, neuropathology, and cognitive status in Alzheimer's disease. *Neurobiol. Aging* **16**, 817–823.

Duyckaerts, C., Delaère, P., Huaw, J. J., Abbamondi-Pinto, A., Sorbi, S., Allen, I., Brion, J. P., Flament-Durand, J., Duchen, L., Kauss, J., Renkawek, K., and Tomlinson, B. (1990). Rating of the lesions in senile dementia of the Alzheimer type: Concordance between laboratories. *J. Neurol. Sci*. **97**, 295–323.

Duyckaerts, C., Bennecib, M., Grignon, Y., Uchihara, T., He, Y., Piette, F., and Hauw, J.-J. (1997). Modeling the relation between neurofibrillary tangles and intellectual status. *Neurobiol. Aging* **18**, 267–273.

Felleman, D. J., and van Essen, D. C. (1991). Distributed hierarchical processing in the primate cerebral cortex. *Cereb. Cortex* **1**, 1–47.

Fisher, A., Hanin, I., and Lachman, C., eds. (1986). ''Proceedings of the Thirtieth OHOLO Conference on Basic and Therapeutic Strategies in Alzheimer's and Other Related Neuropsychiatric Disorders.'' Plenum, New York.

Flood, D. G., and Coleman, P. D. (1988). Neuron numbers and sizes in aging brain: Comparions of human, monkey, and rodent data. *Neurobiol. Aging* **9**, 453–463.

Giaccone, G., Verga, L., Finazzi, M., Pollo, B., Tagliavini, F., Frangione, B., and Bugiani, O. (1990). Cerebral preamyloid deposits and congophilic angiopathy in aged dogs. *Neurosci. Lett*. **114**, 178–183.

Goedert, M. (1993). Tau protein and the neurofibrillary pathology of Alzheimer's disease. *Trends Neurosci*. **16**, 460–465.

Harrison, J., and Buchwald, J. (1982). Auditory brainstem responses in the aged cat. *Neurobiol. Aging* **3**, 163–171.

Harrison, J., and Buchwald, J. (1983). Eyeblink conditioning deficits in the old cat. *Neurobiol. Aging* **4**, 45–51.

Härtig, W., Klein, C., Brauer K., Schüppel, C. F., Asendt, T., Brückner, G., and Bigl, V. (2000). Abnormally phosphorylated protein tau in the cortex of aged individuals of various mammalian orders. *Acta Neuropathol.* **100**, 305–312.

Head, E., Mehta, R., Hartley, J., Kameka, A. M., Cummings, B. J., Cotman, C. W., Ruehl, W. W., and Milgram, N. W. (1995). Spatial learning and memory as a function of age in the dog. *Behav. Neurosci*. **109**: 851–858.

Head, E., Callahan, H., V. Balkissoon, Thirlwell, C., Adam, G. E., Astur, B., Barrington, F., Koerner, A., Weisend, M. P., Sutherland, R. J., Muggenberg, B. A., Cummings, B. J., Cotman, C. W., and Milgram, N. W. (1996). Olfactory discrimination and repeated reversal learning in young and aged dogs. *Soc. Neurosci. Abstr.* **22**, 211.

Head, E., Callahan, H., Muggenburg, B. A., Milgram, N. W., and Cotman, C. W. (1998). Discrimination learning ability and beta amyloid accumulation in the dog. *Neurobiol. Aging* **19**, 415–425.

Head, E., McCleary, R., Hahn, F., Milgram, N. W., and Cotman, C. W. (2000a). Region-Specific age at onset of β-amyloid in dog. *Neurobiol. Aging* **21**, 89–96.

Houpt, K. A., and Beaver, B. (1981). Behavioral problems of geriatric dogs and cats. *Vet. Clin. North Am.: Small Anim. Pract*. **11**, 643–652.

Johnstone, E., Chaney, M., Norris, F., Pascual, R., and Little, S. (1991). Conservation of the sequence of the Alzheimer's disease amyloid peptide in dog, polar bear and five other mammals by cross-species polymerase chain reaction analysis. *Mol. Brain Res*. **10**, 299–305.

Jones, B., and Mishkin, M. (1972). Limbic lesions and the problem of stimulus-reinforcement associations. *Exp. Neurol.* **36**, 362–377.

Kang, J., Lemaire, H. G., Unterbeck A., Salbaum, J. M., Masters, C. L., Grzeschik, K., Multhaup, G., Beyreuther, K., and Müller-Hill, B. (1987). The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature (London)* **325**, 733–736.

Katzman, R., and Terry, R. (1992). Normal aging of the nervous system. *In* ''Principles of Geriatric Neurology'' (R. Katzman and J. W., Rowe, eds.), pp. 37–39. F. A. Davis, Philadelphia.

Kiatipattanasakul, W., Nakamura, S., Hossain, M. M., Nakayama, H., Uchino, T., Shumiya, S., Goto, N., and Doi, K. (1996). Apoptosis in the aged dog brain. *Acta Neuropathol*. **92**, 242–248.

Kiatipattanasakul, W., Nakamura, S., Kuroki, K., Nakayama, H., and Doi, K. (1997). Immunohistochemical detection of anti-oxidative stress enzymes in the dog brain. *Neuropathology* **17**, 307–312.

Kitaguchi, N., Takahashi, Y., Tokushima, Y., Shiojiri, S., and Ito, H. (1988). Novel precursor of Alzheimer's disease amyloid protein shows protease inhibitory activity. *Nature (London)* **331**, 530–532.

Koo, E. H., Sisodia, S. S., Archer, D. R., Martin, L. J., Weidemann, A., Beyreuther, K., Fischer, P., Masters, C. L., and Price, D. L. (1990). Precursor of amyloid protein in Alzheimer's diase undergoes fast anterograde axonal transport. *Proc. Natl. Acad. Sci. U.S.A*. **87**, 1561–1565.

Kuroki, K., Uchida, K., Kiatipattanasakul, W., Nakamura, S., Yamaguchi, R., Nakayama, H., Doi, K., and Tateyama, S. (1997). Immunohistochemical detection of tau proteins in various non-human animal brains. *Neuropathology* **17**, 174–180.

Langui, D., Probst, A., and Ulrich, J. (1995). Alzheimer's changes in non-demented and demented paitents: A statistical approach to their relationships. *Acta Neuropathol*. **89**, 57–62.

Levine, M. S., Lloyd, R. L., Fisher, R. S., Hull, C. D., and Buchwald, N. A. (1987). Sensory, motor and cognitie alterations in aged cats. *Neurobiol. Aging*. **8**, 253–263.

Markowska, A. L., Stone, W. S., Ingram, D. K., Reynolds, J., Gold, P. E., Conti, L. H., Pontecorvo, M. J., Wenk, G. L., and Olton, D. S. (1989). Individual differences in aging: Behavioral and neurobiological correlates. *Neurobiol. Aging* **10**, 31–43.

Mervis, R. (1978). Structural alterations in neurons of aged canine neocortex: A Golgi study. *Exp. Neurol.* **62**, 417–432.

Milgram, N. W., Head, E., Weiner, E., and Thomas, E. (1994). Cognitive functions and aging in the dog: Acquisition of nonspatial visual tasks. *Behav. Neurosci.* **108**, 57–68.

Mishkin, M. (1964). Perseveration of central sets after frontal lesions in monkeys. *In* ''The Frontal Granular Cortex and Behavior'' (J. M. Warren and K., Akert, eds.), pp. 219–241. McGraw-Hill, New York.

Mishkin, M. (1972). Cortical visual areas and their interactions. *In* ''Brain and Human Behavior'' (A. G. Karczman and J. C. Eccles, eds.), pp. 187–206. Springer-Verlag, Berlin.

Mishkin, M., and Delacour, J. (1975). An analysis of short-term visual memory in the monkey. *J. Exp. Psychol. Anim. Behav. Proc*. **1**, 326–334.

Morys, J., Narkiewicz, O., Maciejewska, B., Wegiel, J., and Wisniewski, H. M. (1994). Amyloid deposits and loss of neurones in the claustrum of the aged dog. *NeuroReport* **5**, 1825–1828.

Mosier, J. E. (1988). How aging affects body systems in the dog. *Clin. Insight* **3**, 474–478.

Moss, M. B., Rosene, D. L., and Peters, A. (1988). Effects of aging on visual recognition memory in the rhesus monkey. *Neurobiol. Aging* **9**, 495–502.

Nagata, S. (1997). Apoptosis by death factor. *Cell (Cambridge, Mass.)* **88**, 355–365.

Nakamura, S.-I., Nakayama, H., Uetsuka, K., Sasaki, N., Kazuyuki, U., and Goto, N. (1995). Senile plaques in an aged-two-humped (Bactrian) Camel (*Camelus bactrianus). Acta Neuropathol*. **90**, 415–418.

Nakamura, S., Nakayama, H., Kiatipattanasakul, W., Uetsuka, K., Uchida, K., and Goto, N. (1996). Senile plaques in very aged cats. *Acta Neuropathol*. **91**, 437–439.

Nakamura, S., Tamaoka, A., Sawamura, N., Kiatipattanasakul, W., Nakayama, H., Shoji, S., Yoshikawa, Y., and Doi, K. (1997). Deposition of amyloid β protein (Aβ) subtypes [Aβ40 and Aβ42(43)] in canine senile plaques and cerebral amyloid angiopathy. *Acta Neuropathol*. **94**, 323–328.

Nakayama, H., Katayama, K-I., Ikawa, A., Miyawaki, K., Shinozuka, J., Uetsuka, K., Nakamura, S.-I., Kimura, N., Yoshikawa, Y., and Doi, K. (1999). Cerebral amyloid angiopathy in an aged great spotted woodpecker *(Picoides major). Neurobiol. Aging* **20**, 53–56.

Nelson, P. T., Greenberg, S. G., and Saper, C. B. (1994). Neurofibrillary tangles in the cerebral cortex of sheep. *Neurosci. Lett*. **170**, 187–190.

Okuda, R., Uchida, K., Tateyama, S., Yamaguchi, R., Nakayama, H., and Goto, N. (1994). The distribution of amyloid beta precursor protein in canine brain. *Acta Neuropathol*. **87**, 161–167.

Olmstead, C. E., Villablanca, J. R., Marcus, R. J., and Avery, D. L. (1976). Effects of caudate nuclei or frontal cortical ablation in cats. IV. Bar pressing and maze learning performance. *Exp. Neurol*. **53**, 670–693.

Osmand, A. P., and Switzer, R. C. (1992). Diffuse plaques and cerebrovascular amyloidosis are accompanied by entorhinal perineuronal and hippocampal terminal zone deposition of amyloid in the aging dog. Evidence for a neuronal origin of amyloid. *Neurobiol. Aging*. **13** (Suppl. 1), S104.

Ponte, P., Gonzalaes-DeWhitt, P., Schilling, J., Miller, J., Hsu, D., Greenberg, B., Davis, K., Wallace, W., Lieberburg, I., Fuller, F., and Cordell, B. (1988). A new A4 amyloid mRNA contains a domain homologous to serine protease inhibitors. *Nature (London)* **331**, 525–527.

Price, J. L., Davis, P. B., Morris, J. C., and White, D. L. (1991). The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. *Neurobiol. Aging* **12**, 295–312.

Rapp, P. R. (1990). Visual discrimination and reversal learning in the aged monkey (*Macaca mulatta). Behav. Neurosci*. **6**, 876–884.

Rapp, P. R., and Amaral, D. G. (1989). Evidence for task-dependent memory dysfunction in the aged monkey. *J. Neurosci*. **9**, 3568–3576.

Rapp, P. R., and Amaral, D. G. (1991). Recognition memory deficits in a subpopulation of aged monkeys resemble the effects of medial temporal lobe damage. *Neurobiol. Aging* **12**, 481–486.

Roertgen, K. E., Parisi, J. E., Clark, H. B., Barnes, D. L., O'Brien, T. D., and Johnson, K. H. (1996). Aβ-Associated cerebral angiopathy and senile plaques with neurofibrillary tangles and cerebral hemorrhage in an aged wolverine (*Gulo gulo). Neurobiol. Aging*. **17**, 243–247.

Rowe, J. W., and Kahn, R. L. (1987). Human aging: Usual and successful. *Science* **237**, 143–149.

Ruehl, W. W., Bruyette, D. S., DePaoli, A., Cotman, C. W., Head, E., Milgram, N. W., and Cummings, B. J. (1995). Canine cognitive dysfunction as a model for human dementia and Alzheimer's disease: Clinical presentation cognitive testing, pathology and response to L-deprenyl therapy. *Prog. Brain Res.* **106**, 217–225.

Russell, M. J., White, R., Patel, E., Markesbery, W. R., Watson, C. R., and Geddes, J. W. (1992). Familial influence on plaque formation in the beagle brain. *NeuroReport* **3**, 1093–1096.

Russell, M. J., Bobik, M., White, R. G., Hou, Y., Benjamin, S. A., and Geddes, J. W. (1996). Age-specific onset of beta-amyloid in beagle brains. *Neurobiol. Aging* **17**, 269–273.

Satou, T., Cummings, B. J., Head, E., Nielson, K. A., Hahn, F. F., Milgram, N. W., Velazquez, P., Cribbs, D. H., Tenner, A. J., and Cotman, C. W. (1997). The progression of beta-amyloid deposition in the frontal cortex of the aged canine. *Brain Res.* **774**, 35–43.

Saunders, A. M., Strittmatter, W. J., Schmechel, D., George-Hyslop, P. H., Pericak-Vance, M. A., Joo, S. H., Rosi, B. L., Gusella, J. F., Crapper-McLachlan, D. R., Alberts, M. J., Hulette, C., Crain, B., Goldgaber, D., and Roses, A. D. (1993). Association of apolipoprotein E allele ϵ4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* **43**, 1467–1472.

Scheibel, M. E., Lindsay, R. D., Tomiyasu, U., and Scheibel, A. B. (1975). Progressive dendritic changes in aging human cortex. *Exp. Neurol*. **47**, 392–403.

Scheibel, M. E., Lindsay, R. D., Tomiyasu, U., and Scheibel, A. B. (1976). Progressive dendritic changes in the aging human limbic system. *Exp. Neurol*. **53**, 420–430.

Scheibel, M. E., Tomiyasu, U., and Scheibel, A. B. (1977). The aging human Betz cell. *Exp. Neurol*. **56**, 598–609.

Selkoe, D. J. (1994). Normal and abnormal biology of the beta-amyloid precursor protein. *Annu. Rev. Neurosci*. **17**, 489–517.

Selkoe, D. J. (1996). Amyloid beta-protein and the genetics of Alzheimer's disease. *J. Biol. Chem*. **271**, 18295–18298.

Selkoe, D. J., Bell, D. S., Podlisny, M. B., Price, D. L., and Cork, L. C. (1987). Conservation of brain amyloid proteins in aged mammals and humans with Alzheimer's disease. *Science* **235**, 873–877.

Steward, O. (1976). Topographic organization of the projections from the entorhinal area to the hippocampal formation of the rat. *J. Comp. Neurol*. **167**, 285–314.

Steward, O., and Scoville, S. A. (1976). Cells of origin of entorhinal cortical afferents to the hippocampus and fascia dentata of the rat. *J. Comp. Neurol*. **169**, 347–370.

Su, M.-Y., Head, E., Brooks, W. M., Wang, Z., Muggenberg, B. A., Adam, G. E., Sutherland, R. J., Cotman, C. W., and Nalcioglu, O. (1998). MR Imaging of anatomic and vascular characteristics in a canine model of human aging. *Neurobiol. Aging* **19**, 479–485.

Tanzi, R. E., McClatchey, A. I., Lamperti, E. D., Villa-Komaroff, L., Gusella, J. F., and Neve, R. L. (1988). Protease inhibitor domain encoded by an amyloid protein precursor mRNA associated with Alzheimer's disease. *Nature (London)* **333**, 528–530.

Terry, R. D., Masliah, E., Salmon, D. P., Butters, N., DeTeresa, R., Hill, R., Hansen, L. A., and Katzman, R. (1991). Physical basis of cognitive alterations in Alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. *Ann. Neurol*. **30**, 572–580.

Trojanowski, J. Q., Schmidt, M. L., Shin, R.-W., Brablett, G. T., Rao, D., and Lee, V. M.-Y. (1993a). Altered tau and neurofilament proteins in neurodegenerative diseases: Diagnostic implications for Alzheimer's disease and Lewy body dementias. *Brain Pathol*. **3**, 45–54.

Trojanowski, J. Q., Schmidt, M. L., Shin, R.-W., Bramblett, G. T., Goedert, M., and Lee, V. M.-Y. (1993b). PHFτ (A68): From pathological marker to potential mediator of neuronal dysfunction and degeneration in Alzheimer's disease. *Clin. Neurosci*. **1**, 184–191.

Tucker, R. P. (1990). The roles of microtubule-associated proteins in brain morphogenesis. *Brain Res. Rev.* **15**, 101–120.

Uchida, K., Kuroki, K., Yoshino, T., Yamaguchi, R., and Tateyama, S. (1997). Immunohistochemical study of constituents other than beta-protein in canine senile plaques and cerebral amyloid angiopathy. *Acta Neuropathol*. **93**, 277–284.

Uchida, K., Tani, Y., Uetsuka, K., Nakayama, H., and Goto, N. (1992a). Immunohistochemical studies on canine cerebral amyloid angiopathy and senile plaques. *J. Vet. Med. Sci.* **54**, 659–667.

Uchida, K., Nakayama, H., Tateyama, S., and Goto, N. (1992b). Immunohistochemical analysis of constituents of senile plaques and cerebrovascular amyloid in aged dogs. *J. Vet. Med. Sci.* **54**, 1023–1029.

van Essen, D. C., Anderson, C. H., and Felleman, D. J. (1992). Information processing in the primate visual system: An integrated systems perspective. *Science* **255**, 419–423.

Villablanca, J. R., Olmstead, C. E., and deAndres, I. (1978). Effects of caudate nuceli or fronal ablation in kittens. Responsiveness to auditory stimuli and comparisons with adult operated littermates. *Exp. Neurol*. **61**, 635–649.

Walker, L. C. (1997). Animal models of cerebral beta-amyloid angiopathy. *Brain Res. Rev.* **25**, 70–84.

Wegiel, J., Wisniewski, H. M., Dziewiatkowski, J., Tarnawski, M., Dziewiatkowska, A., Morys, J., Soltysiak, Z., and Kim, K. S. (1996). Subpopulation of dogs with severe brain parenchymal β amyloidosis distinguished with cluster analysis. *Brain Res.* **728**, 20–26.

West, M. J. (1993). Regionally specific loss of neurons in the aging human hippocampus. *Neurobiol. Aging* **14**, 287–293.

West, M. J., Coleman, P. D., Flood, D. G., and Troncoso, J. C. (1994). Differences in the pattern of hippocampal neuronal loss in normal aging and Alzheimer's disease. *Lancet* **344**, 769–772.

Wisniewski, H. M. (1979). The aging brain. *In* "Spontaneous Animal Models of Human Disease" (E. J. Andrews, B. C., Ward, and N. H., Altman, eds.), pp. 148–152. Academic Press, New York.

Wisniewski, H. M., Johnson, A. B., Raine, C. S., Kay, W. J., and Terry, R. D. (1970). Senile plaques and cerebral amyloidosis in aged dogs. *Lab. Invest*. **23**, 287–296.

Wisniewski, H. M., Wegiel, J., Morys, J., Bancher, C., Soltysiak, Z., and Kim, K. S. (1990). Aged dogs: An animal model to study beta-protein amyloidogenesis. *In* "Alzheimer's Disease. Epidemiology, Neuropathology, Neurochemistry and Clinics" (P. R. K. Maurer and H. Beckman, eds.), pp. 151–167. Springer-Verlag, New York.

Wisniewski, T., Lalowski, M., Bobik, M., Russell, M., Strosznajder, J., and Frangione, B. (1996). Amyloid Beta 1-42 deposits do not lead to Alzheimer's neuritic plaques in aged dogs. *Biochem. J*. **313**, 575–580.

Yoshino, T., Uchida, K., Tateyama, S., Yamaguchi, R., Nakayama, H., and Goto, N. (1996). A retrospective study of canine senile plaques and cerebral amyloid angiopathy. *Vet. Pathol*. **33**, 230–234.

Younkin, S. G. (1991). Processing of the Alzheimer's disease A4 amyloid protein precursor (APP). *Brain Pathol*. **1**, 253–262.

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31

Estrogens and Alzheimer's Disease

I. Introduction

Risk factors for the development of Alzheimer's disease (AD) include age, family history, low level of education, head trauma, and gender. Epidemiological studies indicate that AD afflicts women one-and-a-half to three times as frequently as men (Jorm *et al*., 1987; Aronson *et al*., 1990). The prevalence for AD in women remains higher even after adjusting for their longer life span compared to men (Gao *et al*., 1998).

The role of estrogens in the prevention and treatment of osteoporosis is well established (Riggs and Melton, 1992; Lindsay, 1993), and observational studies suggest a reduction of risk for coronary heart disease among women receiving postmenopausal hormone replacement therapy (Grondstein and Stampfer, 1998; Sites, 1998; Oparil, 1999). Investigators have also attempted to gather evidence of a possible effect of estrogens on cognition. Specifically, a presumed role of estrogen deficit in postmenopausal women is a subject of much study. In particular, basic science and clinical studies have suggested an association between estrogens and memory. In this chapter we will review the results from studies that have investigated the role of estrogen in cognition and AD.

II. Estrogen Effects on Cognition and AD

A convergence of evidence demonstrates that a central cholinergic deficiency plays an important role in AD (Bartus *et al*., 1982; Coyle *et al*., 1983). Cholinergic neurons projecting from the nucleus basalis of Meynert to the cortex, amygdala, and hippocampus are critically involved in the performance of learning and memory tasks in humans and animal models (Deutsch, 1971; Drachman and Leavitt, 1974; Fibiger, 1991; Ceyla, 1998). One of the more consistent neurobiological finding is the presence of aromatase (the enzyme that transforms testosterone to 17β-estradiol) and estrogen receptors in the basal forebrain (nucleus basalis of Meynert, septum nuclei, nucleus accumbens), limbic, paralimbic regions, and the gray matter (Terasawa and Timiras, 1968; McLusky *et al*., 1987; McEwen,1988; Loy *et al*., 1988; Toran-Allerand *et al*., 1992; McEwen *et al*., 1997). Experiments have demonstrated that gonadal hormones can affect the activity of both choline acetyltransferase (ChAT) (Luine *et al*., 1980; Luine, 1985; Kaufman *et al*., 1988) and acetylcholinesterase (AChE) (Iraman *et al*., 1980), enzymes involved in the synthesis and degradation of acetylcholine. Moreover, studies with ovariectomized rats have shown that estrogen affects acetylcholine release (Gibbs *et al*., 1997). Colocalization of estrogen receptors with nerve growth factors in cholinergic neurons of the basal forebrain (Toran-Allerand *et al*., 1992) opened a new area of interest regarding the possible importance of estrogen–neurotrophin interaction in neuronal viability (Gibbs, 1994; Singh *et al*., 1995; Mudd *et al*., 1998). Several lines of work have suggested that estrogens play a major role in synaptic formation during the perinatal period and in the plasticity of the adult brain (Matsumoto *et al*., 1985; Matsumoto, 1991; Chung *et al*., 1988; Gould *et al*., 1990; Cordoba-Montoya and Carrer, 1997).

Recent evidence also links estrogen to the metabolism of amyloid precursor protein (APP). The accumulation of β-amyloid protein, which is derived from APP, in the senile plaque cores of the brain is considered a histopathological hallmark of AD (Jaffe *et al*., 1994; Xu *et al*., 1998).

The apolipoprotein E4 (APOE4) allele encoding for plasma apolipoprotein E (apoE) has been associated with increased risk for late-onset, familial, and sporadic AD (Corder *et al*., 1993; Saunders *et al*., 1993; Strittmatter *et al*., 1993). Recent studies have suggested that APOE4 may be a risk factor for early-onset AD (Okuizumi *et al*., 1994; Van Duijn *et al*., 1994). Estrogen modulates the expression of apoE (Srivastava *et al*., 1996) and enhances the synaptic sprouting via an apoE-dependent mechanism (Stone *et al*., 1998).

Other functions of estrogen have also been reported. In experiments with cell cultures it has been demonstrated that estrogens confer protection against oxidative stress-induced neurodegeneration (Behl *et al*., 1995; Goodman *et al*., 1996). In addition, estrogens can increase the cerebral glucose metabolism (Bishop and Simpkins, 1992) and cerebral blood flow, especially in the right lower frontal area suggesting that the increased metabolism may be related to estrogen's enhancement of cholinergic projections (Ohkura *et al*., 1994a,b).

Behavioral studies with animal models have suggested a role for estrogen in facilitating memory and learning. Most animal experiments have used maze task (a spatial memory

task), or avoidance behavior paradigms in ovariectomized rats to determine whether gonadal steroids, notably estrogens, counteract the deficits induced by the anticholinergic agent scopolamine (Farr *et al*., 1995; O'Neal *et al*., 1996; Daniel *et al*., 1997; Fader *et al*., 1998). Estrogen administration has been shown to improve performance in cognitive tasks in ovariectomized rats (Singh *et al*., 1994). Specifically, estrogen replacement not only enhanced performance in the active avoidance tasks, but also accelerated learning. It was consequently hypothesized that chronic exposure to low doses of 17β-estradiol may play a positive role in long-term memory. These studies underscore the importance of estrogen in memory and learning.

III. Clinical Trials of Estrogen Treatment

Several placebo-controlled clinical trials have evaluated the effects of estrogen on the cognitive function of postmenopausal women. Six of the nine most frequently cited studies (Caldwell and Watson, 1952; Campbell and Whitehead, 1977; Fedor-Freybergh, 1977; Hackman and Galbraith, 1977; Sherwin, 1988; Phillips and Sherwin, 1992) concluded that estrogens might benefit the cognitive performance of the participants, whereas three studies (Rauramo *et al*., 1975; Vanhulle and Demol, 1976; Ditkoff *et al*., 1991) failed to detect any hormonal benefits. Although the above studies were very intriguing and suggested a positive role of estrogen in cognition, they were not specifically designed to assess patients with dementia. Other limitations of these studies were the following: First, they did not always compare measure outcomes with the placebo-controlled group. Second, a small number of subjects (< 70) were tested in each study. Third, patients were followed for a brief duration (< 6 months). Fourth, they used a variety of not standardized psychometric instruments. Finally, the authors did not always adjust or match estrogen users with controls for age, education, and depression. Therefore it is not possible to draw general conclusion about the effectiveness of estrogen in postmenopausal women or to translate these results into women with dementia.

Five observational studies (three cross-sectional, one prospective, and one case–control nested in a large prospective study) have been conducted recently with the goal of detecting the purported benefit of estrogen use in healthy postmenopausal women. Three investigations (Kampen and Sherwin, 1994; Robinson *et al*., 1994; Kimura, 1995) suggested a positive association between estrogens and cognitive performance, while the other two (Barrett-Connor and Kritz-Silverstein, 1993; Paganini-Hill and Henderson, 1996a) failed to detect any association. The Barrett-Connor and Kritz-Silverstein (1993) study needs further mention because it was the only prospective study of these five trials with the largest cohort of 800 elderly women and mean duration of 15 years of follow-up. The design of the study provided cognitive assessment of the participants with an array of neuropsychological instruments, which are currently used to evaluate demented patients. The researchers adjusted the scores for age and education but not for depression. The authors concluded that their study offered no evidence that replacement estrogen therapy preserves cognitive function in older postmenopausal women.

Unfortunately, the studies reviewed so far do not unequivocally answer the question whether estrogen enhances cognitive performance in postmenopausal women. Also, it is not clear if positive results represent an independent effect or are secondary to an improvement of mood and somatic relief.

In a meta-analysis (Yaffe *et al*., 1998) of 10 observational studies in which AD, based on any criteria, was associated with estrogen replacement therapy, the summary odds ratio was 0.71, indicating an almost 30% reduction of risk in estrogen users compared with nonusers. The authors commented on the heterogeneity of the above trials (8 case–control, 2 prospective); however, 4 (Broe *et al*., 1986; Henderson *et al*., 1994; Mortel and Meyer, 1995, Paganini-Hill and Henderson, 1996b) of the 8 case–control studies suggested that estrogen therapy lowered the risk of AD (summary odds ratio range 0.3–0.7). In contrast, two studies (Heyman *et al*., 1984; Amaducci *et al*., 1986) noted an almost twofold risk for AD in estrogen users, while two failed to demonstrate any risk reduction (Graves *et al*., 1990; Brenner *et al*., 1994). It is of importance, though, that the two prospective studies (Tang *et al*., 1996; Kawas *et al*., 1986) suggested that estrogen replacement therapy may reduce the risk of AD.

Specifically, the Manhattan study, conducted by Tang and his associates, found 167 patients with probable AD in a cohort of 1124 community-based women with up to 5-year follow-up and 7 years mean duration of estrogen replacement therapy. Women who took estrogen had a reduced relative risk for AD of 0.40. A significant linear trend suggesting that the duration of estrogen use affected the risk for AD was also noted. The Baltimore study, by Kawas and colleagues, identified 34 cases of probable AD in a cohort of 472 community-dwelling women, followed up to 16 years, and reported a reduced relative risk of 0.46, indicating again a protective effect of estrogen therapy. This study was unable to detect any increase in protection depending on the duration of estrogen replacement therapy. These results were replicated in a 3-year longitudinal study of 1568 Italian women who were included in the risk factor analysis (Baldereschi *et al*., 1998). This investigation also reported that the risk of AD for estrogen replacement therapy users was reduced three-fourths below that of women who had never used estrogen (summary odds ratio, 0.24). All prospective studies used standard National Institute for Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for the clinical diagnosis of AD. The protective effect of estrogen remained after adjusting for other covariates (usually age and education).

There have been four trials examining the effect of estrogen in the treatment of AD. Two of the above were uncontrolled and studied only seven subjects. The other two studies were controlled (one of which was double blinded, randomized) and analyzed a total of 58 women. All studies were less than 6 weeks in duration of treatment and varied in the severity of dementia. Specifically, in an open, uncontrolled trial (Fillit *et al*., 1986) with seven women, carrying the diagnosis of AD and being treated with low dosages of estradiol over a 6-week period, significant improvement over baseline in attention, orientation, mood, and social interaction was noted in three patients. In another uncontrolled study (Honjo *et al*., 1989), it was reported that five of seven AD subjects treated

with 1.25 mg/day of conjugated equine estrogen for 6 weeks showed improvement in Hasegawa Dementia Scale and 6 women improved in the New Screening test for dementia. The same group of researchers in a later 3-week, placebo-controlled, double-blind study (Honjo *et al*., 1993) with 14 AD elderly women treated with 1.25 mg/day of conjugated equine estrogen reported an improvement in cognition as measured by the Hasegawa Dementia Scale, but not in the Mini Mental State Exam or the New Screening test. In a 6-week, open, nonrandomized trial (Ohkura *et al*., 1994b) with 15 AD patients, treated with 1.25 mg/day of conjugated equine estrogen and 15 controls, an improvement in the dementia symptoms was noted in the estrogen-treated group. There was a significant elevation of the mean Mini Mental State Exam score in 10 patients and an increase in the Hasegawa Dementia Scale in 11 subjects at both 3 and 6 weeks. The authors reported that the apparent changes were also noted by the caregivers of the patients, and the test scores returned to baseline after dicontinuation of estrogen replacement therapy. Okhura and his associates published in 1995 a case report with seven subjects treated with low-dose estrogen over an extended time period. Four of seven patients improved dramatically, as was shown by psychometric and behavior rating scales, with reversal of benefits upon termination of treatment.

IV. Summary

In summary, the role of estrogens in AD is supported by basic research, observational, epidemiological, and clinical studies. However, the efficacy of estrogen for AD has yet to be confirmed in large-sample, double-blind, controlled studies. Research centers are conducting two ongoing placebo-controlled, double-blind trials (The Women' s Health Initiative Randomized Trial and the Alzheimer's Disease Cooperative Study Unit) in order to clarify the putative role of estrogen replacement therapy in AD.

References

Amaducci, L. A., Fatiglioni, L., Rocca, W. A., *et al*. (1986). Risk factors for clinically diagnosed Alzheimer's disease: A case-control study of an Italian population. *Neurology* **36**, 922–931.

Aronson, M. K., Ooi, W. L., and Morgenstern, H. (1990). Women, myocardial infarction, and dementia in the very old. *Neurology* **40**, 1102–1106.

Baldereschi, M., Di Carlo, A., Lepore, V., *et al*. (1998). Estrogen-replacement therapy and Alzheimer's disease in the Italian Longitudinal Study on Aging. *Neurology* **50**, 996–1002.

Barrett-Connor, E., and Kritz-Silverstein, D. (1993). Estrogen replacement therapy and cognitive function in older women. *JAMA, J. Am. Med. Assoc.* **269**, 2637–2641.

Bartus, R. T., Dean, R. L., Beer, B., and Lippa, A. S. (1982). The cholinergic hypothesis of geriatric memory dysfunction *Science* **217**, 408–417.

Behl, C., Widmann, M., Trapp, T., and Holsboer, F. (1995). 17-beta estradiol protects neurons from oxidative stress-induced cell death in vitro. *Biochem. Biophys. Res. Commun.* **216**, 473–482.

Bishop, J., and Simpkins, J. W. (1992). Roles of estrogens in peripheral and cerebral glucose utilization. *Rev. Neurosci.* **3**, 121–137.

Brenner, D. E., Kukull, W. A., Stergachis, A., *et al*. (1994). Postmenopausal estrogen replacement therapy and the risk of Alzheimer's disease: A population-based case-control study. *Am. J. Epidemiol.* **140**, 262–267.

Broe, G. A., Henderson, A. S., Creasey, H., *et al*. (1986). A case-control study of Alzheimer's disease in Australia. *Neurology* **40**, 1698–1707.

Caldwell, B., and Watson, R. (1952). An evaluation of psychological effects of sex hormone administration in aged women. Results of therapy after six months. *J. Gerontol.* **7**, 228–244.

Campbell, S., and Whitehead, M. (1977). Oestrogen therapy and the menopausal syndrome. *Clin. Obstet. Gynaecol.* **4**, 31–47.

Ceyla, C. (1998). Abnormalities of neuronal circuitry in Alzheimer's disease; Hippocampus and cortical cholinergic innervation. *Neurology* **51** (Suppl. 1), S18–S29.

Chung, S. K., Pfaff, D. W., and Cohen, R. S. (1988). Estrogen induced alterations in synaptic morphology in the midbrain central gray. *Exp. Brain Res.* **69**, 522–530.

Corder, E. H., Saunders, A. M., Strittmatter, W. J., *et al*. (1993). Gene dose of apolipoprotein E type 4 and risk of Alzheimer's disease in late onset families. *Science* **262**, 921–923.

Cordoba-Montoya, D. A., and Carrer, H. F. (1997). Estrogen facilitates induction of long term potentiation in the hippocampus of awake rats. *Brain Res.* **778**, 430–438.

Coyle, J. T., Price, D. C., and Delong, M. R. (1983). Alzheimer's disease: A disorder of cortical cholinergic innervation. *Science* **219**, 1184–1190.

Daniel, J. M., Fader, A. J., Spencer, A. L., and Dohanich, G. P. (1997). Estrogen enhances performance of female rats during acquisition of a radial arm maze. *Horm. Behav.* **32**, 217–225.

Deutsch, J. A. (1971). The cholinergic synapse and the site of memory. *Science* **174**, 788–794.

Ditkoff, E. C., Crary, W. G., Cristo, M., and Lobo, R. A. (1991). Estrogen improves psychological function in asymptomatic postmenopausal women. *Obstet. Gynecol.* **78**, 991–995.

Drachman, D. A., and Leavitt J. (1974). Human memory and the cholinergic system. *Arch. Neurol. (Chicago)* **30**, 113–121.

Fader, A. J., Hendricson, A. W., and Dohanich, G. P. (1998). Estrogen improves performance of reinforced T-maze alternation and prevents the amnestic effects of scopolamine administered systemically or intrahippocampally. *Neurobiol. Learn. Mem.* **69**, 225–240.

Farr, S. A., Flood, J. F., Scherrer, J. F., Kaiser, F. E., Taylor, G. T., and Morley, J. E. (1995). Effect of ovarian steroids on footshock avoidance learning and retention in female mice. *Physiol. Behav.* **58**, 715–723.

Fedor-Freybergh, P. (1977). The influence of oestrogen on the well being and mental performance in climacteric and postmenopausal women. *Acta Obstet. Gynaecol. Scand.* **64**, 5–69.

Fibiger, H. C. (1991). Cholinergic mechanisms in learning, memory and dementia: Preview of recent evidence. *Trends Neurosci.* **14**, 220–223.

Fillit, H., Weinreb, H., Cholst, I., Luine, V., McEwen, B. S., Amader, R., and Zabrisky, J. (1986). Observations in a preliminary open trial of estradiol therapy for senile dementia-Alzheimer's type. *Psychoneuroendocrinology* **11**, 337–345.

Gao, S., Hendrie, H. C., Hall, K. S., and Siu, H. (1998). The relationships between age, sex, and the incidence of dementia and Alzheimer disease. *Arch. Gen. Psychiatry* **55**, 809–815.

Gibbs, R. B. (1994). Estrogen and nerve growth factor related systems in the brain. Effects on basal forebrain cholinergic neurons and implications for learning and memory processes and aging. *Ann. N.Y. Acad. Sci.* **743**, 165–196.

Gibbs, R. B., Hashash, A., and Johnson, P. A. (1997). Effects of estrogen on potassium stimulated acetylcholine release in the hippocampus and overlying cortex of adult rats. *Brain Res.* **749**, 143–146.

Goodman, Y., Bruce, A. J., Cheng, B., and Mattson, M. P. (1996). Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury, and amyloid β-peptide toxicity in hippocampal neurons. *J. Neurochem.* **66**, 1836–184.

Gould, E., Woolley, C. S., Frankfurt, M., and McEwen, B. S. (1990). Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. *J. Neurosci.* **10**, 1286–1291.

Graves, A. B., White, E., Koepsell, T. D., *et al*. (1990). A case-control study of Alzheimer's disease. *Ann. Neurol.* **28**, 766–774.

Grondstein, F., and Stampfer, M. J. (1998). Estrogen for women at varying risk of coronary disease. *Maturitas* **30**, 19–26.

Hackman, B. W., and Galbraith, D. (1977). Six month study of oestrogen therapy with piperazine oestrone sulphate and its effects on memory. *Curr. Med. Res. Opin.* **4**, 21–27.

Henderson, V. W., Paganini-Hill, A., Emanuel, C. K., Dunn, M. E., and Buckwalter, J. G. (1994). Estrogen replacement therapy in older women: Comparisons between Alzheimer's disease cases and non-demented control subjects. *Arch. Neurol. (Chicago)* **51**, 896–900.

Heyman, A., Wilkinson, W. E., Stafford, J. A., *et al*. (1984). Alzheimer's disease: A study of epidemiological aspects. *Ann. Neurol.* **15**, 335–341.

Honjo, H., Ogino, Y., Naitoh, K., *et al*. (1989). In vivo effects by estrone sulfate on the central nervous system-senile dementia (Alzheimer's type). *J. Steroid Biochem.* **34**, 521–525.

Honjo H, Ogino Y, Naitoh K, *et al*. (1993). An effect of conjugated estrogen to cognitive impairment in women with senile dementia-Alzheimer's type: A placebo-controlled double blind study. *J. Jpn. Menopause Soc.* **1**, 167–171.

Iraman, C. A., Owasoyo, J. O., and Egbunike, G. N. (1980). Influence of estradiol on acetylcholinesterase activity in several rat brain areas and adenohypophysis. *Neurosci. Lett.* **16**, 81–84.

Jaffe, A. B., Toran-Allerand, C. D., Greengard, P., and Gandy, S. E. (1994). Estrogen regulates metabolism of Alzheimer amyloid β-precursor protein. *J. Biol. Chem.* **269**, 13065–13068.

Jorm, A. F., Korten, A. E., and Henderson, A. S. (1987). The prevalence of dementia: A quantative integration of literature. *Acta Psychiatr. Scand.* **76**, 465–479.

Kampen, D. L., and Sherwin, B. B. (1994). Estrogen use and verbal memory in healthy postmenopausal women. *Obstet. Gynecol.* **83**, 979–983.

Kaufman, H., Vadasz, C., and Lajtha, A. (1988). Effects of estradiol and dexamethasone on choline acetyltransferase activity in various rat brain regions. *Brain Res.* **453**, 389–392.

Kawas, C., Resnick, S., Morrison, A., *et al*. (1986). A prospective study of estrogen replacement therapy and the risk of developing Alzheimer's disease: The Baltimore Longitudinal Study of Aging. *Neurology* **48**, 1517–1521.

Kimura, D. (1995). Estrogen replacement therapy may protect against intellectual decline in postmenopausal women. *Horm. Behav.* **29**, 312–321.

Lindsay, R. (1993). Prevention and treatment of osteoporosis. *Lancet* **341**, 801–805.

Loy, R., Gerlach, J., and McEwen, B. S. (1988). Autoradiographic localization of estradiol binding neurons in the rat hippocampal formation and entorhinal cortex. *Brain Res.* **467**, 245–251.

Luine, V. N. (1985). Estradiol increases choline acetyltransferase activity in specific basal forebrain nuclei and projection areas of female rats. *Exp. Neurol.* **89**, 484–490.

Luine, V. N., Park, D., Joh, T., Reis, D., and McEwen, B. S. (1980). Immunochemical demonstration of increased choline acetyltransferase concentration in rat preoptic area after estradiol administration. *Brain Res.* **191**, 273–277.

Matsumoto, A. (1991). Synaptogenic action of sex steroids in developing and adult neuroendocrine brain. *Psychoneuroendocrinology* **16**, 25–40.

Matsumoto, A., Yasumasa, A., and Minoru, O. (1985). Estrogen stimulates neuronal plasticity in the deafferented hypothalamic arcuate nucleus in aged female rats. *Neurosci. Res.* **2**, 412–418.

McEwen, B. S. (1988). Steroid hormones in the brain: Linking nature and nurture. *Neurochem. Res.* **13**, 663–669.

McEwen, B. S., Alves, S. E., Bulloch, K, and Weiland, N. G. (1997). Ovarian steroids and the brain: Implications for cognition and aging. *Neurology* **48**(Suppl. 7), S8–S15.

McLusky, N., Clark, A. S., Naftolin, F., and Goldman-Rakic, P. S. (1987). Estrogen formation in the mammalian brain: Possible role of aromatase in sexual differentiation of the hippocampus and neocortex. *Steroids* **50**, 459–474.

Mortel, K. F., and Meyer, J. S. (1995). Lack of postmenopausal estrogen replacement therapy and the risk of dementia. *J. Neuropsychiatry Clin. Neurosci.* **7**, 334–337.

Mudd, L. M., Torres, J., Lopez, T. F., and Montaque, J. (1998). Effects of growth factors and estrogen on the development of septal cholinergic neurons from the rat. *Brain Res. Bull.* **45**, 137–142.

Ohkura, T., Teshima, Y, Isse K, *et al*. (1994a). Estrogen increases cerebral and cerebellar blood flow in postmenopausal women. *Menopause* **2**, 13–18.

Ohkura T, Isse, K., Akazawa, K., *et al*. (1994b). Evaluation of estrogen treatment in female patients with dementia of the Alzheimer's type. *Endocr. J.* **41**, 361–371.

Ohkura, T., Isse, K., Akazawa, K., *et al*. (1995). Long-term estrogen replacement therapy in female patients with dementia of the Alzheimer type: 7 case reports. *Dementia* **6**, 99–107.

Okuizumi, K., Onodera, O., Tanaka, H., *et al*. (1994). ApoE4 and early-onset Alzheimer's disease. *Nat. Genet.* **7**, 10–11.

O'Neal, M. F., Means, L. W., Poole, M. L., and Hamm, R. J. (1996). Estrogen effects performance of ovariectomized rats in a two-choice water-escape working memory task. *Psychoneuroendocrinology* **21**, 51–65.

Oparil, S. (1999). Arthur C. Corcoran Memorial Lecture. Hormones and vasoprotection. *Hypertension* **33**, 170–176.

Paganini-Hill, A., and Henderson, V. W. (1996a). The effects of hormone replacement therapy, lipoprotein cholesterol levels, and other factors on a clock drawing task in older women. *J. Am. Geriatr. Soc.* **44**, 818–822.

Paganini-Hill, A., and Henderson, V. W. (1996b). Estrogen replacement therapy and risk of Alzheimer's disease. *Arch. Intern. Med.* **156**, 2213–2217.

Phillips, S. M., and Sherwin, B. B. (1992). Effects of estrogen on memory function in surgically menopausal women. *Psychoneuroendocrinology* **17**, 485–495.

Rauramo, L., Lagerspetz, K., Engblom, P., and Punnonen, R. (1975). The effect of castration and peroral estrogen therapy on some psychological functions. *Front. Horm. Res.* **3**, 94–104.

Riggs, B. L., and Melton, L. J., III (1992). The prevention and treatment of osteoporosis. *N. Engl. J. Med.* **327**, 620–627.

Robinson, D., Friedman, L., Marcus, R., Tinklenberg, J., and Yesavage, J. (1994). Estrogen replacement therapy and memory in older women. *J. Am. Geriatr. Soc.* **42**, 919–922.

Saunders, A. M., Strittmatter, W. J., Schmechel, D., *et al*. (1993). Association of apolipoprotein E allele E4 with late-onset and sporadic Alzheimer's disease. *Neurology* **43**, 1467–1472.

Sherwin, B. B. (1988). Estrogen and/or androgen replacement therapy and cognitive functioning in surgically menopausal women. *Psychoneuroendocrinology* **13**, 345–357.

Singh, E., Meyer, E. M., and Simpkins, J. W. (1995). The effect of ovariectomy and estradiol replacement on brain derived neurotrophic factor messenger ribonucleic acid expression in cortical and hippocampal brain regions of female Sprague-Dawley rats. *Endocrinology (Baltimore)* **136**, 2320–2324.

Singh, M., Meyer, E. M., Millard, W. J., and Simpkins, J. W. (1994). Ovarian steroid deprivation results in a reversible learning impairment and compromised cholinergic function in female Sprague-Dawley rats. *Brain Res.* **644**, 305–312.

Sites, C. K. (1998). Hormone replacement therapy: Cardiovascular benefit for aging women. *Coronary Artery Dis.* **9**, 789–793.

Srivastava, R. A., Bhasin, N., and Srivastava, N. (1996). Apoliproprotein E gene expression in various tissues of mouse and regulation by estrogen. *Biochem. Mol. Biol. Int.* **38**, 91–101.

Stone, D. J., Rozovsky, I., Morgan, T. E., Anderson, C. P., and Finch, C. E. (1998). Increased synaptic sprouting in response to estrogen via an apolipoprotein E-dependent mechanism: Implications for Alzheimer's disease. *J. Neurosci.* **18**, 3180–3185.

Strittmatter, W. J., Saunders, A. M., Schmechel, D., *et al*. (1993). Apolipoprotein E: High avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 1977–1981.

Tang, M. X., Jacobs, D., Stern, Y., *et al*. (1996). Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease. *Lancet* **348**, 429–432.

Terasawa, E., and Timiras, P. (1968). Electrical activity during the estrous cyclic changes in limbic structures. *Endocrinology (Baltimore)* **83**, 207–216.

Toran-Allerand, C. D., Miranda, R. C., Bentham, W. D. L., *et al*. (1992). Estrogens receptors colocalize with low-affinity growth factor receptors in cholinergic neurons of the basal forebrain. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 4668–4672.

Van Duijn, C. M., de Knijff, P., and Cruts, M. (1994). Apolipoprotein E4 allele in a population-based study of early-onset Alzheimer's disease. *Nat. Genet.* **7**, 74–78.

Vanhulle, R., and Demol, R. (1976). A double blind study into the influence of estriol on a number of psychological tests in postmenopausal women. *In* "Consensus on Menopausal Research" (P. A. Van Keep, R. B. Greenblatt, and M. Albeaux-Fernet, eds.), pp. 94–99. MTP Press, London.

Xu, H., Gouras, G. K., Greeenfield, J. P., *et al*. (1998). Estrogen reduces neuronal generation of Alzheimer β-amyloid peptides. *Nat. Med.* **4**, 447–451.

Yaffe, K., Sawaya, G., Lieberburg, I., and Grady, D. (1998). Estrogen therapy in postmenopausal women; effects on cognitive function and dementia. *JAMA, J. Am. Med. Assoc.* **279**, 688–695.

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Cholinergic Treatments of Alzheimer's Disease

I. Introduction

A gradual degenerative process of the central nervous system characterizes Alzheimer's disease (AD). Although the pathogenesis of AD is not completely understood, advances in research have led to the development of several therapeutic strategies. The conceptualization of treatments for AD is in accordance with preclinical and basic science findings. In this chapter we review the evidence supporting the use of therapies targeting the cholinergic system. Several lines of evidence suggest a critical role of the cholinergic mechanisms involved in AD.

1. Cholinergic innervation and neurotransmission are pivotal in the execution of complex cognitive tasks (Deutsch, 1971; Bartus *et al*., 1982, 1987).

2. Rodents, monkeys, and humans subjected to anticholinergic treatments, such as scopolamine (Ostfeld and Aruguette, 1962; Crow and Grove-White, 1973; Drachman and Leavitt, 1974; Bartus *et al*., 1987) or cholinergic lesions (Irle and Markowitsch, 1987; Berger-Sweeney *et al*., 1994; Leanza *et al*., 1996; Walsh *et al*., 1996), manifest cognitive impairments.

3. These deficits can be effectively restored with cholinergic agents (Davis *et al*., 1978; Bartus, 1979; Aigner *et al*., 1987; Bartus *et al*., 1987; Dokla and Thal, 1988). Experiments with cholinergic-rich fetal tissue transplants, grafted in rats with lesions of the nucleus basalis of Meynert, support the role of acetylcholine (ACh) in restoring learning and memory deficits following basal forebrain damage (Winkler *et al*., 1995).

4. Postmortem studies of patients with AD demonstrate a significant reduction of cholinergic neurons in the basal forebrain (Davies and Maloney, 1976; Whitehouse *et al*., 1981) and a profound decline in the activity of choline acetyltransferase (ChAT) in the neocortex and hippocampus (Davies and Maloney, 1976; Perry *et al*., 1977).

5. AD brains demonstrate no change or an increased number of muscarinic receptors and a decreased number of high-affinity nicotinic receptors (Nordberg and Winblad, 1986; Nordberg, 1992; Shimohama *et al*., 1986).

6. Significant correlation exists between cholinergic deficits and the severity of dementia (Perry *et al*., 1978).

All these findings suggest the potential therapeutic value of agents that enhance cholinergic neurotransmission for AD.

II. Acetylcholinesterase Inhibitors

Different strategies, including ACh precursor loading (lecithin, choline), ACh releasers, cholinometics, and acetylcholinesterase (AChE) inhibitors have been actively pursued as therapeutic options over the past decades. Trials with choline or lecithin have had equivocal outcomes (Etienne *et al*., 1978, 1981; Thal *et al*., 1981). Most promising results have been observed with cholinesterase inhibitors, several of which have been tested in phase III clinical trials in the United States and Europe. Currently, two of the above drugs (tacrine and donepezil) have been approved by the Food and Drug Administration (FDA). However, several others AChE inhibitors (rivastigmine, galanthamine, metrifonate, and physostigmine) are at different stages of FDA authorization. The FDA guidelines recommend that the clinical studies accompanying new drug applications for AD use the Alzheimer's Disease Assessment Scale, Cognitive Subscale (ADAS-Cog), the Clinician Interview-Based Impression of Change (CIBIC) or the Clinical Global Impression of Change (CGIC) scale as primary efficacy variables. The ADAS-Cog is an 11-item psychometric instrument assessing different aspects of cognitive performance. Scores in ADAS-Cog range from 0 (no errors) to 70 (severe impairment) with an expected average decline of 8 points over a 1-year period. An improvement of four points or more in ADAS-Cog with drugs would be considered a clinically significant effect (U.S. Department of Health and Human Services, 1989). Both the CIBIC and the CGIC are seven-point ordinal scales ranging from 1 (marked improvement) to 7 (marked deterioration), which provide a more global assess-

ment of the patient's clinical condition, and unlike the ADAS-Cog are not standardized. A drug to be considered for approval should result in a clinically and statistically significant improvement of the core cognitive symptoms of AD compared to placebo.

A. Tacrine (THA)

Tacrine (9-amino-1,2,3,4-tetrahydroacridine) was the first cholinesterase inhibitor approved by the FDA for the treatment of AD. Tacrine is a synthetic compound, known since 1949, that has a structural similarity to the K^+ channel blocker 4-aminopyridine (Osterrieder, 1987). This agent is a mixed-type (competitive/noncompetitive) (Catalan *et al*., 1993), reversible inhibitor of cholinesterase that binds close to the catalytically active serine in the active site of the enzyme. In addition to its inhibition of AChE and butyrylcholinesterase, tacrine has an effect on muscarinic (M1, M2) and nicotinic receptors (Flynn and Mash, 1989; Adem *et al*., 1990; Xiao *et al*., 1993). Tacrine is rapidly absorbed from the gastrointestinal tract and easily crosses the blood–brain barrier. It is metabolized in the liver by the P450 system (CYP1A2, CYP2D6) and excreted by the kidneys with a 3 hr elimination half-life (Parnetti, 1995; Physician's Desk Reference, 1999a). Tacrine has nonlinear pharmacokinetics, with significant variability in pharmacokinetic parameters after oral, intravenous, and rectal administration. This compound has potential drug interactions with cimetidine and theophylline.

Following the pilot trials with tacrine (Summers *et al*., 1981, 1986), more than a dozen (Chatellier and Lacomblez, 1990; Gauthier *et al*., 1990; Ahlin *et al*., 1991; Molloy *et al*., 1991; Davis *et al*., 1992; Farlow *et al*., 1992; Wilcock *et al*., 1993; Knapp *et al*., 1994; Maltby *et al*., 1994; Wood and Castleden, 1994; Forette *et al*., 1995; Foster *et al*., 1996) randomized, double-blind, placebo-controlled trials have been published. The majority of them used standardized psychometric instruments and demonstrated that tacrine in a dose above 80 mg/day improves the cognition and function in patients with mild to moderate-stage AD. A large percentage of patients had to be discontinued from the studies because of the development of liver function tests elevations (Knapp *et al*., 1994; Watkins *et al*., 1994; Samuels and Davis, 1997; Gracon *et al*., 1998). Many of these abnormalities may have been due to forced uptitration. More than 25% of patients developed clinically significant hepatic enzyme elevations (>3 times the upper limit of normal) and severe liver enzyme abnormalities (>20 times normal) occurred in 2% of patients. These elevations occurred mostly during the first 9 weeks of treatment and they were reversible, with patients recovering within 3–4 weeks after discontinuation of treatment. They were almost always asymptomatic, more common in women, and related to the duration of the treatment. Fatal hepatotoxicity has not been reported yet. It is of interest that 88% of patients were successfully rechallenged with tacrine. Other common adverse effects related to the cholinomimetic profile of tacrine include gastrointestinal symptoms (nausea, vomiting, diarrhea, abdominal pain) and rarely myalgia and dizziness.

Because of tacrine's liver toxicity, monitoring of the liver function is required during the course of treatment and careful uptitration of the medication is recommended (Samuels and Davis, 1997). Tacrine has been widely used after its FDA approval and pharmacoeconomic studies (Lubeck *et al*., 1994; Knopman *et al*., 1996) have demonstrated that tacrine delays institutionalization, suggesting the economic utility of this medication. Moreover tacrine has provided a benchmark against which other cognitive enhancers can be compared. The need for liver function monitoring and four-time dosing schedule of tacrine has led to its limited use.

B. Donepezil (E2020)

Donepezil is the second AChE inhibitor approved in 1996 for the treatment of AD (Physician's Desk Reference, 1999b). Donepezil is a noncovalent, highly selective inhibitor of AChE (donepezil's inhibition of AChE is 1250 times more than that of butyrylcholinesterase). It is a reversible inhibitor of mixed type with a favorable pharmacokinetic profile (Ohnishi *et al*., 1993; Shintani and Uchida, 1997).

Donepezil is well absorbed after oral administration with an almost 100% bioavailability, and it is not affected by the presence of food. It has a 70 hr elimination half-life (60 hr in young adults, 104 hr in elderly) and it is metabolized by CYP2D6, CYP3A4 hepatic enzymes, without evidence of liver toxicity—in contrast to acridine based AChE inhibitors. It manifests linear pharmacokinetics with a plateau of inhibition at plasma concentration >50 ng/ml and the recommended dose is 5–10 mg/day. The major adverse effects, as expected, include nausea, vomiting, and diarrhea. Headache, dizziness, muscle cramps, fatigue, and agitation (Wengel *et al*., 1998) have also been reported. Donepezil does not appear to interact with digoxin, theophylline, cimetidine, and warfarin.

In the first phase II/III, double-blind, placebo-controlled study (Rogers and Friedhoff, 1996), a sample of 161 AD patients were randomized to different fixed doses (1, 3, and 5 mg daily) versus placebo. The trial was 14 weeks in duration (12 weeks double-blind donepezil or placebo, and 2 weeks placebo washout phase). More than 80% of the cohort completed the study with the 5 mg group showing statistically significant improvement on ADAS-Cog and MMSE relative to the placebo group. Comparable incidences of adverse effects among the three dose groups and placebo (68% vs 65%) were noted. In an open-label study, with 133 patients who had completed the previous 14 week trial and were followed up to 192 weeks, a long-term benefit of donepezil was noted (Rogers and Friedhoff, 1998). Donepezil was well tolerated without liver abnormalities. A 30 week, randomized, double-blind, placebo-controlled trial (24 week active medication and 6 week placebo washout phase) of 473 patients suggested statistically significant improvements in cognitive performance outcomes with both 5 and 10 mg/day doses, mostly in favor of the 10 mg dose at the end point (Rogers *et al*., 1998a). This study confirmed that there is a dose–response effect with 16% of patients in the 10 mg group discontinuing the study because of serious adverse effects compared to 7% in the placebo or 6% to the 5 mg group, possibly related to the greater degree of AChE inhibition. It is worthwhile to mention here that 68% of the 10 mg group completed the study and half of these patients achieved at least four-point improvement in ADAS-Cog scores. These findings compare very favorably

with the high doses of tacrine (160 mg/day), where the dropout percentage reached 75 at the end of the 30 week trial (Knapp *et al*., 1994).

A 15 week (12 week active and 3 week washout phase), double-blind, placebo-controlled, parallel group study (Rogers *et al*., 1998b) with 468 patients replicated the results of previous trials with donepezil. Statistically significant correlations were detected between donepezil plasma concentrations and AChE inhibition, ADAS-cog, and CIBC with caregiver input (CIBIC-plus) scores. The adverse effects with both 5 and 10 mg dosages were comparable to the ones observed with placebo. Transient nausea, diarrhea, and insomnia were more common with the 10 mg dose and there was no association with liver toxicity. The efficacy of donepezil has been reevaluated in a multinational 24 week, placebo-controlled study of 818 patients, which demonstrated significant improvement in cognitive and global function, suggesting also a greater benefit in the 10 mg daily regimen, for the treatment of mild to moderate AD (Burns *et al*., 1999).

Donepezil's once-a-day dosing schedule and its mild side-effect profile has provided a safe alternative to tacrine. No direct studies comparing tacrine with donepezil have been performed as yet. Currently, the potential utility of donepezil for patients who exhibit mild cognitive impairment, but do not meet criteria for frank dementia, is under investigation.

C. Galanthamine

Galanthamine is a naturally occurring alkaloid of the common snowdrop plant and it is structurally related to codeine (Cozanitis *et al*., 1983). Galanthamine is a selective, reversible, competitive, carbamate inhibitor of AChE (Thomsen and Kewitz, 1990). This compound, like other homologous alkaloids, can act as a noncompetitive agonist of nicotinic acetylcholine receptors (nAChR) by binding to sites that are different from the ACh receptors (Storch *et al*., 1995). It has two major metabolites (epigalanthamine and galanthaminone) with negligible potency (>130 times) in comparison with the parent compound (Bickel *et al*., 1991). The drug has a mean half-life of 5.7 hr and is safe. The most common adverse effects are nausea and vomiting; hepatotoxicity has not been reported to date. Galanthamine has been used in anesthesia to reverse the paralytic effect of tubocurarine-like muscle relaxants (Cozanitis *et al*., 1981). Its long half-life and its proven efficacy in reversing the cognitive deficit of rats with basal forebrain lesions (Sweeney *et al*., 1988) suggested its role, as a cognitive enhancer, for patients with AD (Dal-Bianco *et al*., 1991). A recent phase II trial suggested a beneficial role for this agent with clinical improvement on the ADAS-cog (Wilcock and Wilkinson, 1997). Multicenter clinical trials are currently conducted in the United States and in Europe and the results have not been published yet.

D. Physostigmine

Physostigmine was one of the first agents studied as treatment for the cognitive impairment produced by the cholinergic deficit in AD (Drachman and Leavitt, 1974; Davis *et al*., 1978; Bartus *et al*., 1982). Physostigmine, a natural alkaloid, is a reversible, nonselective cholinesterase inhibitor, being more selective for AChE than butyrylcholinesterase. It can be administered orally and parenterally with a variable dose response and a narrow therapeutic window. Physostigmine, when taken orally, exhibits a high but variable bioavailability with an elimination half-life of 20–30 min (Whelpton, 1983; Whelpton and Hurst, 1985; Johansson and Nordberg, 1993). Due to the very short half-life, administration every 2 hr was required in the initial trials (Beller *et al*., 1985; Stern *et al*., 1987). Earlier reports relied on the intravenous administration of physostigmine (Christie *et al*., 1981; Davis and Mohs, 1982). Studies with the oral route of administration (Mohs *et al*., 1985; Beller *et al*., 1985, 1988; Jenike *et al*., 1990) reported cognitive enhancement with this agent. Several of these pilot studies were not controlled; they tested a small number of subjects and used different routes of physostigime administration, and a variety of psychometric tools. Yet, investigators consistently demonstrated cognitive improvement in a subgroup (30%) of patients. Variability in the absorption, metabolism, central nervous system penetration, and plasma concentration, along with a short half-life with an inverted U-shaped response curve have hampered the use of physostigmine as a viable choice in the treatment of AD. However, a long-acting physostigmine is currently under investigation and the reported results are encouraging. Specifically, in a 6 week, placebo-controlled, double-blind study (Thal *et al*., 1996) with an initial cohort of 1111 subjects treated with controlled-release physostigmine, a subset (366) of AD patients, showing some improvement during the initial dose titration period, entered the active phase and were randomized to placebo versus their ''best dose'' of physostigmine. At the end of the 6 week period physostigmine-treated patients scored higher than placebo-treated patients on the ADAS (1.75 points) and CGIC (0.26 points) scores. In a 24 week, parallel-group trial (Thal *et al*., 1999) with 475 patients randomized to three groups—placebo and controlled-release physostigmine at 30 or 36 mg daily—a 2.9-point difference in ADAS-Cog and a 0.3-points difference in favor of this agent versus placebo was noted. In both trials a significant number of patients withdrew prior to completion of the study, while more than 40% reported nausea and vomiting, raising questions about the clinical utility of this drug. Hepatotoxicity or blood dyscrasias did not occur. A recently published, 24 week, placebo-controlled study of 204 patients treated with transdermal administration of two different doses of physostigmine (30 and 60 mg) failed to show any beneficial effect (Moller *et al*., 1999).

Data, suggesting that pharmacological manipulations of the noradrenergic system may improve cognition, have led in studies investigating a possible role of α2-agonists—such as clonidine or guanfacine—and the α2-antagonist, yohimbine, in the cognitive processes of learning and memory (Coull, 1994). In accordance with the above, trials with physostigmine in combination with noradrenergic system modulators as clonidine (Davidson *et al*., 1989), or selegiline (Marin *et al*., 1995) have been conducted, in order to achieve augmentation of both cholinergic and noradrenergic neurotransmission. These studies proved the efficacy and safety of combination treatments. However, they were too small to provide a definite answer regarding the potential utility of combination treatments. The efficacy of the above treatments is still being explored.

E. Eptastigmine

Eptylphysostigmine (eptastigmine) is a carbamate, derivative of physostigmine. The medication after oral administration has rapid distribution, with an inverted U-shaped dose–response curve and good penetration of the blood–brain barrier (Parnetti, 1995), although food can reduce its bioavailability (Bjornsson *et al*., 1998). It causes a long-lasting, dose-dependent, sigmoidal red blood cell AChE inhibition of the competitive type and its action on butyrylcholinesterase is weak and not dose dependent (Imbimbo *et al*., 1995). Phase I studies in healthy elderly subjects have shown that oral doses up to 32 mg are safe and well tolerated (Auteri *et al*., 1993; Sramek *et al*., 1995; Mant *et al*., 1998). In a multicenter, double-blind, placebo-controlled, parallel group, 4 week study (Canal and Imbimbo, 1996) of 103 subjects receiving 40–60 mg/day of eptastigmine, 34% of AD patients versus 0% in the placebo group improved on the CGIC and the Instrument of Activities of Daily Living Scales (IADLS). Marginal improvements were shown in other cognitive tests. The drug was well tolerated and moderate cholinergic side-effects were noted in one-third of patients, predominantly in patients with 50% acetylcholinesterase inhibition. In 25 week, controlled trial (Imbimbo *et al*., 1998) of 320 patients receiving placebo versus 10 or 15 mg of eptastigmine three times daily a statistically significant difference of 2.0 points on ADAS-Cog was noted only in the 15 mg three times daily group, while the CIBIC-plus score did not reach statistical significance in any group. The drug was well tolerated and more than 90% completed the protocol. A recently published, controlled study (Imbimbo *et al*., 1999) of 491 patients, randomized in three groups (placebo and 15 and 20 mg three times daily) demonstrated that 20 mg three times daily of eptastigmine can reach statistically meaningful improvement on the ADAS-Cog, CIBIC-plus, and IADL. The majority of patients (>85%) completed the trial and only 8% of patients on eptastigmine versus 7% on placebo had to discontinue the study due to adverse effects. The researchers noted that these results compared favorably with tacrine, donepezil, and metrifonate but a dose-dependent neutropenia in about 5% of treated patients may limit the clinical utility of this agent.

F. Rivastigmine

Rivastigmine (ENA, 713, carbamoylatine) is a novel, ''pseudoirreversible,'' selective, noncompetitive, carbamate AChE inhibitor (Enz and Floersheim, 1996; Polinsky, 1998). This agent preferentially inhibits in a dose-dependent fashion (Cutler *et al*., 1998a) the G1 enzymatic form of AChE, which predominates in the central nervous system. It has a long duration of action and results in up to 10 hr of enzyme blockade after a single dose. The drug demonstrates nonlinear pharmacokinetics with rapid and almost complete absorption and low bioavailability (35% of the administered dose), with primarily renal metabolism. Rivastigmine lacks hepatic toxicity or interaction with medications metabolized by CYP-450 isoenzymes. Rivastigmine has been shown to improve cognitive performance in animal models with basal forebrain lesions (Niigawa *et al*., 1995). Phase I/II trials have shown the efficacy and safety of the medication (Anand *et al*., 1996; Sramek *et al*., 1996; Forette *et al*., 1999). In phase II/III clinical studies it appeared that rivastigmine 6–12 mg/day may confer statistically significant cognitive improvement to recipients of the drug compared to placebo (Spencer and Noble, 1998). A randomized, double-blind, placebo-controlled, 26 week study (Rosler *et al*., 1999) with 1–4 mg/day or 6–12 mg/day doses of rivastigmine in 725 AD patients demonstrated that higher doses resulted in cognitive improvement as measured by ADAS, CIBIC-plus, MMSE, and the Global Deterioration Scale (GDS). The adverse effects were cholinergic in nature, occurred in a dose-dependent fashion, and affected predominantly the gastrointestinal system. Headaches, malaise, fatigue, and dizziness have also been reported. Specifically, half of patients in the higher dose (6–12 mg) group developed nausea and one-third from the same group vomiting. A statistically significant decrease in body weight (mean change, -1.39 kg) was noted in the higher rivastigmine group. Apart from nausea there was no significant difference in adverse effects between placebo and the low-dose group. The percentage of patients who discontinued treatment for any reason reached 33% in the higher dose arm versus 14% for the other two groups, whereas the proportion of patients who dropped out because of adverse effects was 23% versus 7%, respectively. Similar results have been reported by an earlier randomized study in North America (Corey-Bloom *et al*., 1998). The medication has already been approved in some countries in Europe and South America.

G. Velnacrine (HP-029)

Velnacrine maleate, or L-hydroxytacrine, is an acridine-based derivative and a principal metabolite of tacrine (Truman *et al*., 1991). Velnacrine is a reversible, lipophilic cholinesterase agent that exerts both AChE and butyrylcholinesterase inhibition. It can be taken orally (Puri *et al*., 1989, 1990), is metabolized primarily by the liver, has limited renal excretion, and manifests nonlinear pharmacokinetics (Turcan *et al*., 1993; Parnetti, 1995). This compound has been associated with improvement of memory in primates (Jackson *et al*., 1995). In two, double-blind, placebo-controlled studies conducted by the Mentane Study Group (Antuono, 1995; Zemlan, 1996), patients achieved better scores in the ADAS-cog and CGIC scales. Most improvement was observed in doses greater than 150 mg per day. The most common adverse effects were consistent with the cholinergic properties of the drug (diarrhea, nausea, vomiting). A few patients developed white blood cell abnormalities. Velnacrine caused a reversible, dose-dependent, asymptomatic elevation of liver transaminases in about 30% of patients versus 3% in the placebo group. Liver enzyme elevations occurred between 6 and 8 weeks and led to termination of treatment in a third of the studied patients. These findings invite a comparison between velnacrine and tacrine, given the structure similarities of these agents.

H. Metrifonate

Metrifonate is a nonselective almost irreversible phosphorylating agent, which has been used for decades against schistosomiasis (Cerf *et al*., 1962). Its therapeutic efficacy for the treatment of schistosomiasis is due to the fact that AChE is present in abundance in the muscle system of the schistosomes

(Camacho *et al*., 1994). Metrifonate is a long-acting cholinesterase inhibitor, with no anticholinesterase activity, that exerts its action through a slow transformation to 2,2′-dichlorovinyldimethyl phosphate (DDVP or dichlorvos) (Hinz *et al*., 1996). This compound inhibits butyrylcholinesterase more than AChE (Pacheco *et al*., 1995). The administration of metrifonate achieves a high degree of cholinesterase inhibition (70%) without the need for dose adjustment. The active compound is released in a slow sustained mode, and steady state takes 6–8 weeks in the absence of loading dose (Cutler *et al*., 1998b). Metrifonate is not dependent on the P450 enzyme system and undergoes less than 15% protein binding, which limits the possibility of drug interactions. Phase I/II studies have demonstrated that the drug has a safe profile with minimal drug interactions and is well tolerated. Clinical trials with patients diagnosed with mild to moderate AD have demonstrated the role of this agent in enhancing cognitive performance. In a 3 month double-blind, placebo-controlled trial (Becker *et al*., 1996) with 50 patients who completed the study, the authors reported a mean 50% inhibition of red blood cell AChE activity and a 2.6-point difference in ADAS-Cog. No difference was found in other outcome measures and the authors questioned the clinical value of their findings. Adverse effects were uncommon, although two patients were diagnosed with lymphoma but reviews of use of metrifonate have not linked this compound with increased risk of malignancy (World Health Organization, 1989). In a 6 month double-blind study, conducted by the same researchers in a cohort of 47 AD patients the results suggested a clinically meaningful cognitive improvement in the metrofinate group (Becker *et al*., 1998).

In a 12 week, placebo-controlled, double-blind study (Cummings *et al*., 1998) of 480 patients, of whom more than 90% completed the trial, improvement in cognition was demonstrated in ADAS-cog and the CIBIC-plus. In a 26 week, placebo-controlled study (Morris *et al*., 1998) of 408 subjects, a beneficial effect of metrofinate was observed in both ADAS-Cog and CIBIC-plus subscales. Again, more than 80% of patients completed the active period and 12% discontinued due to adverse effects. The low attrition reflects the favorable safety and tolerance profile of the drug. The above results have been replicated in a 26 week, double-blind study of 264 patients (Raskind *et al*., 1999). The later two studies suggested that metrifonate benefits not only the cognition and global function but may also attenuate the behavioral disturbances of AD patients (Morris *et al*., 1998, Raskind *et al*., 1999). Similar findings have been noted in a preliminary report of the Metrifonate in Alzheimer Trial study (McKeith, 1998). Due to the unique pharmacokinetic profile of metrifonate, with delay in reaching steady-state, patients in clinical trials are dosed initially with a loading regimen of the drug. It appears though that the no-loading dose regimen is equally efficacious and better tolerated in comparison to the loading dose regimen (Jann *et al*., 1999). Overall, the adverse events have been mild and primarily gastrointestinal in nature, and less than 10% of patients discontinued the treatment because of adverse effects. The most common adverse events associated with the drug included diarrhea, nausea, abdominal pain, leg cramps, and rhinitis. Liver toxicity or elevations of hepatic enzymes have not been observed and the drug is in the process of FDA approval.

I. MSF

MSF (methanesulfonyl fluoride) is a highly selective, long-lasting AChE inhibitor with low toxicity, which was able to attenuate scopolamine-induced in Sprague–Dawley rats (Palacios-Esqivel *et al*., 1993). In the first double-blind, placebo-controlled, phase II/III study of 21 patients (Moss *et al*., 1999), this agent demonstrated a safe pharmacodynamic profile without gastrointestinal side-effects and a statistical improvement in ADAS-cog and MMSE compared to placebo, which endured for 8 weeks after termination of treatment. The small number of patients and the short duration of treatment (8 weeks double-blind, placebo-controlled and 8 weeks washout period) do not permit generalization of the above results. Further clinical trials with larger cohorts are needed to support the notion of less toxicity and equal or better efficacy of this agent, compared to placebo.

III. Cholinergic Agonists

Another potential strategy for boosting cholinergic function in patients with AD involves the administration of selective cholinergic receptor agonists. It is known that the cholinergic receptors fall into two major categories, muscarinic and nicotinic. Four muscarinic receptor subtypes (M1, M2, M3, M4) (Waelbroeck *et al*., 1990) have been identified pharmacologically and five receptor subtypes (M1–M5) have been detected through cloning (Bonner *et al*., 1988; Hulme *et al*., 1990; Dorje *et al*., 1991). Studies with antisera (Yasuda *et al*., 1992) demonstrate a substantial overlap between the pharmacologically and the molecularly defined subtypes of muscarinic receptors. The muscarinic receptors are slow, and they bind to G proteins. Specifically the M1, M3, and M5 are coupled to phosphoinositide hydrolysis and cause cell excitation, whereas M2 and M4 are coupled to cyclic AMP and cause inhibition (Peralta *et al*., 1988; Cooper *et al*., 1996). Localization studies have shown that M1 receptors are found preferentially in the neocortex and hippocampus (Levey *et al*., 1991; Flynn *et al*., 1995), areas crucial for cognition and memory. M2 receptors are found in the cardiac tissue and M3 subtype receptors are detected in secretory glands (Waelbroeck *et al*., 1991). M4 receptors have a distribution almost similar to M1 and their pharmacological properties have not been defined very well (Yasuda *et al*., 1992). M5 receptors levels are very low in rat brains (Yasuda *et al*., 1992) and their role is not clear yet. In most studies with postmortem brain tissue from AD patients it was demonstrated that the density of M1 receptors remains unaltered or even increased in AD (Whitehouse and Kellar, 1987; Giacobini, 1990; Nordberg, 1992), whereas M2 receptors are relatively decreased (Mash *et al*., 1985). Stimulation of the presynaptic M2 receptors inhibits Ach release. Because M1 is the more abundant receptor in the neocortex and hippocampus and most of the peripheral adverse effects are caused by activation of M2 and M3 subtypes, the development of agents with high specificity for the M1 receptors has a potential clinical value. In addition, *in vitro* studies have shown that M1 agonists increase the synthesis of nonamyloidogenic forms of APP (Nitsch *et al*., 1992) and regulate tau phosphorylation (Sadot *et al*., 1996). These agents may be involved in neurotrophic mechanisms in the brain (Pinkas-Kramarski *et al*.,

1992), affecting the development, plasticity, and preservation of brain tissue. Muscarinic agonists without specificity have been studied in the past with little or no success. Moreover, since M2 receptor agonists can inhibit Ach release, the blockage of M2 receptors would result in increased levels of acetylcholine. At present studies with selective muscarinic agonists are being conducted with the objective of restoring the cholinergic deficit and possibly succeeding in modifying the course of this disease. However, the tonic stimulation by the above agents does not reflect the phasic physiological mechanism of AChE release and may pose limitations to the benefits gained by these agents.

A. Bethanechol

Bethanechol is a synthetic β-methyl analog of Ach with M1 and M2 agonist properties. This compound has been studied extensively in the past decades and has demonstrated limited efficacy (Harbaugh *et al*., 1989; Wilson and Martin, 1988), a narrow therapeutic window (Penn *et al*., 1988), and variable response with severe adverse effects in higher doses (Read *et al*., 1990). A major limitation has been the inability of bethanechol to penetrate the blood–brain barrier, thereby requiring intrathecal administration with a high risk of perioperative complications (seizures, hematoma, and infections).

B. Arecoline

Arecoline is a natural alkaloid of the Taiwanese betel nut. The compound has cytomodulating effects and has been implicated in the pathogenesis of oral cancer and oral submucous fibrosis (Van Wyck *et al*., 1994; Tsai *et al*., 1997). This agent has been also used in *Echinococcus* screening testing due to its strong purging activity (Craig *et al*., 1995). Arecoline is a nonselective M1 and M2 agonist with nicotinic properties. It has been evaluated for enhancing memory and learning in rats (Molinengo *et al*., 1995) and primates (Buccafusco *et al*., 1995a) and for its ability to increase brain glucose utilization (Maiese *et al*., 1994). Arecoline infusion in patients with probable AD produced a dose-dependent response in cognitive tests (Rafaele *et al*., 1996). In a randomized, double-blind study (Tariot *et al*., 1988) with 12 AD patients, no improvement was found in an array of cognitive psychometric tests. However, with a low-dose infusion (1–2 mg/hr), an arousal effect and psychomotor activation was observed, whereas infusions with a higher dose (4 mg/hr) produced psychomotor retardation, dysphoria, and difficulty with verbal expression. It was postulated that the lower dose activation was a muscarinic phenomenon and the higher dose retardation was a nicotinic phenomenon. Overall, this agent has not been recently studied as a cognitive enhancer for AD.

C. RS-86

RS-86 (2-ethyl-8-methyl-2,8-diazospiro-4,5-decart-1,3-dianhydrobromide) is a nonselective muscarinic agonist with higher affinity for M1 than M2 receptors. It can be taken orally, penetrates the blood–brain barrier, has a long half-life, and has the tendency to induce hypothermia in mice (Freedman *et al*., 1989). RS-86 has been administered in sleep studies as it has been hypothesized that REM sleep disinhibition in depression is associated with muscarinic hypersensitivity (Berger *et al*., 1989; Gann *et al*., 1992). A double-blind, crossover study (Foster *et al*., 1989) of 10 patients with progressive supranuclear palsy who were treated with RS-86 did not improve motor activity or cognition. In a controlled study (Wettstein and Spiegel, 1984) with RS-86, a subset of patients with AD showed minimal changes in mood, social behavior, and cognition. These results have not been replicated in other trials (Bruno *et al*., 1985; Hollander *et al*., 1987).

D. AF Compounds

AF-series compounds, are structurally rigid, synthetic analogs of Ach (Fisher *et al*., 1993, 1996). They were designed to act on specific muscarinic subtype receptors and they have been shown to exert both agonistic and antagonistic activity. The action of AF agents on muscarinic receptors depends on the tissue, the muscarinic receptor subtype, and the functional assays. They demonstrate variable degree of receptor selectivity and efficacy (Fisher *et al*., 1993, 1996). AF102B, AF150, and AF151 are selective M1 agonists and AF125 is mostly a M2 agonist. AF compounds have demonstrated a beneficial effect on cognition in experiments performed with various animal models with a relatively wide safety margin. The activity of AF compounds is coupled with phosphoinositides hydrolysis and enhances the secretion of APP. Observations in cultured cells have suggested that these drugs may act in concert with nerve growth factor to enhance APP processing, since the secreted form of APP are instrumental in decreasing the production of β-amyloid (Nitsch *et al*., 1992; Haring *et al*., 1995). Other *in vitro* data (Sadot *et al*., 1996) suggest that these muscarinic ligands decrease the amount of phosphorylated tau protein, the principal component of neurofibrillary tangles.

E. AF102B

AF102B is highly selective for M1 receptor agonist. The compound has a long-lasting activity and in experiments with rats (Fisher *et al*., 1989; Nakahara *et al*., 1989; Vincent and Sepinwall, 1992) and primates (O'Neill *et al*., 1998) has been shown to enhance cognitive performance with a safe therapeutic index. In a 6 week, single-blind, placebo-controlled study (Fisher *et al*., 1994), AF102B, administered in 20 to 60 mg three times daily doses, was found to improve cognitive performance, measured with the ADAS-cog and ADAS-word recognition scales. Diaphoresis and excessive salivation were the most common side-effects in the higher dose (60 mg three times daily) group. Double-blind studies will provide more definitive evidence regarding the efficacy of AF102B.

F. Xanomeline

Xanomeline is a functionally selective M1/M4 agonist that has shown a promising therapeutic profile in preclinical trials (Shannon *et al*., 1994). Xanomeline is well absorbed orally, crosses the blood–brain barrier, and undergoes extensive liver metabolism with at least six metabolites (Andersen and Hansen, 1997). In earlier phase II studies (Medina *et al*., 1997) the oral formulation was found to reduce the blood pres-

sure and was associated with an increased risk for syncope. A 6-month, double-blind, placebo-controlled study (Bodick *et al*., 1997) of 343 AD patients, randomized to receive 75, 150, or 225 mg/day versus placebo, demonstrated a significant improvement in ADAS-cog and CIBIC-plus, with the higher dose of xanomeline (225 mg/day). The analysis of Treatment Emergent Signs and Symptoms (TESS) disclosed a dose-dependent reduction in behavioral disturbances and psychotic symptoms. Favorable effects were reported in the Nurse's Observation Scale for Geriatric Patients (NOSFGP). These findings were more pronounced in the high-dose group. However 59% of patients in the 225 mg group discontinued therapy compared to 35% in the placebo group. Half of them dropped out predominantly due to gastrointestinal adverse effects, while 12.5% patients in this group experienced transient syncope. Overall 34 patients (13%) receiving xanomeline developed moderate, reversible elevations of transaminases. As a result of the hypotensive episodes and other side-effects the oral formulation has been discontinued. The efficacy of xanomeline through transdermal delivery is now being investigated.

G. Sabcomeline

Sabcomeline is a functionally selective M1 partial agonist, which has been tested in rats and marmoset monkeys and show improved cognitive performance in doses that did not cause the common cholinergic adverse effects (Harries *et al*., 1998; Hatcher *et al*., 1998).

H. Milameline (CI-979)

Milameline is a nonselective M1/M4 partial agonist of muscarinic receptors, which in animal models has shown to reverse scopolamine-induced amnesia (M'Harzi *et al*., 1995). It appears that milameline has a safe cholinergic profile in doses used in both healthy subjects and probable AD. A multicenter clinical study of the role of milameline in patients with mild, moderate AD is currently under way.

IV. Cholinergic Agonists with Nicotinic Affinity

Muscarinic receptors have been the target of the cholinergic replacement strategy due to their abundance in comparison to the nicotinic receptors and the well known cognitive impairment produced by muscarinic antagonists. Nonetheless, there is convergence of evidence that nicotinic acetylcholine receptors play an important role in the cognitive deficits that characterize AD (Vidal, 1996; Levin and Simon, 1998). Postmortem studies of AD brains have shown a significant reduction of nicotinic receptors in both neocortex and hippocampus (Perry *et al*., 1987; Nordberg *et al*., 1992). Basic research has shown that nicotine receptor stimulation might protect neurons from a β-amyloid (Kihara *et al*., 1997) and glutamate toxicity (Seguela *et al*., 1993; Akaike *et al*., 1994).

A. Nicotine

Nicotine has been repeatedly found to improve cognitive performance of aged rats (Levin and Torry, 1996) and primates (Terry *et al*., 1993). It has also been shown that the nicotinic antagonist mecamylamine induces significant impairment of working memory performance of rats (Decker and Majchrzac, 1992) and cognitive dysfunction in humans (Newhouse *et al*., 1992).

Preliminary studies in humans also suggest the potential efficacy of nicotine as a cognitive enhancer (Rusted *et al*., 1995; Foulds *et al*., 1996). Metaanalysis (Lee, 1994) of 19 case–control studies demonstrated a significant negative association between AD and smoking, with an overall estimate of 40% risk reduction of AD in smokers compared to nonsmokers. It has been argued though that this protective effect of smoking reflects a survival disadvantage for nonsmokers (Riggs, 1996).

A pilot study (Newhouse *et al*., 1988) with intravenous administration of nicotine in six nonsmokers with probable AD demonstrated a reduction of intrusion errors. Unfortunately, significant behavioral effects occurred, such as anxiety, depression, and dysphoria. Trials with subcutaneous (Sahakian *et al*., 1989; Jones *et al*., 1992) or dermal (Wilson *et al*., 1995; Snaedal *et al*., 1996) administration of nicotine have been inconclusive, and more selective agents are being investigated.

B. ABT-418

ABT-418 is a selective, channel ligand for nicotinic acetycholine receptors, which has been tested with success in rats with lesions in the septal nuclei (Decker *et al*., 1994a) and in macaque monkeys (Buccafusco *et al*., 1995b), improving performance on cognitive tasks. This compound has anxiolytic effects (Brioni *et al*., 1994; Decker *et al*., 1994b). It has also been shown to protect human cell lines against glutamate toxicity and might slow the rate of neurodegeneration of the aged brain (Arneric *et al*., 1995; Donnelly-Roberts *et al*., 1996). Tests with humans are necessary to determine the efficacy of ABT-418 as a cognitive enhancer.

V. Summary

In summary, several compounds that target the potentiation of cholinergic neurotransmission have been studied with AD patients. Acetylcholinesterase inhibitors are associated with significant cognitive improvement in a subgroup of patients with dementia. These drugs have been tested in well designed clinical trials in patients meeting inclusion criteria for mild to moderate dementia. The potential value of these agents in patients with incipient dementia is currently under investigation. Less is known, however, about the effects of selective muscarinic agonists and nicotinic ligands on the neuropsychiatric manifestations of AD, and studies with these agents are ongoing.

References

Adem, A., Mohammed, A. K., and Winblad, B. (1990). Multiple effects of tetrahydroamino- acridine on the cholinergic system: Biochemical and behavioral aspects. *J. Neural Transm. [Park. Dis. Dement. Sect]*. **2**, 113–128.

Ahlin, A., Nyback, H., Junthe, T., *et al*. (1991). THA in Alzheimer's dementia: Clinical, biochemical and pharmacokinetic findings. *In* "Alzheimer's Disease: Basic Mechanisms, Diagnosis and Therapeutic

Strategies'' (K. Iqbal, D. C. R. McLachlan, B. Winblad, and H. M. Wisniewski, eds.), pp. 522–532. Wiley, New York.

Aigner, T. G., Mitchell, S. J., Aggleton, J. P., *et al*. (1987). Effects of scopolamine and physostigmine on recognition memory in monkeys with ibotenic-acid lesions of the nucleus basalis of Meynert. *Psychopharmacology* **92**, 292–300.

Akaike, A., Tamura, Y., Yokaota, T., *et al*. (1994). Nicotine-induced protection of cultural cortical neurons against *N*-methyl-D-aspartate receptor-mediated glutamate cytotoxicity. *Brain Res*. **644**, 181–187.

Anand, R., Gharabawi, G., and Enz, A. (1996). Efficacy and safety results of the early phase studies with Exelon (ENA 713) in Alzheimer's disease: An overview. *J. Drug Dev. Clin. Pract*. **8**, 109–116.

Andersen, J. V., and Hansen, K. T. (1997). Normal-phase liquid chromatography-particle-beam mass spectrometry in drug metabolism studies of the dopamine receptor antagonist Odapipam and the muscarine M1 receptor agonist Xanomeline. *Xenobiotica* **27**, 901–912.

Antuono, P. G. (1995). Effectiveness and safety of velnacrine for the treatment of Alzheimer's disease: A double-blind, placebo-controlled study. Mentane Study Group. *Arch. Intern. Med*. **155**, 1766–1772.

Arneric, S. P., Sullivan, J. M., Decker, M. W., *et al*. (1995). Potential treatment of Alzheimer disease using cholinergic channel activators (ChCAs) with cognitive enhancement, anxiolytic-like, and cytoprotective properties. *Alzheimer Dis. Assoc. Disord*. **9** (Suppl. 2), 50–61.

Auteri, A., Mosca, A., Lattuada, N., *et al*. (1993). Pharmacodynamics and pharmacokinetics of eptastigmine in elderly subjects. *Eur. J. Clin. Pharmacol*. **45**, 373–376.

Bartus, R. T. (1979). Physostigmine and recent memory: Effects in young and aged nonhuman primates. *Science* **206**, 1087–1089.

Bartus, R. T., Dean, R. L., III, Beer B., and Lippa, A. S. (1982). The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217**, 408–417.

Bartus, R. T., Dean, R. L., and Flicker, C. (1987). Cholinergic psychopharmacology: An integration of human and animal research on memory. *In* ''Psychopharmacology: The third Generation of Progress'' (H. Y. Meltzer, ed.), pp. 219–232. Raven Press, New York.

Becker, R. E., Colliver, J. A., Markwell, S. J., *et al*. (1996). Double-blind, placebo-controlled study of metrofinate, an acetylcholinesterase inhibitor, for Alzheimer's disease. *Alzheimer Dis. Assoc. Disord*. **10**, 124–131.

Becker, R. E., Colliver, J. A., Markwell, S. J., *et al*. (1998). Effects of metrifonate on cognitive decline in Alzheimer disease: A double-blind, placebo-controlled, 6-month study. *Alzheimer Dis. Assoc. Disord*. **12**, 54–57.

Beller, S. A., Overall, J. E, and Swann, A. C. (1985). Efficacy of oral physostigmine in primary degenerative dementia: A double-blind study of response to different dose level. *Psychopharmacology* **87**, 147–151.

Beller, S. A., Overall, J. E., Rhoades, H. M., and Swann, A. C. (1988). Long-term outpatient treatment of senile dementia with oral physostigmine. *J. Clin. Psychiatry* **49**, 400–404.

Berger, M., Riemann, D., Hochli, D., and Spiegel, R. (1989). The cholinergic rapid eye movement sleep induction test with RS-86. State or trait marker of depression? *Arch. Gen. Psychiatry* **46**, 421–428.

Berger-Sweeney, J., Heckers, S., Mesulam, M. M., *et al*., (1994). Differential effects on spatial navigation of immunotoxin-induced cholinergic lesions of the medial septal area and nucleus basalis magnocellularis. *J. Neurosci*. **14**, 4507–4519.

Bickel, U., Thomsen, T., Weber, W., *et al*. (1991). Pharmacokinetics of galanthamine in humans and corresponding cholinesterase inhibition. *Clin. Pharmacol. Ther*. **50**, 420–428.

Bjornsson, T. D., Troetel, W. M., and Imbimbo, B. P. (1998). Effect of food on the absorption of eptastigmine *Eur. J. Clin. Pharmacol* **54**, 243–247.

Bodick, N. C., Offen, W. W., Levey, A. I., *et al*. (1997). Effects of xanomeline, a selective muscarinic receptor agonist, on cognitive function and behavioral symptoms in Alzheimer disease. *Arch. Neurol. (Chicago)* **54**, 465–473.

Bonner, T. I., Young, A. C., Brann, M. R., and Buckley, N. J. (1988). Cloning and expression of the human and rat m5 acetylcholine receptor genes. *Neuron* **1**, 403–410.

Brioni, J. D., O'Neill, A. B., Kim, D. J., *et al*. (1994). Anxiolytic effects of the novel cholinergic channel activator, ABT-418. *J. Pharmacol. Exp. Ther*. **271**, 353–361.

Bruno, G., Mohr, E., Gillespie, M., *et al*. (1985). RS-86 therapy of Alzheimer's disease. *Arch. Neurol. (Chicago)* **43**, 659–661.

Buccafusco, J. J., Jackson, W. J., Gattu, M., and Terry, A. V., Jr. (1995a). Isoarecoline-induced enhancement of delayed matching to sample performance in monkeys: Role of nicotinic receptors. *NeuroReport* **6**, 1223–1227.

Buccafusco, J. J., Jackson, W. J., Terry, A. V., *et al*. (1995b). Improvement in performance of a delayed matching-to-sample task by monkeys following ABT-418: A novel cholinergic channel activator for memory enhancement. *Psychopharmacology* **120**, 256–266.

Burns, A., Rossor, M., Hecker, J. *et al*. (1999). The effects of donepezil in Alzheimer' disease — results from a multinational trial. *Dement. Geriatr. Cogn. Disord*. **10**, 237–244.

Camacho, M., Tarrab-Hazdai, R., Espinoza, B., Arnon, R., and Agnew, A. (1994). The amount of acetylcholinesterase on the parasite surface reflects the differential sensitivity of schistosome species to metrifonate. *Parasitology* **108**, 153–160.

Canal, N., and Imbimbo, B. P. (1996). Relationship between pharmacodynamic activity and cognitive effects of eptastigmine in patients with Alzheimer's disease. Eptastigmine Study Group. *Clin. Pharmacol. Ther*. **60**, 218–228.

Catalan, R. E., Martinez, A. M., Aragones, M. D., *et al*. (1993). Tetrahydroaminoacridine affects the cholinergic function of blood-brain barrier. *Life Sci*. **53**, 1165–1172.

Cerf, J., Lebrun, A., and Dietrich, J. (1962). A new approach to helminthiasis control: The use of an organophosphorus compound. *Am. J. Trop. Med. Hyg*. **11**, 514–517.

Chatellier, G., and Lacomblez, L. (1990). Tacrine (tetrahydroaminoacridine; THA) and lecithin in senile dementia of the Alzheimer's type: A multicentre trial. *Br. Med. J*. **300**, 495–499.

Christie, J. E, Shering, A., Ferguson, J., and Glen, A. I. M. (1981). Physostigmine and arecoline: Effects of intravenous infusions in Alzheimer presinile dementia. *Br. J. Psychiatry* **138**, 46–50.

Cooper, J. R., Bloom, F. E., and Roth, R. H. (1996). Acetylcholine. *In* ''The Biochemical Basis of Neuropharmacology,'' 7th ed., pp. 194–225. Oxford University Press, New York.

Corey-Bloom, J., Anand, R., and Veach, J. (1998). A randomized trial evaluating the efficacy and safety of ENA 713 (rivastigmine tartrate), a new acetylcholinesterase inhibitor, in patients with mild to moderately severe Alzheimer's disease. *J. Geriatr. Psychopharmacol*. **1**, 55–65.

Coull, J. T. (1994). Pharmacological manipulation of the a2- noradrenergic system: Effects on cognition. *Drugs Aging* **5**, 116–126.

Cozanitis, D. A., van de Pol, F., van Wezel, H., and Crul, J. F. (1981). Antagonistic activity of galanthamine on constant infusion of pancuronium in rats. *Experientia* **37**, 1326–1327.

Cozanitis, D. A., Friedmann, T., and Furst, S. (1983). Study of the analgesic effects of galanthamine, a cholinesterase inhibitor. *Arch. Int. Pharmacodyn. Ther*. **266**, 229–238.

Craig, P. S., Casser, R. B., Parada, L., *et al*. (1995). Diagnosis of canine echinococcosis: Comparison of coproantigen and serum antibody tests with arecoline purgation in Uruguay. *Vet. Parasitol*. **56**, 293–301.

Crow, T. J., and Grove-White, I. (1973). An analysis of the learning deficit following hyoscine on human learning capacity. *Br. J. Pharmacol*. **49**, 322–327.

Cummings, J. L., Cyrus, P. A., Bieber, F., *et al*. (1998). Metrifonate treatment of the cognitive deficits of Alzheimer's disease. Metrifonate Study Group. *Neurology* **50**, 1214–1221.

Cutler, N. R., Polinski, R. J., *et al*. (1998a). Dose dependent CSF acetylcholinesterase inhibition by SDZ ENA 713 in Alzheimer's disease. *Acta Neurol. Scand*. **97**, 244–250.

Cutler, N. R., Jhee, S. S., Cyrus, P., *et al*. (1998b). Safety and tolerability of metrifonate in patients with Alzheimer's disease: Results of a maximum tolerated dose study. *Life Sci*. **62**, 1433–1441.

Dal-Bianco, P., Maly, J., Wöber, C., *et al*. (1991). Galanthamine treatment in Alzheimer's disease. *J. Neural Transm. Suppl*. **33**, 59–63.

Davidson, M., Bierer, L. M., Kaminsky, R., *et al*. (1989). Combined administration of physostigmine and clonidine to patients with dementia of the Alzheimer type: A pilot safety study. *Alzheimer Dis. Assoc. Disord*. **3**, 224–227.

Davies, P., and Maloney, A. J. F. (1976). Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet* **2**, 1403.

Davis, K. L., and Mohs, R. C. (1982). Enhancement of memory processes in Alzheimer's disease with multiple-dose intravenous physostigmine. *Am. J. Psychiatry* **139**, 1421–1424.

Davis, K. L., Mohs, R. C., Tinklenberg, J. R., *et al*. (1978). Physostigmine: Improvement of long term memory processes in normal humans. *Science* **201**, 272–274.

Davis, K. L., Thal, L. J., Gamzu, E., *et al*. (1992). Tacrine in patients with Alzheimer's disease: A double-blind, placebo-controlled multicenter study. *N. Engl. J. Med*. **327**, 1253–1259.

Decker, M. W., and Majchrzac, M. J. (1992). Effects of systemic and intracerebroventricular administration of mecamylamine, a nicotinic cholinergic antagonist, on spatial memory rats. *Psychopharmacology* **107**, 530–536.

Decker, M. W., Curzon, P., Brioni, J. D., and Arneric, S. P. (1994a). Effects of ABT-418, a novel cholinergic channel ligand, on place learning in septal-lesioned rats. *Eur. J. Pharmacol* **261**, 217–222.

Decker, M. W., Brioni, J. D., Sullivan, J. P., *et al*. (1994b). (S)-3-methyl-5-(1-methyl-2-pyrrolidinyl)-isoxazole (ABT 418): A novel cholinergic ligand with cognition-enhancing and anxiolytic activities: II. In vivo characterization. *J. Pharmacol. Exp. Ther*. **270**, 319–328.

Deutsch, J. A. (1971). The cholinergic synapse and the site of memory. *Science* **174**, 788–794.

Dokla, C. P., and Thal, L. J. (1988). Effect of cholinesterase inhibitors on Morris water task behavior following lesions of the nucleus basalis magnocellularis. *Behav. Neurosci*. **102**, 861–871.

Donnelly-Roberts, D. L., Xue, I. C., Arneric, S. P., *et al*. (1996). In vitro neuroprotective properties of the novel cholinergic channel activator (ChCA), ABT-418. *Brain Res*. **719**, 36–44.

Dorje, F., Levey, A. I., and Brann, M. R. (1991). Immunological detection of muscarinic receptor subtype proteins (m1–m5) in rabbit peripheral tissues. *Mol. Pharmacol*. **40**, 459–462.

Drachman, D. A., and Leavitt, J. L. (1974). Human memory and the cholinergic system. A relationship to aging? *Arch. Neurol. (Chicago)* **30**, 113–121.

Enz, A., and Floersheim, P. (1996). Cholinesterase inhibitors: An overview of their mechanism of action in Alzheimer's disease. *In* ''Altzheimer's Disease: From Molecular Biology to Therapy'' (R. Becker, and E. Giacobini, eds.), pp. 211–215. Birkhaeuser, Boston.

Etienne, P., Gauthier, S., Johnson, G., *et al*. (1978). Clinical effects of choline in Alzheimer's disease. *Lancet* **1**, 508–509.

Etienne, P., Dastoor, D., Gauthier, S. *et al*. (1981). Alzheimer disease: Lack effect of lecithin treatment for 3 months. *Neurology* **31**, 1552–1554.

Farlow, M., Gracon, S. I., Hershey, L. A., *et al*. (1992). A 12-week, double-blind, placebo-controlled, parallel-group study of tacrine patients with probable Alzheimer's disease. *JAMA, J. Am. Med. Assoc*. **268**, 2523–2529.

Fisher, A., Brandeis, R., Pittel, Z., *et al*. (1989). (+)-cis-2-methyl-spiro-(1,3-oxathiolane-5,3')-quinuclidine (AF102B): A new M1 agonist attenuates cognitive dysfunction in AF64A-treated rats. *Neurosci. Lett*. **102**, 325–331.

Fisher, A., Karton, Y., Heldman, E., *et al*. (1993). Progress in medicinal chemistry of novel selective muscarinic agonists. *Drug Des. Discovery* **9**, 221–235.

Fisher, A., Heldman, E., Gurwitz, D., *et al*. (1994). Selective signaling via novel muscarinic agonists: Implications for Alzheimer's disease treatments and clinical update. *3rd Springfield Intl. Symp. Alzheimer's Dis*., Springfield, Il, *1994*.

Fisher, A., Heldman, E., Gurwitz, D., *et al*. (1996). M1 agonists for the treatment of Alzheimer's disease. Novel properties and clinical update. *Ann. N.Y. Acad. Sci*. **777**, 189–196.

Flynn, D. D., and Mash, D. C. (1989). Multiple in vitro interactions with and differential in vivo regulation of muscarinic receptor subtypes by tetrahydroaminoacridine. *J. Pharmacol. Exp. Ther*. **250**, 573–581.

Flynn, D. D., Ferrari-DiLeo, G., Mash, D. C., and Levey, A. I. (1995). Rapid communication: Differential regulation of molecular subtypes of muscarinic receptors in Alzheimer's disease. *J. Neurochem*. **64**, 1888–1891.

Forette, F., Hoover, T., Gracon, S., *et al*. (1995). A double-blind, placebo-controlled, enriched population study of tacrine in patients with Alzheimer's disease. *Eur. J. Neurol*. **2**, 1–10.

Forette, F., Anand, R., and Gharabawi, G. (1999). A phase II study in patients with Alzheimer's disease to assesss the preliminary efficacy and maximum tolerated dose of rivastigmine (Exeloninfinity). *Eur. J. Neurol*. **6**, 423–429.

Foster, N. L., Aldrich, M. S., Bluemlein, L., *et al*. (1989). Failure of cholinergic agonist RS–86 to improve cognition and movement in PSP despite effects on sleep. *Neurology* **39**, 257–261.

Foster, N. L., Peterson, R. C., Gracon, S. I., Lewis, K., for the Tacrine 970-6 Study Group. (1996). An enriched population, double-blind, placebo-controlled, cross-over study of tacrine and lecithin in Alzheimer's disease. *Dementia* **7**, 260–266.

Foulds, J., Stapleton, J., Swettenham, J., *et al*. (1996). Cognitive performance effects of subcutaneous nicotine in smokers and never smokers. *Psychopharmacology* **127**, 31–38.

Freedman, S. B., Harley, E. A., and Patel, S. (1989). Direct measument of muscarinic agents in the central nervous system of mice using ex vivo binding. *Eur. J. Pharmacol.* **174**, 253–260.

Gann, H., Riemann, D., Hohagen, F., *et al*. (1992). The sleep structure of patients with anxiety disorder in comparison to that of healthy controls and depressive patients under baseline conditions and after cholinergic stimulation. *J. Affect. Disord*. **26**, 179–189.

Gauthier, S., Bouchard, R., Lamontagne, A., *et al*. (1990). Tetrahydroaminoacridine-lecithin combination treatment in patients with intermediate-stage Alzheimer's disease. Results of a Canadian double-blind, crossover, multicentre study. *N. Engl. J. Med*. **322**, 1272–1276.

Giacobini, E. (1990). Cholinergic receptors in human brain: Effects of aging and Alzheimer's disease. *J. Neurosci. Res*. **27**, 548–560.

Gracon, S. I., Knapp, M. J., Berghoff, W. G., *et al*. (1998). Safety of tacrine: Clinical trials, treatment IND, and postmarketing experience. *Alzheimer Dis. Assoc. Disord*. **12**, 93–101.

Harbaugh, R. E., Reeder, T. M., Senter, H. J., *et al*. (1989). Intracerebroventricular bethanechol chloride administration in Alzheimer's disease: Results of a collaborative double-blind study. *J. Neurosurg*. **71**, 481–486.

Haring, R., Gurwitz, D., Barg, J., *et al*. (1995). NGF promotes amyloid precursor protein secretion via muscarinic receptor activation. *Biochem. Biophys. Res. Commun*. **213**, 15–23.

Harries, M. H., Samson, N. A., Cilia, J., and Hunter, A. J. (1998). The profile of sabcomeline (SB-202026), a functionally selective agonist in the marmoset. *Br. J. Pharmacol*. **124**, 409–415.

Hatcher, J. P., Loudon, J. M., Hagan, J. J., and Clark, M. S. (1998). Sabcomeline (SB-202026), a functionally selective M1 receptor partial agonist, reverses delay-induced deficits in the T-maze. *Psychopharmacology* **138**, 275–282.

Hinz, V. C., Grewig, S., and Schmidt, B. H. (1996). Metrifonate induces cholinesterase inhibition exclusively via slow release of dichlorvos. *Neurochem. Res*. **21**, 331–337.

Hollander, E., Davidson, M., Mohs, R. C., *et al*. (1987). RS-86 in the treatment of Alzheimer's disease: Cognitive and biological effects. *Biol. Psychiatry* **22**, 1067–1078.

Hulme, E. C., Birdsall, N. J. M., and Buckley, N. J. (1990). Muscarinic receptor subtypes. *Annu. Rev. Pharmacol. Toxicol*. **30**, 633–673.

Imbimbo, B. P., Licini, M., Schettino, M., *et al*. (1995). Relationship between pharmacokinetics and pharmacodynamics of eptastigmine in young healthy volunteers. *J. Clin. Pharmacol*. **35**, 285–290.

Imbimbo, B. P., Lucca, U., Lucchelli, F., *et al*. (1998). A 25-week placebo-controlled study of eptastigmine in patients with Alzheimer's disease. Eptastigmine Study Group. *Alzheimer Dis. Assoc. Disord*. **12**, 313–322.

Imbimbo, B. P., Martelli, P., Troetel, W. M., *et al*. (1999). Efficacy and safety of eptastigmine for the treatment of patients with Alzheimer's disease. *Neurology* **52**, 700–708.

Irle, E., and Markowitsch, H. L. (1987). Basal forebrain-lesioned monkeys are severely impaired in tasks of association and recognition memory. *Ann. Neurol*. **22**, 735–743.

Jackson, W. J., Buccafusco, J. J., Terry, A. V., *et al*. (1995). Velnacrine maleate improves delayed matching performance by aged monkeys. *Psychopharmacology* **119**, 391–398.

Jann, M. W., Cyrus, P. A., Eisner, L. S., *et al*. (1999). Efficacy and safety of a loading dose-regimen versus a no-loading-dose regimen of metrifonate in the symptomatic treatment of Alzheimer's disease: A randomized, double-masked, placebo-controlled trial. Metrifonate Study Group. *Clin. Ther*. **21**, 88–102.

Jenike, M. A., Albert, M., Baer, L., and Gunther, J. (1990). Oral physostigmine as treatment for primary degenerative dementia: A double-blind placebo-controlled inpatient trial. *J. Geriatr. Psychiatry Neurol*. **3**, 13–16.

Johansson, M., and Nordberg, A. (1993). Pharmacokinetic studies of cholinesterase inhibitors. *Acta Neurol. Scand., Suppl*. **149**, 22–25.

Jones, G. M. M., Sahakian, B. J., Levy, R., Warburton, D. M., and Gray, J. A. (1992). Effects of acute subcutaneous nicotine on attention, information processing and short term memory in Alzheimer's disease. *Psychopharmacology* **108**, 485–494.

Kihara, T., Shimohama, S., Sawada, H., *et al*. (1997). Nicotinic receptor stimulation protects neurons against β-amyloid toxicity. *Ann. Neurol*. **42**, 159–163.

Knapp, M. J., Knopman, D. S., Solomon, P. R., *et al*. for the Tacrine Study Group. (1994). A 30-week randomized controlled trial of high dose tacrine in patients with Alzheimer's disease. *JAMA, J. Am. Med. Assoc*. **271**, 985–991.

Knopman, D., Schneider, L., Davis, K., *et al*. (1996). Long term tacrine (Cognex) treatment: Effects on nursing home placement and mortality. *Neurology* **47**, 166–177.

Leanza, G., Muir, J., Nilsson, O. G., *et al*. (1996). Selective immunolesioning of the basal forebrain cholinergic system disrupts short-term memory in rats. *Eur. J. Neurosci*. **8**, 1535–1544.

Lee, P. N. (1994). Smoking and Alzheimer's disease: A review of the epidemiological evidence. *Neuroepidemiology* **13**, 131–144.

Levey, A. I., Kitt, C. A., Simonds, W. F., *et al* (1991). Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype specific antibodies. *J. Neurosci*. **11**, 3218–3226.

Levin, E. D., and Simon, B. B. (1998). Nicotinic acetylcholine involvement in cognitive function in animals. *Psychopharmacology* **138**, 217–230.

Levin, E. D., and Torry, D. (1996). Acute and chronic nicotine effects on working memory in aged rats. *Psychopharmacology* **123**, 88–97.

Lubeck, D. P., Mazonson, P. D., and Bowe, T. (1994). Potential effects of tacrine on expenditures for Alzheimer's disease. *Med. Interface* **7**, 130–138.

Maiese, K., Holloway, H. H., Larson, D. M., and Soncrant, T. T. (1994). Effect of acute and chronic arecoline treatment on cerebral metabolism and blood flow in the conscious rat. *Brain Res*. **641**, 65–75.

Maltby, N., Broe, G. A., Creasey, H., *et al*. (1994). Efficacy of tacrine and lecithin in mild to moderate Alzheimer's disease: Double blind trial. *Br. Med. J*. **308**, 879–883.

Mant, T., Troetel, W. M., and Imbimbo, B. P. (1998). Maximum tolerated dose of eptastigmine in elderly healthy volunteers. *J. Clin. Pharmacol*. **38**, 610–617.

Marin, D. B., Bierer, L. M., Lawlor, B. A., *et al*. (1995). L-Deprenyl and physostigmine for the treatment of Alzheimer's disease. *Psychiatry Res*. **58**, 181–189.

Mash, D. C., Flynn, D. D., and Potter, L. T. (1985). Loss of M2 muscarine recepotors in the cerebral cortex in Alzheimer's disease and experimental cholinergic denervation. Science **228**, 1115–1117.

McKeith, I. G. (1998). The clinical trial protocol of the Metrifonate in Alzheimer's trial (MALT). *Dement. Geriatr. Cogn. Disord*. **9**(Suppl. 2), 2–7.

Medina A, Bodick, N., Goldberger, A. L., *et al*. (1997). Effects of central muscarinic-1 receptor stimulation on blood pressure regulation. *Hypertension* **29**, 828–834.

M'Harzi, M., Palou, A. M., Oberlander, C., and Barzaghi, F. (1995). Antagonism of scopolamine-induced memory impairments in rats by the muscarinic agonist RU 35,926 (CI-979). *Pharmacol. Biochem. Behav*. **51**, 119–24.

Mohs, R. C., Davis, B. M., Johns, C. A., *et al*. (1985). Oral physostigmine treatment of patients with Alzheimer's disease. *Am. J. Psychiatry* **142**, 28–33.

Molinengo, L., Orsetti, M., Pastorello, B., *et al*. (1995). The action of arecoline on retrieval and memory storage evaluated in the staircase maze. *Neurobiol. Learn. Mem*. **63**, 167–173.

Moller, H.-J., Hampel, H., Hegerl, U., *et al*. (1999). Double-blind, randomized, placebo-controlled clinical trial on the efficacy and tolerability of a physostigmine patch in patients with senile dementia of the Alzheimer type. *Pharmacopsychiatry* **32**, 99–106.

Molloy, D. W., Guyatt, G. H., Wilson, D. B., *et al*. (1991). Effects of tetrahydroaminoacridine on cognition, function and behavior in Alzheimer's disease. *Can. Med. Assoc. J*. **144**, 29–34.

Morris, J. C., Cyrus, P. A., Orazem, J., *et al*. (1998). Metrifonate benefits cognitive, behaviorial, and global function in patients with Alzheimer's disease. *Neurology* **50**, 1222–1230.

Moss, D. E., Berlanga, P., Hagan, M. M., *et al*. (1999). Methanesulfonyl fluoride (MSF): A double-blind, placebo-controlled study of safety and efficacy in the treatment of senile dementia of the Alzheimer type. *Alzheimer Dis. Assoc. Disord*. **13**, 20–25.

Nakahara, N., Iga, Y., Saito, Y., Mizobe, F., and Kawanishi, G. (1989). Beneficial effects of FKS-508 (AF102B), a selective M1 agonist, on the impaired working memory in AF64A treated rats. *Jpn. J. Pharmacol*. **51**, 539–547.

Newhouse, P. A., Sundeland, T., Tariot, P. N., *et al*. (1988). Intravenous nicotine in Alzheimer's disease: A pilot study. *Psychopharmacology* **95**, 171–175.

Newhouse, P. A., Potter, A., Corwin, J., and Lenox, R. (1992). Acute nicotinic blockade produces cognitive impairment in normal humans. *Psychopharmacology* **108**, 480–487.

Niigawa, H., Tanimukai, S., Hariguchi, S., and Nishimura, T. (1995). Effects of SDZ ENA713, a novel acetylcholinestrase inhibitor, on learning of rats with basal forebrain lesions. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **19**, 171–186.

Nitsch, R. M., Slack, B. E., Wurtman, R. J., and Growdon, J. H. (1992). Release of Alzheimer's amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors. *Science* **258**, 304–307.

Nordberg, A. (1992). Neuroreceptor changes in Alzheimer disease. *Cerebrovasc. Brain Metab. Rev*. **4**, 303–328.

Nordberg, A., and Winblad, B. (1986). Reduced number of [^{3}H]nicotine and [^{3}H]acetylcholine binding sites in the frontal cortex of Alzheimer brains. *Neurosci. Lett*. **72**, 115–119.

Nordberg, A., Alafuzoff, I., and Winblad, B. (1992). Nicotinic and muscarinic subtypes in the human brain changes with aging and dementia. *J. Neurosci. Res*. **31**, 103–111.

Ohnishi, A., Mihara, M., Kamakura, H., *et al*. (1993). Comparison of the pharmacokinetics of E2020, a new compound for Alzheimer's disease, in healthy young and elderly subjects. *J. Clin. Pharmacol*. **33**, 1086–1091.

O'Neill, J., Fitten, L. J., Siembienda, D., *et al*. (1998). Effects of AF102B and tacrine on delayed match to sample in monkeys. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry.* **22**, 665–678.

Osterrieder, W. (1987). 9-Amino-1,2,3,4-tetrahydroacridine (THA) is a potent blocker of cardiac potassium channels. *Br. J. Pharmacol*. **92**, 521–525.

Ostfeld, A., and Aruguette, A. (1962). Central nervous system effects of hyoscine in man. *J. Pharmacol. Exp. Ther.* **137**, 133–139.

Pacheco, G., Palacios-Esquivel, R., and Moss, D. E. (1995). Cholinesterase inhibitors proposed for treating dementia in Alzheimer's disease: Selectivity toward human brain acetylcholinesterase compared with butyrylcholinesterase. *J. Pharmacol. Exp. Ther.* **274**, 767–770.

Palacios-Esqivel, R. L., Pacheco, G., and Moss, D. E. (1993). Methanesulfonyl fluoride (MSF) blocks scopolamine-induced amnesia in rats. *Neurobiol. Aging* **14**, 93–96.

Parnetti, L. (1995). Clinical pharmacokinetics of drugs for Alzheimer's disease. *Clin. Pharmacokinet*. **29**, 110–129.

Penn, R. D., Martin, E. M., Wilson, R. S., Fox, J. H., and Savoy, S. M. (1988). Intraventicular bethanechol infusion for Alzheimer's disease: Results of double-blind and escalating-dose trials. *Neurology* **38**, 219–222.

Peralta, E. G., Ashkenazi, A., Winslow, J. W., *et al*. (1988). Differential regulation of PI hydrolysis and adenyl cyclase by muscarinic receptor subtypes. *Nature (London)* **334**, 434–437.

Perry, E. K., Gibson, P. H., Blessed, G., *et al*. (1977). Neurotransmitter enzyme abnormalities in senile dementia. *J. Neurol. Sci*. **34**, 247–265.

Perry, E. K., Tomlinson, B. E., Blessed, G., *et al*. (1978). Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *Br. Med. J*. **2**, 1457–1459.

Perry, E. K., Perry, R. H., Smith, C. J., *et al*. (1987). Nicotinic receptor abnormalities in Alzheimer's and Parkinson's diseases. *J. Neurol., Neurosurg. Psychiatry* **50**, 806–809.

"Physician's Desk Reference." (1999a). Cognex (tacrine). *In* "Physician's Desk Reference," 53rd ed., pp. 2274–2278.

"Physician's Desk Reference." (1999b). Aricept (donepezil). *In* "Physician's Desk Reference," 53rd ed., pp. 2364–2367.

Pinkas-Kramarski, R., Stein, R., Lindenboim, L., and Sokolovsky, M. (1992). Growth factor-like effects mediated by muscarinic receptors in PC12M1 cells. *J. Neurochem*. **59**, 2158–2166.

Polinsky, R. J. (1998). Clinical pharmacology of rivastigmine: A new generation acetylcholinesterase inhibitor for the treatment of Alzheimer's disease. *Clin. Ther.* **20**, 637–647.

Puri, S. K., Hsu, R., Ho, I., and Lassman, H. B. (1989). The effect of food on the bioavailability of velnacrine (HP-029) in healthy elderly men: A potential Alzheimer agent. *J. Clin. Pharmacol*. **29**, 956–960.

Puri, S. K., Ho, I., Hsu, R., and Lassman, H. B. (1990). Multiple dose pharmacokinetics, safety and tolerance of velnacrine (HP 029) in healthy elderly subjects: A potential therapeutic agent for Alzheimer's disease. *J. Clin. Pharmacol*. **30**, 948–955.

Rafaele, K. C., Asthana, S., Berardi, A., *et al*. (1996). Differential response to the cholinergic agonist arecoline among different cognitive modalities in Alzheimer's disease. *Neuropsychopharmacology* **15**, 163–170.

Raskind, M. A., Cyrus, P. A., Ruzicka, B. B., and Gulanski, B. I. (1999). The effects of metrofinate on the cognitive, behaviorial, and functional performance of Alzheimer's disease. Metrifonate Study Group. *J. Clin. Psychiatry* **60**, 318–325.

Read, S. L., Frazee, J., Shapira, J., *et al*. (1990). Intracerebroventricular bethanechol for Alzheimer's disease: Variable dose-related responses. *Arch. Neurol. (Chicago)* **47**, 1025–1030.

Riggs, J. E. (1996). The "protective" influence of cigarette smoking on Alzheimer's and Parkinson diseases. Quagmire or opportunity for neuroepidemiology? *Neurol. Clin*. **14**, 353–358.

Rogers, S. L., and Freidhoff, L. T. (1996). The efficacy and safety of donepezil in patients with Alzheimer's disease: Results of a US multicentre, randomized, double-blind, placebo-controlled trial. The Donepezil Study Group. *Dementia* **7**, 293–303.

Rogers, S. L., and Friedhoff, L. T. (1998). Long-term efficacy and safety of donepezil in the treatment of Alzheimer's disease: An interim analysis of the results of a US multicentre open label extension study. *Eur. Neuropsychopharmacol*. **8**, 67–75.

Rogers, S. L., Farlow, M. R., Doody, R. S., *et al*. (1998a). A 24-week, double-blind, placebo-controlled trial of donepezil in patients with Alzheimer's disease. Donepezil Study Group. *Neurology* **50**, 136–145.

Rogers, S. L., Doody, R. S., Mohs, R. C., and Friedhoff, L. T. (1998b). Donepezil improves cognition and global function in Alzheimer's disease; a 15-week, double-blind, placebo-controlled study. Donepezil Study Group. *Arch. Intern. Med*. **158**, 1021–1031.

Rosler, M., Anand, R., Cicin-Sain, A., *et al*. (1999). Efficacy and safety of rivastigmine in patients with Alzheimer's disease: International randomized controlled trial. *Br. Med. J*. **318**, 633–638.

Rusted, J., Graupner, L., and Warburton, D. (1995). Effects of post-trial administration of nicotine on human memory evaluating the conditions for improving memory. *Psychopharmacology* **119**, 405–413.

Sadot, E., Gurwitz, D., Barg, J., *et al*. (1996). Activation of m1 muscarinic acetylcholine receptor regulates τ-phosphorylation in transfected PC12 cells. *J. Neurochem.* **66**, 877–880.

Sahakian, B. J, Jones, G. M. M., Levy, R., *et al*. (1989). The effects of nicotine on attention, information processing and short-term memory in patients with dementia of the Alzheimer's type. *Br. J. Psychiatry* **154**, 797–800.

Samuels, S. C., and Davis, K. L. (1997). A risk-benefit assessment of tacrine in the treatment of Alzheimer's disease. *Drug Safety* **16**, 66–77.

Seguela, P., Wadiche, J., Dineley-Miller, K., *et al*. (1993). Molecular cloning, functional properties and distribution of rat brain a7: A nicotinic action channel highly permeable to calcium. *J. Neurosci*. **13**, 596–604.

Shannon, H. E., Bymaster, F. P., Calligaro, D. O., *et al*. (1994). Xanomeline: A novel muscarinic receptor agonist with functional selectivity for M1 receptors. *J. Pharmacol. Exp. Ther.* **269**, 271–281.

Shimohama, S., Taniguchi, T., Fujiwara, M., and Kameyama, M. (1986). Changes in nicotinic and muscarinic cholinergic receptors in Alzheimer-type dementia. *J. Neurochem*. **46**, 288–293.

Shintani, E. Y., and Uchida, K. M. (1997). Donepezil: An anticholinesterase inhibitor for Alzheimer's disease. *Am. J. Health Syst. Pharmacol*. **54**, 2805–2810.

Snaedal, J., Johannesson, T., Jonsson, J. E., and Gylfadottir, G. (1996). The effects of nicotine in dermal plaster on cognitive functions in patients with Alzheimer's disease. *Dementia* **7**, 47–52.

Spencer, C. M., and Noble, S. (1998). Rivastigmine: A review of its use in Alzheimer's disease. *Drugs Aging* **13**, 391–411.

Sramek, J. J., Block, G. A., Reines, S. A., *et al*. (1995). A multiple-dose safety trial of eptastigmine in Alzheimer's disease, with pharmacodynamic observations of red blood cell cholinesterase. *Life Sci*. **56**, 319–326.

Sramek, J. J, Anand, R., Wardle, T. S, *et al*. (1996). Safety/tolerability trial of SDZ ENA713 in patients with probable Alzheimer's disease. *Life Sci*. **58**, 1201–1207.

Stern, Y., Sano, M., and Mayeux, R. (1987). Effects of oral physostigminein Alzheimer's disease. *Ann. Neurol*. **22**, 306–310.

Storch, A., Schrattenholtz, A., Cooper, J. C., *et al*. (1995). Physostigmine, galanthamine and codeine act as 'non- competitive nicotinic receptor agonists' on clonal rat pheochromocytoma cells. *Eur. J. Pharmacol*. **290,** 207–219.

Summers, W. K., Viesselman, J. O., Marsh, G. M., and Candelora, K. (1981). Use of THA in treatment of Alzheimer-like dementia: Pilot study in twelve patients. *Biol. Psychiatry* **16,** 145–153.

Summers, W. K., Majovski, L. V., Marsh, G. M., *et al*. (1986). Oral tetrahydroaminoacridine in long-term treatment of senile dementia, Alzheimer type. *N. Engl. J. Med*. **315**, 1241–1245.

Sweeney, J. E., Hohmann, C. F., Moran, T. H., and Coyle, J. T. (1988). A long lasting cholinesterase inhibitor reverses spatial memory deficits in mice. *Pharmacol., Biochem. Behav.* **31**, 141–147.

Tariot, P. N., Cohen, R. M., Welkowitz, J. A., *et al*. (1988). Multiple-dose arecoline infusions in Alzheimer's disease. *Arch. Gen. Psychiatry* **45**, 901–905.

Terry, A. V., Jr., Buccafusco, J. J., and Jackson, W. J. (1993). Scopolamine reversal of nicotine enhanced delayed matching to sample performance in monkeys. *Pharmacol. Biochem. Behav.* **45**, 925–929.

Thal, L. J., Rosen, W., Sharpless, N. S., and Crystal, H. (1981). Choline chloride fails to improve cognition of Alzheimer's disease. *Neurobiol. Aging* **2**, 205–208.

Thal, L. J., Swartz, G., Sano, M., *et al*. (1996). A multicenter double-blind study of controlled release physostigmine for the treatment of symptoms secondary to Alzheimer's disease. Physostigmine Study Group. *Neurology* **47**, 1389–1395.

Thal, L. J., Ferguson, J. M., Mintzer, J., *et al*. (1999). A 24-week randomized trial of controlled-release physostigmine in patients with Alzheimer's disease. *Neurology* **52**, 1146–1152.

Thomsen, T., and Kewitz, H. (1990). Selective inhibition of human acetylcholinesterase by galanthamine in vitro and in vivo. *Life Sci*. **46**, 1553–1558.

Truman, C. A., Ford, J. M., and Roberts, C. J. (1991). Comparison of the chromatographic characteristics of metabolites of tacrine hydrochloride in human serum and urine with those of in vitro metabolic products from hepatic microsomes. *Biochem. Pharmacol*. **42**, 956–959.

Tsai, C. L., Kuo, M. Y., Hahn, L. J., *et al*. (1997). Cytotoxic and cytostatic effects of arecoline on oral mucosal fibroblasts. *Proc. Natl. Sci. Counc., Repub. China B* **21**, 161–167.

Turcan, R. G., Hillbeck, D., Hartley, T. E., *et al*. (1993). Disposition of [14C] velnacrine maleate in rats, dogs, and humans. *Drug Metab. Dispos*. **21**, 1037–1047.

U.S. Department of Health and Human Services (USDHHS). (1989). ''Peripheral and Central Nervous System Drugs Advisory Committee Meeting, July 7, 1989,'' p. 227. USDHHS, Public Health Service, Food and Drug Administration, Rockville, MD.

Van Wyk, C. W., Olivier, A., De Miranda, C. M., *et al*. (1994). Observations on the effect of areca nut extracts on oral fibroblast proliferation. *J. Oral Pathol. Med*. **23**, 145–148.

Vidal, C. (1996). Nicotinic receptors in the brain. Molecular biology, function, and therapeutics. *Mol. Chem. Neuropathol*. **28**, 3–11.

Vincent, G., and Sepinwall, J. (1992). AF102B, a novel M1 agonist, enhanced spatial learning in C57BL/10 mice with a long duration of action. *Brain Res*. **597**, 264–268.

Waelbroeck, M., Tastenoy, M., Camus, J., and Cristophe, J. (1990). Binding of selective antagonists to four muscarinic receprors (M1 to M4) in rat forebrain. *Mol. Pharmacol*. **38**, 267–273.

Waelbroeck, M., Camus, J., Tastenoy, M., *et al*. (1991). Stereoselectivity of (R)- and (S)-hexaydro-difenidol binding to neuroblastoma M1, cardiac M2, pancreatic M3, and striatum M4 muscarinic receptors. *Chirality* **3**, 118–123.

Walsh, T. J., Herzog, C. D., Gandhi, C., *et al*. (1996). Injection of IgC 192-saporin into the medial septum produces cholinergic hypofunction and dose-dependent working memory deficits. *Brain Res*. **726**, 69–79.

Watkins, P. B., Zimmerman, H. J., Knapp, M. J., *et al*. (1994). Hepatotoxic effects of tacrine administration in patients with Alzheimer's disease. *JAMA, J. Am. Med. Assoc*. **271**, 992–998.

Wengel, S., *et al*. (1998). Behavioral complications associated with donepezil. *Am. J. Psychiatry* **155**, 1632–1633.

Wettstein, A., and Spiegel, R. (1984). Clinical trials with the cholinergic drug RS-86 in Alzheimer's disease (AD) and senile dementia of the Alzheimer type (SDAT). *Psychopharmacology* **84**, 572–573.

Whelpton R. (1983) Analysis of plasma physostigmine concentrations by liquid chromatography. *J. Chromatogr.* **272**, 216–220.

Whelpton, R., and Hurst, P. (1985). Bioavailability of oral physostigmine. *N. Engl. J. Med*. **313**, 1293–1294.

Whitehouse, P. J., and Kellar, K. J. (1987). Nicotinic and muscarinic cholinergic receptors in Alzheimer's disease and related disorders. *J. Neural Transm., Suppl*. **24**, 175–182.

Whitehouse, P. J., Price, D. L., Clark, A. W., *et al*. (1981). Alzheimer disease: Evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann. Neurol*. **10**, 122–126.

Wilcock, G., and Wilkinson, D. (1997). Galanthamine hydrobromide: Interim results of a group comparative, placebo-controlled study of efficacy and safety in patients with a diagnosis of senile dementia of the Alzheimer type. *In* ''Alzheimer's Disease: Biology, Diagnosis and Therapeutics.'' (K. Iqbal, B. Winblad, T. Nishimura, *et al*., eds.), pp. 661–664. Wiley, Chichester.

Wilcock, G. K., Surmon, D. J., Scott, M., *et al*. (1993). An evaluation of the efficacy and safety of tetrahydroaminoacridine (THA) without lecithin in the treatment of Alzheimer's disease. *Age Ageing* **22**, 316–324.

Wilson, A. L., Langley, L. K., Monley, J., *et al*. (1995). Nicotine patches in Alzheimer's disease; pilot study on learning, memory, and safety. *Pharmacol., Biochem. Behav.* **51**, 509–514.

Wilson, R. S., and Martin, E. M. (1988). New intrathecal drugs in Alzheimer's disease and psychometric testing. *Ann. N.Y. Acad. Sci*. **531**, 180–186.

Winkler, J., Suhr, S. T., Gage, F. H., *et al*. (1995). Essential role of neocortical acetylcholine in spatial memory. *Nature (London)* **375**, 484–487.

Wood, P. C., and Castleden, C. M. (1994). A double-blind, placebo-controlled, multicentre study of tacrine for Alzheimer's disease. *Int. J. Geriatr. Psychiatry* **9**, 649–654.

World Health Organization (WHO). (1989). ''Dichlorvos,'' Environ. Health Criteria 79. WHO, Geneva.

Xiao, W. B., Nordberg, A., and Zhang, X. (1993). Effect of in vivo microdialysis of 1,2,3,4-tetrahydro-9-aminoacridine (THA) on the extracellular concentration of acetylcholine in the striatum of anesthetized rats. *J. Pharmacol. Exp. Ther.* **265**, 759–764.

Yasuda, R. P., Ciesla, W., Flores, L. R., *et al*. (1992). Development of antisera selective for m4 and m5 muscarinic cholinergic receptors in rat brain. *Mol. Pharmacol*. **43**, 149–157.

Zemlan, F. P. (1996). Velnacrine for the treatment of Alzheimer's disease: A double-blind, placebo-controlled trial. The Mentane Study Group. *J. Neural Transm*. **103**, 1105–1116.

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Anti-inflammatory and Antioxidant Therapies in Alzheimer's Disease

I. Introduction

The primary treatment of Alzheimer's disease (AD) may be considered to consist of two categories of therapy: symptomatic therapy that boosts cognitive performance and disease-modifying therapy that prevents or slows the disease process. The drugs approved thus far by the Food and Drug Administration (FDA) for the treatment of AD fit into the first category; they provide modest but measurable symptomatic benefit, without altering the rate of long-term decline. Much effort at present is devoted to seeking treatments in the second category to favorably alter the process of neurodegeneration. The development of transgenic mouse models of amyloid deposition in brain offer methods for screening potential anti-amyloid therapies; the result is likely to be human trials of agents that alter processing of the amyloid precursor protein into amyloidogenic fragments or otherwise interfere with the generation or deposition of β-amyloid peptide (Aβ). The recent report of dramatic effect of Aβ immunization in blocking plaque formation in a mouse model (Schenk *et al.*, 1999) represents one exciting example.

Symptomatic treatment, such as cholinesterase inhibitors, augment neurotransmission to improve cognitive performance. Disease-modifying treatment strategies, on the other hand, promote neuronal survival. While anti-amyloid strategies target events specific to the pathogenesis of AD, it may also be fruitful to seek non-disease-specific neuroprotective therapy. For example, treatments that increase the generation or effectiveness of neurotrophic factors may promote neuronal survival in the face of multiple stressors.

Anti-inflammatory and antioxidant treatment strategies fall into this latter category: approaches to improving neuronal survival in the face of nonspecific neurodegenerative stress. This chapter reviews evidence that inflammatory mechanisms and oxidative stress contribute to neurodegeneration in AD and outlines current efforts to develop anti-inflammatory and antioxidant treatment strategies.

II. The Inflammatory Hypothesis of AD

Though clinical features of inflammation are absent, on a molecular level it is apparent that an inflammatory response accompanies the neuropathologic features of AD (Aisen and Davis, 1994; Rogers *et al.*, 1996). Amyloid plaques are surrounded by reactive microglia and astrocytes; the former have the characteristics of antigen-presenting tissue macrophages, including HLA-DR surface markers. There is clear evidence of an acute phase response, with upregulation of inflammatory cytokines such as interleukins 1 and 6 and tumor necrosis factor α (TNF-α) accompanied by an increase in acute-phase proteins such as α1 antichymotrypsin and α2 macroglobulin. The complement system is active in the AD brain (Pasinetti, 1996), with generation of the lytic membrane attack complex, and presumably with release of anaphylatoxins. Upregulation of cyclooxygenase-2 in AD neurons (Pasinetti and Aisen, 1998) suggests that inflammatory lipids may also be involved in the pathogenesis of the disease. On the other hand, there is no acute neutrophilic inflammation in the AD brain, and little evidence of lymphocytic involvement or a humoral response.

Do inflammatory processes represent an ''innocent bystander'' reaction to AD neurodegeneration, induced by neuronal damage without contributing to it? Or do inflammatory mechanisms actually combat amyloid deposition and promote neuronal survival? There is no definitive answer to these pivotal questions, but evidence suggests that inflammation may play an active role in the disease process. Certainly, inflammatory cytokines, microglial activation, and complement activation are potentially neurotoxic, *in vitro* and *in vivo*. Inflammatory cytokines (Fagarasan and Aisen, 1996) and complement components (Oda *et al.*, 1995) augment the toxicity of Aβ protein in cell culture systems. Activated microglia secrete neurotoxins (Giulian *et al.*, 1994) and also augment amyloid toxicity in hippocampal slice cultures (London *et al.*, 1996). Inflammatory cytokines may be directly neurotoxic: overexpression of

IL-6 in the brain of a transgenic mouse leads to neurodegeneration (Campbell *et al.*, 1993). However, recent studies indicate that the role of inflammatory mediators may be complex. It has been assumed that complement activation in the AD brain is destructive, with anaphylatoxin release contributing to the recruitment and activation of microglia, and with generation of the membrane attack complex leading to neuronal membrane lysis. Indeed, suppression of anaphylatoxin release has been considered as a therapeutic goal in AD (Fagarasan *et al.*, 1997). Evidence derived from mice deficient in the complement component C5 (Pasinetti *et al.*, 1996) and studies of C5a receptor knockout mice (Osaka *et al.*, 1999) suggest that the anaphylatoxin C5a may actually play a neuroprotective role. Thus, it is not clear whether broad suppression of complement system activity is beneficial or harmful in AD. It may, therefore, be necessary to develop targeted anti-inflammatory strategies. Specific inflammatory processes that contribute to neuronal failure must be identified, so that selective interventions can be designed.

III. Cyclooxygenase and Brain Inflammation

Some evidence suggests that cyclooxygenase (COX)-2 may be such a specific target for anti-inflammatory therapy. COX catalyzes the conversion of membrane-derived arachidonic acid to prostanoids, a number of which are potent inflammatory mediators (Abramson and Weissmann, 1989). There are two distinct isoforms of COX, called COX-1 and COX-2. COX-2, the inducible form of the enzyme, is most important in inflammatory pathways. Inhibition of the constitutive COX-1, on the other hand, may be responsible for the adverse effects of NSAIDs (Vane and Botting, 1995).

Surprisingly, COX-2 apparently plays a role in mechanisms of neuronal death apart from inflammatory pathways (Pasinetti, 1998). Thus, in animal models of neurodegeneration (using lesions produced by kainic acid and by colchicine, for example) there is marked neuronal upregulation of COX-2 associated with apoptotic mechanisms (Tocco *et al.*, 1997; Ho *et al.*, 1999; Kelley *et al.*, 1999). In cell culture studies, amyloid toxicity is associated with induction of COX-2, and selective COX-2 inhibitors are cytoprotective (G. M. Pasinetti, unpublished data). COX-2 inhibitors also protect against ischemic and excitotoxic neuronal injury (Graham *et al.*, 1996; Nakayama *et al.*, 1996). It appears that COX-2 is involved in programmed cell death and that COX-2 inhibitors are neuroprotective. There is neuronal expression of COX-2 in the AD brain (Pasinetti, 1998). While an initial report indicated reduced levels of COX-2 mRNA in AD cortex (Chang *et al.*, 1996), we have demonstrated upregulation of COX-2 mRNA and protein, primarily associated with neurons, in AD frontal cortex (Pasinetti and Aisen, 1998). Recent studies also show COX-2 upregulation in AD hippocampus (Ho *et al.*, 1999; Yasojima *et al.*, 1999).

Selective COX-2 inhibitors have been developed by several pharmaceutical companies, because these drugs share anti-inflammatory efficacy with older nonselective COX inhibitors (such as indomethacin) with fewer side-effects. A selective COX-2 inhibitor with central nervous system penetration would be a good candidate for therapeutic trials in AD. While NSAIDs inhibit the enzyme's catalytic activity, glucocorticoids downregulate COX-2 expression. If the hypothesis that COX-2 contributes to apoptotic neuronal death in AD is correct, then a glucocorticoid might have synergistic benefit with a selective COX-2 inhibitor. It should be noted that COX-1 may also play a role in the pathophysiology of AD. Although it may not be upregulated, COX-1 is expressed in neurons and glia in the AD brain (Pasinetti and Aisen, 1998; Yasojima *et al.*, 1999). Both COX-1 and COX-2 may be a source of prostanoids that contribute to inflammation and neurodegenerative stress, and both may be targets for pharmacologic intervention. A cartoon depicting the possible role COX isoforms in neuronal–glial interactions in AD is shown in Fig. 33.1.

IV. Oxidative Stress and AD

Free radicals are molecules with unpaired electrons, formed as byproducts of metabolic processes (Halliwell and Gutteridge, 1989). They are highly reactive with macromolecules including lipids and thus can readily damage cell membranes. When the balance between free radical production and cellular anti-oxidant defenses is lost, cellular damage may ensue; such an imbalance has been invoked to explain age-related deterioration in many tissues. The theory that oxidative stress contributes substantially to neuronal damage in AD has received substantial attention (Simonian and Coyle, 1996; Markesbery, 1997; Behl, 1999). Nitrotyrosine, a marker of oxidative damage to proteins, has been demonstrated in association with neurofibrillary tangles in AD (Good *et al.*, 1996). Lipid peroxidation may be most notable in the AD temporal cortex (Marcus *et al.*, 1998). Oxidative damage to nuclear DNA occurs in the AD brain (Lyras *et al.*, 1997), particularly in neocortex (Gabbita *et al.*, 1998), along with a reparative response (Love *et al.*, 1999). Altered expression of superoxide dismutases, antioxidant enzymes, in areas of AD neurodegeneration has been demonstrated (Furuta *et al.*, 1997). Regional increase in iron levels in AD hippocampus could contribute to oxidative damage (Deibel *et al.*, 1997; LeVine, 1997). Malonaldehyde

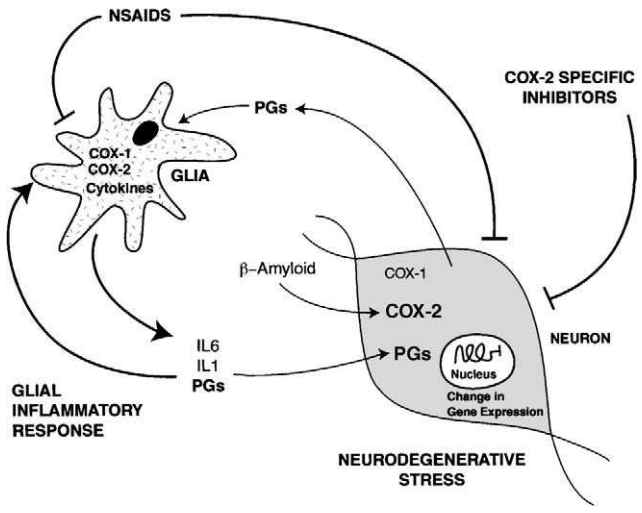

FIG. 33.1. Hypothetical role of COX isoforms in neuronal–glial interactions in Alzheimer's disease neurodegeneration.

levels in erythrocytes are elevated in AD compared to that seen in age-matched controls (Bermejo *et al.*, 1997), while levels of antioxidant enzymes and plasma antioxidant capacity are altered (Famulari *et al.*, 1996; De Leo *et al.*, 1998), consistent with systemic oxidative stress in the disease.

In vitro studies also support a possible link between free radicals and AD. Aβ protein generates free radicals in cell culture and in cell-free solution (Hensley *et al.*, 1994), and the toxicity of Aβ *in vitro* can be limited by antioxidants (Bruce *et al.*, 1996; Tomiyama *et al.*, 1996; Hirai *et al.*, 1998). Mitochondrial dysfunction, with cytochrome oxidase deficiency and impaired function of the electron transport chain, may contribute to free radical accumulation (Beal, 1998; Cassarino and Bennett, 1999). If free radicals play a significant role in neuronal loss in AD, then antioxidants that penetrate cell membranes within the brain would be expected to slow the neurodegeneration (Hall, 1992; Schulz *et al.*, 1996). The results of the multicenter trial of vitamin E and selegiline in AD (Sano *et al.*, 1997) provide encouraging support to this hypothesis.

V. Specific Interventions

A. Glucocorticoids

Glucocorticoids are the most potent anti-inflammatory/immunosuppressive drugs available, and are the primary treatment of many inflammatory disorders of brain. If the hypothesis that suppression of inflammation in brain will slow the progression of AD is correct, glucocorticoid therapy should be considered. The major concern with this approach has been the potential toxicity in the AD population. High-dose steroid regimens (e.g., prednisone 60 mg daily or more) are typically used to suppress brain inflammation in disorders such as multiple sclerosis, lupus cerebritis and vasculitis; such regimens are associated with significant toxicity in elderly subjects, and AD patients may be particularly susceptible to adverse effects on bone and behavior. It is known that glucoroticoids are actually toxic to the hippocampus in rodents (Sapolsky *et al.*, 1985), though perhaps not in primates (Leverenz *et al.*, 1999). Cognitive testing in normal volunteers suggests that glucocorticoids may adversely affect declarative memory (Newcomer *et al.*, 1994), though interestingly this effect diminishes with age (Newcomer *et al.*, 1995). On the other hand, in the presence of an inflammatory disease such as systemic lupus erythematosus, glucocorticoid therapy may improve cognitive function (Denburg *et al.*, 1994).

The Alzheimer's Disease Cooperative Study group (ADCS), a consortium of academic centers conducting clinical trials in AD with support from the National Institute on Aging, conducted the first multicenter trial of anti-inflammatory therapy for AD (Aisen *et al.*, 2000). Based on pilot studies that demonstrated the tolerability (Aisen *et al.*, 1996a) of low-dose prednisone in the AD population, and the efficacy of this treatment in suppressing peripheral markers of inflammation in AD (Aisen *et al.*, 1996a; Fagarasan *et al.*, 1997), the ADCS opted to study the effect of low-dose prednisone treatment on cognitive decline in subjects with mild to moderate AD. In this double-blind placebo-controlled parallel design study, 138 subjects were randomized to receive a regimen of low-dose prednisone or matching placebo. Subjects received an initial dose of 20 mg daily for 4 weeks, tapered to a maintenance dose of 10 mg daily for the duration of 1 year. After 1 year of treatment, medication was slowly tapered over an additional 16 weeks. The primary outcome measure for this study was the cognitive component of the Alzheimer's Disease Assessment Scale (Rosen *et al.*, 1984). The results of the prednisone trial were negative: the low-dose prednisone regimen did not influence the rate of cognitive decline. This may indicate that the regimen of prednisone studied is insufficient to suppress destructive brain inflammatory activity. Much higher doses are used to treat inflammatory diseases of brain such as lupus cerebritis and central nervous system vasculitis. However, higher doses may not be tolerable for prolonged treatment, particularly in the elderly, as indicated by the serious toxicity associated with high-dose glucocorticoids in the treatment of temporal arteritis. Adverse effects on behavior, and on blood sugar and bone density, of the prednisone regimen studied in the ADCS trial suggests that higher doses of glucocorticoids will cause serious toxicity in the AD population.

It is also possible that the diverse actions of glucocorticoids produced deleterious effects that nullified a beneficial anti-inflammatory effect. Thus, hippocampal toxicity may have canceled out a palliative effect on the disease. Further, recent evidence suggests that some inflammatory mediators, such as the anaphylatoxin C5a (Pasinetti, 1996; Osaka *et al.*, 1999) and the cytokine TNF-α (Mattson *et al.*, 1997), may have both neurodegenerative and neuroprotective effects. A more specific, targeted anti-inflammatory strategy may prove successful despite the nagative result of the prednisone trial.

B. Nonsteroidal Anti-inflammatory Drugs (NSAIDs)

The most widely discussed class of anti-inflammatory drugs with regard to AD is the NSAIDs. This interest is fueled by a large number of epidemiologic studies that support the theory that exposure to NSAIDs offers some degree of protection with regard to AD. Rheumatoid arthritis and AD are rarely diagnosed in the same individual (McGeer *et al.*, 1990); one interpretation of this finding is that NSAIDs, the most commonly used drugs to treat rheumatoid arthritis, reduce the subsequent incidence of AD. Studies of twins (Breitner *et al.*, 1994) and sibling pairs (Breitner *et al.*, 1995) discordant for AD also suggest a protective effect of NSAIDs. Numerous other cross-sectional and longitudinal surveys provide further evidence for NSAID neuroprotection (McGeer *et al.*, 1996). A small pilot study of indomethacin treatment in AD suggested that this NSAID slowed the rate of disease progression (Rogers *et al.*, 1993). This study generated a great deal of interest, but the results have been interpreted with caution because of the small number of subjects involved and a large number of drop-outs in the active drug group. Indeed, concern about the tolerability of traditional NSAIDs in the AD population has delayed the development of confirmatory studies. A study using a similar design, but testing the combination of diclofenac and misoprostol (a prostaglandin that reduces the risk of NSAID-induced ulceration), suffered from a similar high drop-out rate (50% in the active drug group) (Scharf *et al.*, 1999).

If long-term treatment with full anti-inflammatory doses of traditional NSAIDs is not feasible in population at risk for AD, there are at least two alternative approaches. One is to test a

low-dose NSAID, reducing the toxicity; the epidemiologic studies cited above suggest that casual use of low-dose NSAIDs may be neuroprotective. The second approach is to use new classes of COX inhibitors, which appear to be safer than older NSAIDs. Two pharmaceutical companies are currently conducting studies of new selective COX-2 inhibitors for the prevention or treatment of AD. In addition, National Institute on Aging-funded studies of both low-dose NSAIDs and selective COX-2 inhibitors have been initiated. Finally, newly developed drugs which combine NSAID activity with nitric oxide release appear to be safer than traditional NSAIDs and may represent candidates for testing in AD.

C. Other Anti-inflammatory Agents

There are other classes of anti-inflammatory drugs that merit consideration for trials in AD. Selection of agents for such trials will depend on evaluation of the contribution of specific mechanisms to neuronal stress. Activated microglia appear to play a prominent role in plaque maturation and may contribute to neuronal damage. If this is the case, agents which target mononuclear cell function may be useful. Colchicine and chloroquine both may be more effective than glucocorticoids in suppressing microglial accumulation and activation (Giulian and Robertson, 1990). Colchicine is also anti-amyloidogenic, highly effective in the secondary amyloidosis of familial Mediterranean fever (Zemer *et al.*, 1992), and perhaps effective in other amyloidoses. A pilot study of colchicine in AD demonstrated excellent tolerability (Aisen *et al.*, 1996b). A multicenter trial of hydroxychloroquine in AD is in progress in the Netherlands (van Gool, personal communication). The activity of propentofylline, a compound under development for the treatment of AD as well as vascular dementia, may work in part by suppressing microglial activity (Rother *et al.*, 1998). On the other hand, some evidence suggests that microglial function plays a beneficial role in the AD brain, scavenging amyloid deposits (Schenk *et al.*, 1999); drugs which inhibit microglial activity could prove harmful.

D. Antioxidants

Inflammatory and oxidative mechanisms overlap, and some therapeutic modalities have both anti-inflammatory and antioxidant properties. For example, potentially toxic free radicals may be generated during the activity of COX, and COX inhibitors may have antioxidant effects. However, interest in the agents discussed below for the treatment or prevention of AD is based primarily on putative antioxidant effects.

E. Vitamin E/Selegeline

The ADCS has reported the results of a multicenter trial of the antioxidants selegeline and vitamin E (α-tocopherol) for the treatment of moderate stage AD (Sano *et al.*, 1997). This trial investigated the effect of treatment of subjects at an initial Clinical Dementia Rating (Morris, 1993) of 2 with vitamin E (1000 IU twice daily), selegeline (5 mg twice daily), or both for 2 years. While there was no evidence of an effect of either agent on cognitive measures, treatment with either (or both) agents was associated with a delay in clinical markers of disease progression (death, institutionalization, progression of and loss of activities of daily living). Interestingly, there was no additive benefit of administering the two agents together. These results support the theory that antioxidant therapy can favorably influence the progression of AD.

F. Ginkgo Biloba

Substantial interest has developed in the use of ginkgo biloba, a botanical antioxidant, in the treatment of AD since publication of a study showing a small but statistically significant cognitive benefit with treatment for 12 months (Le Bars *et al.*, 1997). The study has been criticized over methodologic concerns, such as a very high dropout rate and the use of a last observation carried forward analysis. A recent review suggested that there is a small cognitive benefit to ginkgo treatment (Oken *et al.*, 1998), and the NIH is currently supporting a trial of ginkgo for the prevention of AD.

G. Idebenone

Interest in idebenone as a treatment for AD is based on both its antioxidant properties (Yamada *et al.*, 1999) and its capacity to increase brain production of nerve growth factor (Nitta *et al.*, 1993; Yamada *et al.*, 1997). One trial has reported that treatment of AD patients with idebenone for 6 months results in a slowing of cognitive decline (Weyer *et al.*, 1997). A controlled trial of idebenone to slow the progression of Huntington's disease did not demonstrate benefit (Ranen *et al.*, 1996).

VI. Conclusion

It is likely that specific inflammatory mechanisms, along with oxidative stress, contribute to neuronal damage in AD. Available agents which penetrate into brain tissue and inhibit these mechanisms are now undergoing testing for prevention and treatment of the disease. Further research into the inflammatory and oxidative pathways that influence neuronal survival in neurodegenerative conditions will increase the likelihood that these treatment strategies will succeed.

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References

Abramson, S. B., and Weissmann, G. (1989). The mechanism of action of non-steroidal anti-inflammatory drugs. *Arthritis Rheum.* **32**, 1.

Aisen, P. S., and Davis, K. L. (1994). Inflammatory mechanisms in Alzheimer's disease: Implications for therapy. *Am. J. Psychiatry* **151**, 1105–1113.

Aisen, P. S., Marin, D, Altstiel, L. *et al.* (1996a). A pilot study of prednisone in Alzheimer's disease. *Dementia* **7**, 201–206.

Aisen, P. S., Marin, D., Fusco, M., Baruch, B., Ryan, T., and Davis, K. L. (1996b). A pilot study of colchicine in Alzheimer's disease. *Alzheimer's Res.* **2**, 153–156.

Aisen, P. S., Davis, K. L., Berg, J. D., *et al.* (2000). A randomized controlled trial of prednisone in Alzheimer's disease. *Neurology* **54**, 585–593.

Beal, M. F. (1998). Mitochondrial dysfunction in neurodegenerative diseases. *Biochim. Biophys. Acta Bio-Energ.* **1366**, 211–223.

Behl, C. (1999). Alzheimer's disease and oxidative stress: Implications for novel therapeutic approaches. *Prog. Neurobiol.* **57**, 301–323.

Bermejo, P., Gomez-Serranillos, P., Santos, J., Pastor, E., Gil, P., and Martin-Aragon, S. (1977). Determination of malonaldehyde in Alzheimer's disease: A comparative study of high-performance liquid chromatography and thiobarbituric acid test. *Gerontology* **43**, 218–222.

Breitner, J. C., Gau, B. A., Welsh, K. A. *et al*. (1994). Inverse association of anti-inflammatory treatments and Alzheimer's disease: Initial results of a co-twin control study. *Neurology* **44**, 227–232.

Breitner, J. C. S., Welsh, K. A., Helms, M. J. *et al*. (1995). Delayed onset of Alzheimer's disease with nonsteroidal anti-inflammatory and histamine H2 blocking drugs. *Neurobiol. Aging* **16**, 523–530.

Bruce, A. J., Malfroy, B., and Baudry, M. (1996). β-Amyloid toxicity in organotypic hippocampal cultures: Protection by EUK-8, a synthetic catalytic free redical scavenger. *Proc. Nat. Acad. Sci. U.S.A.* **93**, 2312–2316.

Campbell, I. L., Abraham, C. R., Masliah, E. *et al.* (1993). Neurologic disease induced in transgenic mice by cerebral overexpression of interleukin 6. *Proc. Nat. Acad. Sci. U.S.A.* **90**, 10061–10065.

Cassarino, D. S., and Bennett, J. P., Jr. (1999). An evaluation of the role of mitochondria in neurodegenerative diseases: Mitochondrial and oxidative pathology, protective nuclear responses, and cell death in neurodegeneration. *Brain Res. Rev.* **29**, 1–25.

Chang, J. W., Coleman, P. D., and O'Banion, M. K. (1996). Prostaglandin G/H synthase-2 (cyclooxygenase-2) mRNA expression is decreased in Alzheimer's disease. *Neurobiol. Aging* **17**, 801–808.

Deibel, M. A., Ehmann, W. D., and Markesbery, W. R. (1997). Copper, iron, and zinc imbalances in severely degenerated brain regions in Alzheimer's disease. Possible relation to oxidative stress. *J. Neurol. Sci.* **143**, 137–142.

De Leo, M. E., Borrello, S., Passantino, M., *et al.* (1998). Oxidative stress and overexpression of manganese superoxide dismutase in patients with Alzheimer's disease. *Neurosci. Lett.* **250**, 173–176.

Denburg, S. D., Carbotte, R. M., and Denburg, J. D. (1994). Corticosteroids and neuropsychological functioning in patients with systemic lupus erythematosus. *Arthritis Rheum.* **37**, 1311–1320.

Fagarasan, M. O., and Aisen, P. S. (1996). Il–1 and anti-inflammatory drugs modulate Aβ cytotoxicity in PC12 cells. *Brain Res.* **723**, 231–234.

Fagarasan, M. O., Sevilla, D., Baruch, B., Santoro, J., Marin, D., and Aisen, P. S. (1997). Plasma C3a levels in Alzheimer's disease. *Alzheimer's Res.* **3**, 137–140.

Famulari, A. L., Marschoff, E. R., Llesuy, S. F. *et al.* (1996). The antioxidant enzymatic blood profile in Alzheimer's and vascular diseases. Their association and a possible assay to differentiate demented subjects and controls. *J. Neurol. Sci.* **141**, 69–78.

Furuta, A., Price, D. L., Pardo, C. A., *et al.* (1997). Localization of superoxide dismutases in Alzheimer's disease and Down's syndrome neocortex and hippocampus. *Am. J. Pathol.* **146**, 357–367.

Gabbita, S. P., Lovell, M. A., and Markesbery, W. R. (1998). Increased nuclear DNA oxidation in the brain in Alzheimer's disease. *J. Neurochem.* **71**, 2034–2040.

Giulian, D., and Robertson, C. (1990). Inhibition of mononuclear phagocytes reduces ischemic injury in the spinal cord. *Ann. Neurol.* **27**, 33–42.

Giulian, D., Li, J., Leara, B., and Keenen, C. (1994). Phagocytic microglia release cytokines and cytotoxins that regulate the survival of astrocytes and neurons in culture. *Neurochem. Int.* **25**, 227–233.

Good, P. F., Warner, P., Hsu, A., Olanow, C. W., and Perl, D. P. (1996). Evidence for neuronal oxidative damage in Alzheimer's disease. *Am. J. Pathol.* **149**, 21–28.

Graham, S. H., Kawaguchi, L., Zhu, L., and Chen, J. (1996). Cyclooxygenase 2 is induced in rat brain after kainate induced seizures and promotes neuronal death in CA3 hippocampus. *Soc. Neurosci. Abstr.* **22**, 2088.

Hall, E. D. (1992). Novel inhibitors of iron-dependent lipid peroxidation for neurodegenerative disorders. *Ann. Neurol.* **32**, S137–S142.

Halliwell, B., and Gutteridge, J. M. C. (1989). ''Free Radicals in Biology and Medicine,'' Vol 3. Clarendon Press, Oxford.

Hensley, K., Carney, J. M., Mattson, M. P., *et al.* (1994). A model for beta-amyloid aggregation and neurotoxicity based on free radical generation by the peptide: Relevance to Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 3270–3274.

Hirai, K., Hayako, H., Kato, K., and Miyamoto, M. (1998). Idebenone protects hippocampal neurons against amyloid beta-peptide-induced neurotoxicity in rat primary cultures. *Proc. Natl. Acad. Sci. U. S. A.* **358**, 582–585.

Ho, L., Osaka, H., Aisen, P. S., and Pasinetti, G. M. (1998). Induction of cyclooxygenase (COX)-2 but not COX-1 gene expression in apoptotic cell death. *J. Neuroimmunol.* **89**, 142–149.

Ho, L., Pieroni, C., Winger, D., Purohit, D. P., Aisen, P. S., and Pasinetti, G. M. (1999). Regional distribution of cyclooxygenase-2 in the hippocampal formation in Alzheimer's disease. *J. Neurosci. Res.* **57**, 295–303.

Kelley, K. A., Ho, L., Winger, D., Freire-Moar, J., Blanco-Borelli, C., Aisen, P. S., and Pasinetti, G. M. (1999). Potentiation of excitotoxicty in transgenic mice overexpressing neuronal cyclooxygenase-2. *Am. J. Pathol.* **155**, 995–1004.

Le Bars, P. L., Katz, M. M., Berman, N., Itil, T. M., Freedman, A. M., and Schatzberg, A. F. (1997). A placebo-controlled, double-blind, randomized trial of an extract of Ginkgo biloba for dementia. North American Egb Study Group. *JAMA, J. Am. Med. Assoc.* **278**, 1327–1332.

Leverenz, J. B., Wilkinson, C. W., Wamble, M. *et al.* (1999). Effect of chronic high-dose exogenous cortisol on hippocampal neuronal number in aged nonhuman primates. *J. Neurosci.* **19**, 2356–2361.

LeVine, S. M. (1997). Iron deposits in multiple sclerosis and Alzheimer's disease brains. *Brain Res.* **760**, 298–303.

London, J. A., Biegel, D., and Pachter, J. S. (1996). Neurocytopathic effects of Beta-amyloid-stimulated monocytes: A potential mechanism for central nervous system damage in Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 4147–4152.

Love, S., Barber, R., and Wilcock, G. K. (1999). Increased poly (ADP-ribosyl)ation of nuclear proteins in Alzheimer's disease. *Brain* **122**, 247–253.

Lyras, L., Cairns, N. J., Jenner, A., Jenner, P., and Halliwell, B. (1997). An assessment of oxidative damage to proteins, lipids, and DNA in brain from patients with Alzheimer's disease. *J. Neurochem.* **68**, 2061–2069.

Marcus, D. L., Thomas, C., Rodriguez, C. *et al.* (1998). Increased peroxidation and reduced antioxidant enzyme activity in Alzheimer's disease. *Exp. Neurol.* **150**, 40–44.

Markesbery, W. R. (1997). Oxidative stress hypothesis in Alzheimer's disease. *Free Radical Biol. Med.* **23**, 134–147.

Mattson, M. P., Barger, S. W., Furukawa, K. *et al.* (1997). Cellular signaling roles of TGFβ, TNFα and βAPP in brain injury responses and Alzheimer's disease. *Brain Res. Rev.* **23**, 47–61.

McGeer, P. L., McGeer, E., Rogers, J., and Sibley, J. (1990). Anti-inflammatory drugs and Alzheimer disease. *Lancet* **335**, 1037.

McGeer, P. L., Schulzer, M., and McGeer, E. G. (1996). Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: A review of 17 epidemiologic studies. *Neurology* **47**, 425–432.

Morris, J. C. (1993). The Clinical Dementia Rating (CDR): Current version and scoring rules. *Neurology* **43**, 2412–2414.

Nakayama, M., Uchimura, K., Zhu, L. *et al.* (1996). Cyclooxygenase 2 promotes neuronal death after global ischemia in rat CA1 hippocampus. *Soc. Neurosci. Abstr.* **22**, 1670.

Newcomer, J. W., Craft, S., Hershey, T., Askins, K., and Bardgett, M. E. (1994). Glucocorticoid-induced impairment in declarative memory performance in adult humans. *J. Neurosci*. **14**, 2047–2053.

Newcomer, J. W., Selke, G., Kelly, A. K., Paras, L., and Craft, S. (1995). Age-related difference in effects of glucocorticoid on memory in humans. *Am. Coll. Neuropsychopharmaco. Sci. Abstr.* **34**, 211.

Nitta, A., Hasegawa, T., and Nabeshima, T. (1993). Oral administration of idebenone, a stimulator of NGF synthesis, recovers reduced NGF content in aged rat brain. *Neurosci. Lett*. **163**, 219– 222.

Oda, T., Lehrer-Graiwer, J., Finch, C. E., and Pasinetti, G. M. (1995). Complement and beta-amyloid neurotoxicity in vitro: A model for Alzheimer disease. *Alzheimer's Res*. **1**, 29–34.

Oken, B. S., Storzbach, D. M., and Kaye, J. A. (1998). The efficacy of Ginkgo biloba on cognitive function in Alzheimer disease. *Arch. Neurol. (Chicago)* **55**, 1409–1415.

Osaka, H., Mukherjee, P., Aisen, P. S., and Pasinetti, G. M. (1999). Complement-derived anaphylatoxin C5a protects against glutamate-mediated neurotoxicity. *J. Cell. Biochem.* **73**, 303–311.

Pasinetti, G. M. (1996). Inflammatory mechanisms in neurodegeneration and Alzheimer's disease: The role of the complement system. *Neurobiol. Aging* **17**, 707–716.

Pasinetti, G. M. (1998). Cyclooxygenase and inflammation in Alzheimer's disease: Experimental approaches and clinical interventions *J. Neurosci. Res.* **54**, 1–6.

Pasinetti, G. M., and Aisen, P. S. (1998). Cyclooxygenase-2 expression is increased in frontal cortex of Alzheimer's disease brain. *Neuroscience* **87**, 319–324.

Pasinetti, G. M., Tocco, G., Sakhi, S. *et al.* (1996). Hereditary deficiencies in complement C5 are associated with intensified neurodegenerative responses that implicate new roles for the C-system in neuronal and astrocytic functions. *Neurobiol. Dis.* **3**, 197–204.

Ranen, N. G., Peyser, C. E., Coyle, J. T. *et al.* (1996). A controlled trial of idebenone in Huntington's disease. *Mov. Disord.* **11**, 549–554.

Rogers, J., Kirby, L. C., Hempelman, S. R. *et al.* (1993). Clinical trial of indomethacin in Alzheimer's disease. *Neurology* **43**, 1609–1611.

Rogers, J., Webster, S., Lue, L. F. *et al.* (1996). Inflammation and Alzheimer's disease pathogenesis. *Neurobiol. Aging* **17**, 681–686.

Rosen, W. G., Mohs, R. C., and Davis, K. L. (1984). A new rating scale for Alzheimer's disease. *Am. J. Psychiatry* **141**, 1356–1364.

Rother, M., Erkinjuntti, T., Roessner, M., Kittner, B., Marcusson, J., and Karlsson, I. (1998). Propentofylline in the treatment of Alzheimer's disease and vascular dementia: A review of phase III trials. *Dementia* **9**(Suppl. 1), 36–43.

Sano, M., Ernesto, C., Thomas, R. G. *et al.* (1997). A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. *N. Engl. J. Med.* **336**, 1216–1222.

Sapolsky, R. M., Krey, L. C., and McEwen, B. S. (1985). Prolonged glucocorticoid exposure reduces hippocampal neuron number: Implications for aging. *J. Neurosci.* **5**, 1222–1227.

Scharf, S., Mander, A., Ugoni, A., Vajda, F., and Christophidis, N. (1999). A double-blind, placebocontrolled trial of diclofenac/misoprostol in Alzheimer's disease. *Neurology* **53**, 197–201.

Schenk, D., Barbour, R., Dunn, W. *et al.* (1999). Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature (London)* **400**, 173–177.

Schulz, J. B., Matthews, R. T., Henshaw, D. R., and Beal, M. F. (1996). Neuroprotective strategies for treatment of lesions produced by mitochondrial toxins: Implications for neurodegenerative diseases. *Neuroscience* **71**, 1043–1048.

Simonian, N. A., and Coyle, J. T. (1996). Oxidative stress in neurodegenerative diseases. *Annu. Rev. Pharmacol. Toxicol.* **36**, 83–106.

Tocco, G., Freire, O., Schreiber, S. S., Sakhi, S. H., Aisen, P. S., and Pasinetti, G. M. (1997). Maturational regulation and regional induction of cyclooxygenase-2 in rat brain: Implications for Alzheimer's disease. *Exp. Neurol.* **144**, 339–349.

Tomiyama, T., Shoji, A., Kataoka, K. *et al.* (1996). Inhibition of amyloid β protein aggregation and neurotoxicity by rifampicin—Its possible function as a hydroxyl radical scavenger. *J. Biol. Chem.* **271**, 6839–6844.

Vane, J. R., and Botting, R. M. (1995). New insights into the mode of action of anti-inflammatory drugs. *Inflammation Res.* **44**, 1–10.

Weyer, G., Babej-Doelle, R. M., Hadler, D., Hofmann, S., and Herrmann, W. M. (1997). A controlled study of 2 doses of idebenone in the treatment of Alzheimer's disease. *Neuropsychobiology* **36**, 73–82.

Yamada, K., Nitta, A., Hasegawa, T. *et al.* (1997). Orally active NGF synthesis stimulators: Potential therapeutic agents in Alzheimer's disease. *Behav. Brain Res.* **83**, 117–122.

Yamada, K., Tanaka, T., Han, D., Senzaki, K., Kameyama, T., and Nabeshima, T. (1999). Protective effects of idebenone and alpha-tocopherol on β-amyloid-(1–42)-induced learning and memory deficits in rats: Implication of oxidative stress in beta-amyloid-induced neurotoxicity in vivo. *Eur. J. Neurosci.* **11**, 83–90.

Yasojima, K., Schwab, C., McGeer, E. G., and McGeer, P. L. (1999). Distribution of cyclooxygenase-1 and cyclooxygenase-2 mRNAs and proteins in human brain and peripheral organs. *Brain Res.* **830**, 226–236.

Zemer, D., Livneh, A., and Langevitz, P. (1992). Reversal of the nephrotic syndrome by colchicine in amyloidosis of familial Mediterranean fever. *Ann. Intern. Med.* **116**, 426.

SECTION III

Senses: Sensory Cortices and Primary Afferent Functions

A. Vision
(CHAPTERS 34–36)

B. Hearing
(CHAPTERS 37–44)

C. Chemical Senses
(CHAPTER 45)

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Rodrigo O. Kuljis

34

The Retina in Aging and in Alzheimer's Disease

I. Changes in the Retina

Defective vision and visually guided behavior is a frequent complaint in the elderly and in patients with neurodegenerative disorders, which may be due to eye pathology—including the retina—and a variety of processes affecting the central visual pathways. Elucidating the underlying mechanisms is a complex task, especially under conditions where several regions mediating vision are involved, such as Alzheimer's disease. In fact, impairments of visual perception and eye movements have been repeatedly demonstrated in many patients with Alzheimer's disease (AD). These defects include, but are not limited to, anomalies in color vision, spatial contrast sensitivity, susceptibility to visual masks, electroretinographic and visual evoked potential abnormalities, saccadic and eye tracking dysfunction, and deficits in stereoacuity (Hutton *et al*., 1984; Fletcher and Sharpe, 1988; Katz and Rimmer, 1989; Cronin-Golomb *et al*., 1991). The precise basis for these deficits remains elusive, since senile plaques, neurofibrillary tangles, and neuropil threads, among other lesions, occur in multiple regions that collectively mediate vision (Kuljis, 1994), but not in the retina itself (Hinton *et al*., 1986; Blanks *et al*., 1989; Sadun, 1989; Sadun and Bassi, 1990). Here we focus on one of the putative mechanisms for some of the above clinical manifestations, i.e., retinal ganglion cell loss, postulated on the basis of electroretinographic abnormalities, reduction in the numbers of these cells, and axonal loss in the optic nerve of patients with AD (Hinton *et al*., 1986; Blanks *et al*., 1989; Katz *et al*., 1989). However, this is still a controversial field because virtually all of the postulated Alzheimer-specific histopathological abnormalities in the retina and optic nerve have been found by others to be indistinguishable from age-associated degenerative changes and may not be specific to AD (Price *et al*., 1990; Curcio and Drucker, 1993).

The general problem of visual defects in AD exhibits several levels of complication because traditional accounts of AD have tended to neglect the involvement of the visual system in this condition. However, many different types of visuoperceptive and visuomotor manifestations—and histopathologically demonstrable lesions—have been increasingly recognized or claimed in recent years (Hutton *et al*., 1984; Hutton, 1985; Hinton *et al*., 1986; Beach and McGeer, 1988; Fletcher and Sharpe, 1988; Beach *et al*., 1989; Hof *et al*., 1989, 1990a,b, 1993; Katz and Rimmer, 1989; Katz *et al*., 1989; Méndez *et al*., 1990a,b; Sadun and Bassi, 1990; Cronin-Golomb *et al*., 1991; Hof and Bouras, 1991; Kuljis, 1992, 1994; Fletcher, 1994; Hof and Morrison, 1994). It has since become apparent that involvement of the visual system is both common and multifocal in AD, raising important questions about the precise substrates underlying each of the specific visual manifestations in this condition. The marked heterogeneity in the clinical, neuropsychological, and histopathological manifestations among individual patients, however, have conspired against the elucidation of the precise relationship between these various aspects of involvement and visual impairment in AD.

One of the areas in which efforts at elucidating these mechanisms have concentrated is the possibility of retinal pathology in AD. This is a relatively new area of inquiry, in which most of the relevant work has taken place within a decade and a half. In 1986, it was stated that "whether neuritic plaques are present in the axonal terminations of [retinal] ganglion cells in the lateral geniculate nucleus or the superior colliculus is not known" (Hinton *et al*., 1986). This statement was motivated by focused attention on retinal and optic nerve anbormalities, suggesting possible Alzheimer-specific pathology (Hinton *et al*., 1986; Blanks *et al*., 1989; Sadun, 1989; Sadun and Bassi, 1990). Specific abnormalities originally reported in AD patients include: (1) increase in the glia/neuron ratio throughout the retina, (2) 25–40% ganglion cell loss, (3) "frothy" degeneration in ganglion cells, (4) thinning of the optic nerve fiber layer, and (5) degeneration of predominantly large-diameter axons in the optic nerve. These findings appeared compatible with clinical and electrophysiological abnormalities supporting a retinal disturbance in patients with AD, such as electroretinographic abnormalities and funduscopic changes in the retina and optic nerve (Katz *et al*., 1989; Trick *et al*., 1989; Sadun and Bassi, 1990; Tsai *et al*., 1991; Fletcher, 1994). However, subsequent examinations

have cast doubt on some of these early claims on retinal histopathology, as some investigators have been unable to distinguish quantitatively and qualitatively between the preceived abnormalities in patients with AD and age-matched, presumably normal elderly individuals (Price *et al*., 1990; Curcio and Drucker, 1993).

More recent studies, however, have supported some aspects of the original reports of both neuronal and glial alterations in AD, with proper comparisons with age-matched, presumably eye-disease-free controls (Blanks *et al*., 1996a,b). In particular, these improved reassessments indicate that there is a 25–43% decrease in the total number of ganglion cells, and that the percentage of cell loss is regionally variable. Thus, neuronal loss is greatest in the central retina (52%), especially in its temporal region, and decreases away from the fovea (24%). This phenomenon seems to affect all classes of ganglion cells, and not predominantly large ones as previously suggested (Sadun and Bassi, 1990). There is also loss of additional neuron types throughout the retina (an average of 36%), which is most severe in the peripheral regions (up to 59%). Furthermore, while there is a modest and perhaps insignificant increase in absolute astrocyte numbers (16%), there is a statistically highly significant ($P < 0.0008$) increase in the ratio of astrocytes to neurons (82%). There are also indications of additional changes in Müller glial cells (e.g., increased labeling in their end-feet) in patients with AD.

II. Summary

Virtually all of the histopathological studies in the retina of patients with AD to date have been performed with quite small number of patients, and many of the initial studies did not employ stereological methodology. This, coupled with disconfirming evidence from other laboratories led to controversy as to whether retinal lesions occur or not in AD. There seems to be a consensus, however, that: (1) retinal ganglion cell loss—and other retinal changes—occur in normal aging, which must be distinguished from any changes attributable to AD or any neurodegenerative disorder potentially affecting the brain, and (2) no changes resembling those in the brain—such as senile plaques, neurofibrillary tangles, and amyloid protein deposits—have ever been reported in the retina of patients with AD. Nevertheless, the most recent histopathological findings (Blanks *et al*., 1996a,b), summarized above, lend credence to those postulating that AD results in retinal degeneration.

Future work in this field will hopefully include larger numbers of patients to ascertain to what extent retinal pathology is truly characteristic of AD. In addition, since increasing attention is being paid to AD with visual manifestations—clearly a subgroup of patients with the disorder (Hof *et al*., 1989, 1993; Levine *et al*., 1993; Blanks *et al*., 1996a,b)—it would be most helpful to have information about eye abnormalities in these patients in whom unusually severe involvement of association visual cortices has been recognized. This information is important not only to improve our understanding of the pathobiology of the disease, but is also relevant to the understanding and management of both visual deficits as well as hallucinations frequently observed in patients with AD (Chapman *et al*., 1999).

References

Beach, T. G., and McGeer, E. G. (1988). Lamina-specific arrangement of astrocytic and gliosis and senile plaques in Alzheimer's disease visual cortex. *Brain Res*. **463**, 357–361.

Beach, T. G., Walker, R., and McGeer, E. G. (1989). Lamina-selective A68 immunoreactivity in primary visual cortex of Alzheimer's disease patients. *Brain Res*. **501**, 171–174.

Blanks, J. C., Hinton, D. R., Sadun, A. A., and Miller, C. A. (1989). Retinal ganglion cell degeneration in Alzheimer's disease. *Brain Res*. **501**, 364–372.

Blanks, J. C., Torigoe, Y., Hinton, D. R., and Blanks, R. H. (1996a). Retinal pathology in Alzheimer's disease. I. Ganglion cell loss in foveal/parafoveal retina. *Neurobiol. Aging* **17**, 377–384.

Blanks, J. C., Schmidt, S. Y., Torigoe, Y., Porrello, K. V., Hinton, D. R., and Blanks, R. H. (1996b). Retinal pathology in Alzheimer's disease. II. Regional neuron loss and glial changes in CGL. *Neurobiol. Aging* **17**, 385–395.

Chapman, F. M., Dickinson, J., McKeith, I., and Ballard, C. (1999). Association among visual hallucinations, visual acuity, and specific eye pathologies in Alzheimer's disease: Treatment implications. *Am. J. Psychiatry* **156**, 1983–1985.

Cronin-Golomb, A., Rizzo, J. F., Corkin, S., and Growdon, J. H. (1991). Visual function in Alzheimer's disease and normal aging. *Ann. N. Y. Acad. Sci*. **640**, 28–35.

Curcio, C. A., and Drucker, D. N. (1993). Retinal ganglion cells in Alzheimer's disease and aging. *Ann. Neurol*. **33**, 248–257.

Fletcher, W. A. (1994). Ophthlamological aspects of Alzheimer's disease. *Curr. Opin. Ophthalmol*. **5**, 38–44.

Fletcher, W. A. and Sharpe, J. A. (1988). Smooth pursuit dysfunction in Alzheimer's disease. *Neurology* **38**, 272–277.

Hinton, D. R., Sadun, A. A., Blanks, J. C., and Miller, C. A. (1986). Optic-nerve degeneration in Alzheimer's disease. *N. Engl. J. Med*. **315**, 485–487.

Hof, P. R., and Bouras, C. (1991). Object recognition deficit in Alzheimer's disease: possible disconnection of the occipito-temporal component of the visual system. *Neurosci. Lett*. **122,** 53–56.

Hof, P. R., and Morrison, J. H. (1994). The cellular basis of cortical disconnection in Alzheimer's disease and related dementing conditions. In "Alzheimer's Disease" (R. D. Terry, R. Katzman, and K. L. Bick, eds.), pp. 197–229. Raven Press, New York.

Hof, P. R., Bouras, C., Constantinidis, J., and Morrison, J. H. (1989). Balint's syndrome in Alzheimer's disease: Specific disruption of the occipito-parietal visual pathway. *Brain Res*. **493**, 368–375.

Hof, P. R., Bouras, C., Constantinidis, J., and Morrison, J. H. (1990a). Selective disconnection of specific visual association pathways in cases of Alzheimer's disease presenting with Balint's syndrome. *J. Neuropathol. Exp. Neurol*. **49**, 168–184.

Hof, P. R., Cox, K., and Morrison, J. H. (1990b). Quantitiative analysis of a vulnerable subset of pyramidal neurons in Alzheimer's disease. I. Superior frontal and inferior temporal cortex. *J. Comp. Neurol*. **301**, 44–54.

Hof, P. R., Archin, N., Osmand, A. P., Dougherty, J. H., Wells, C., Bouras, C., and Morrison, J. H. (1993). Posterior cortical atrophy in Alzheimer's disease: analysis of a new case and re-evaluation of an historical report. *Acta Neuropathol*. **86**, 215–223.

Hutton, J. T. (1985). Eye movements and Alzheimer's disease: Significance and relationship to visuospatial confusion. *In* "Senile Dementia of the Alzheimer Type" (J. T. Hutton and A. D. Kenny, eds.). pp. 3–33. Alan R. Liss, New York.

Hutton, J. T., Nagel, J. A., and Loewenson, R. B. (1984). Eye tracking dysfunction in Alzheimer-type dementia. *Neurology* **34**, 99–102.

Katz, B., and Rimmer, S. (1989). Ophthalmologic manifestations of Alzheimer's disease. *Surv. Ophthalmol*. **34**, 31–43.

Katz, B., Rimmer, S., Iragui, V., and Katzman, R. (1989). Abnormal pattern electroretinogram in Alzheimer's disease: Evidence for retinal ganglion cell degeneration? *Ann. Neurol*. **26**, 221–225.

Kuljis, R. O. (1992). Differential distribution of Alzheimer's disease lesions in the striate cortex and visual nuclei. *Neurology* **42** (Suppl.), 444 (1007P).

Kuljis, R. O. (1994). Lesions in the pulvinar in patients with Alzheimer's disease. *J. Neuropathol. Exp. Neurol*. **53**, 202–211.

Levine, D. N., Lee, J. M., and Fisher, C. M. (1993). The visual variant of Alzheimer's disease. *Neurology* **43**, 305–313.

Méndez, M. F., Méndez, M. A., Martin, R., Smyth, K. A., and Whitehouse, P. J. (1990a). Complex visual disturbances in Alzheimer's disease. *Neurology* **40**, 439–443.

Méndez, M. F., Turner, J., Gilmore, G. C., Remler, B., and Tomask, R. L. (1990b). Bálint's syndrome in Alzheimer's disease: Visuospatial functions. *Int. J. Neurosci*. **54**, 339–346.

Price, D. L., Silva, J. C., Martin, L. J., and Troncoso, L. J. (1990). The retina and optic nerve in Alzheimer's disease (AD) and aging. *Neurobiol. Aging* **11**, 275–295.

Sadun, A. A. (1989). The optic neuropathy of Alzheimer's disease. *Metab. Pediatr. Syst. Ophthalmol*. **12**, 64–68.

Sadun, A. A., and Bassi, C. J. (1990). Optic nerve damage in Alzheimer's disease. *Ophthalmology* **97**, 9–17

Trick, G. L., Barris, M. C., and Bickler-Bluth, M. (1989). Abnormal pattern electroretinograms in patients with senile dementia of the Alzheimer type. *Ann. Neurol*. **26**, 226–231.

Tsai, C. S., Ritch, R., Schwartz, B., Lee, S. S., Miller, N. R., Chi, T., and Hsieh, F. Y. (1991). Optic nerve head and nerve fiber layer in Alzheimer's disease. *Arch. Ophthalmol. (Chicago)* **109**, 199–204.

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M. Rosario Hernandez and Arthur H. Neufeld

35

Pathogenesis of Glaucomatous Optic Neuropathy

I. Introduction

A. Primary Open-Angle Glaucoma

Glaucoma is an optic neuropathy characterized by loss of retinal ganglion cells, cupping or excavation of the optic disc, and a characteristic defect and progressive changes in the visual field. Loss of vision in glaucoma is due to the progressive and irreversible loss of the axons of the retinal ganglion cells. Glaucoma is usually, but not always, associated with impaired aqueous humor outflow, which results in elevated intraocular pressure (IOP). Elevated IOP is considered the major risk factor for glaucoma. Depending on the mechanism of aqueous outflow obstruction, glaucoma is subdivided into open-angle, closed-angle, and congenital or developmental glaucoma. The causes of glaucoma can be primary or secondary, depending on the nature of the pathophysiological events leading to impairment of the flow of aqueous humor. In primary open-angle glaucoma, there is no visible obstruction in the trabecular meshwork; whereas, in secondary glaucoma or in angle closure glaucoma, there are clinically observable causes that impede the flow of aqueous humor.

Primary open-angle glaucoma (POAG), the most common form of glaucoma, is a major cause of blindness worldwide. The prevalence of POAG increases with age markedly, with an estimated age-adjusted prevalence of 1.8% in Caucasians and 5.2% in persons of African descent over the age of 40 (Quigley and Vitale, 1997). POAG is often bilateral, although it may develop in one eye first, and is asymptomatic until later in the disease when patients lose central vision.

The retinal ganglion cells are found in the ganglion cell layer that is the innermost neural layer of the retina bordering the vitreous humor. The retinal ganglion cells have large somas that send dendritic processes into the inner plexiform layer and synapse with bipolar cells and amacrine cells. The retinal ganglion cells carry visual information that has been processed by the retina into the central nervous system by way of long axons that arise from the soma to make up the nerve fiber layer of the retina. These axons run centripetally along the vitreous surface in the nerve fiber layer and, when reaching the optic nerve head, make a right-angle turn to form the optic nerve. In humans, the optic nerve is made up of approximately one million axons from the retinal ganglion cells.

In glaucoma, the loss of neural tissue is believed to be exclusively that of retinal ganglion cells. Clinically, this is seen by biomicroscopy as progressive loss of tissue in the optic nerve head (cupping or excavation) and loss of birefringence in the nerve fiber layer. By visual field testing, the functional loss of retinal ganglion cells can be followed. The statement that only retinal ganglion cells are lost in glaucoma is based on pathological examination of retinas from patients with glaucoma that were done many years ago. However, this question has not been revisited with contemporary techniques. Nevertheless, we will focus on the loss of retinal ganglion cells in this disease.

There is general agreement in the field that damage to the optic nerve axons occurs at the level of the lamina cribrosa in the anterior, nonmyelinated portion of the optic nerve known as the optic nerve head (for comprehensive reviews, see Hayreh, 1978; Kolker and Hetherington, 1983; Quigley, 1992; Hernandez and Pena, 1997). The lamina cribrosa is composed of stacks of connective tissue plates, the cribriform plates, with holes that are aligned in register to form channels through which the axons pass as they exit the globe. In the glaucomatous optic nerve, cupping of the optic disc and compression, stretching and rearrangement of the cribriform plates of the lamina cribrosa occur in response to elevated IOP (Quigley *et al.*, 1983). The changes in the optic disk can be seen clinically by ophthalmoscopy.

The relationship between IOP and glaucomatous optic neuropathy has been extensively studied. Optic nerve changes occur throughout the entire range of IOP levels and damage to the optic nerve shows individual variability. There are many patients with elevated IOP in which optic nerve damage does not occur (ocular hypertension) and there are patients with IOP within the normal range in which optic nerve head damage does occur (normal pressure glaucoma) (Werner, 1996).

Nevertheless, the tendency to develop optic nerve damage and the diagnosis of glaucoma increase with increasing IOP and with each decade over the age of 40. Thus, there seems to be a relationship between age and optic nerve damage, and elevated IOP and optic nerve damage. A less frequent form of primary open-angle glaucoma may occur before age 40 and is referred as a separate entity: juvenile primary open-angle glaucoma. In addition to increasing age and IOP, other important risk factors for glaucoma are race and family history. Persons of African descent are at higher risk for developing more severe glaucoma and at a younger age (Tielsch *et al*., 1991; Leske *et al*., 1995).

B. Human Genetics of Glaucoma

Some forms of early-onset glaucoma are inherited as autosomal-dominant, autosomal-recessive traits or as complex traits involving multiple gene defects and environmental factors. Undoubtedly, many of the risk factors underlying the pathogenesis of glaucoma are also inherited. Ocular parameters such as optic cup size, level of IOP, outflow facility, ocular dimensions, and sensitivity to steroids appear to have a genetic component (Johnson *et al*., 1996). Major advances in glaucoma genetics have led to the discovery and chromosomal location of several genes that cause early-onset glaucoma.

Of interest in this chapter are genes associated with adult onset POAG. The first major gene loci for POAG, GLC1A, and GLC1B, were described by Stone and collaborators (1997) and by Sarfarazi and collaborators (Stoilova *et al*., 1996). GLC1A, also known as myocilin or Trabecular Meshwork Inducible Glucocorticoid Responsive (TIGR), is a gene that is localized on chromosome 1 and expressed in the trabecular meshwork of the outflow pathway. Many families affected with juvenile open-angle glaucoma (JOAG) and 2–4% of patients that are affected with adult-onset POAG show mutations in GLC1A (Alward *et al*., 1996, 1998; Fingert *et al*., 1999). In the laboratory, expression of this gene is induced by chronic treatment of cultured trabecular meshwork cells with glucocorticoids, thus an early name of this gene was TIGR (Polansky *et al*., 1997). The sequence of myocilin suggests a protein with multiple leucine zipper motifs, PEST regions susceptible to intracellular degradation, and regions with homology to the myosin heavy chain. Studies are underway to determine whether there is an association of myocilin with the cytoskeleton and/or the microtubules (Takahashi *et al*., 1998; Mertts *et al*., 1999).

The expression pattern of myocilin in ocular tissues indicates that the outflow pathway is probably the site where the mutation causes the elevated IOP found in these patients (Polansky *et al*., 1997; Takahashi *et al*., 2000). However, the function of myocillin in the outflow tissue is unknown. Myocilin is also expressed in many tissues of the body, including the optic nerve head. Nevertheless, to date there is no evidence that correlates mutations in myocilin with retinal ganglion cell loss or optic nerve damage, the hallmarks of the optic neuropathy in glaucoma.

The GLC1B locus is located on chromosome 2 and is associated with normal to moderately elevated IOP and with optic nerve damage (Stoilova *et al*., 1996). The GLC1C locus was mapped to chromosome 3 and is associated clinically with elevated IOP, cup/disc ratios larger than 0.7, and visual fields defects (Wirtz *et al*., 1999). Other loci for adult onset POAG have been reported in the literature (GLC1D–GLC1F); however, the genes have not been identified. To date, only the GLC1A gene has been identified as causative for glaucoma. Future work in this field may uncover genes that affect retinal ganglion cell or optic nerve susceptibility to elevated IOP.

II. The Optic Nerve Head as the Site of Glaucomatous Damage

A. Structure

The optic nerve head is the intraocular portion of the optic nerve and extends from the vitreal surface of the optic disc to the posterior scleral surface. The axons of the retinal ganglion cells are not myelinated as they traverse through this region. The prelaminar region of the nerve head is where the axons of the retinal ganglion cells converge and turn 90° to form the optic nerve. Columns of astrocytes, which contain blood vessels, separate the nonmyelinated axons into bundles (Anderson, 1969).

The lamina cribrosa is posterior to the prelaminar region and is composed of about 10 fibroelastic plates, cribriform plates, stretched across the scleral canal. The cribriform plates are perforated by several hundred openings, aligned in register to permit the exit of the nerve bundles (Anderson, 1969).

In normal human optic nerve heads, the lamina cribrosa is fibroelastic and formed by stacks of connective tissue, the cribriform plates, which are organized in lamellae perpendicular to the axons and are lined by astrocytes (Elkington *et al*., 1990; Hernandez and Gong, 1996). The extracellular matrix (ECM) of the lamina cribrosa, together with the astrocytes, provides mechanical and biological support to the axons from the retinal ganglion cells as they exit the eye (Hernandez and Pena, 1997).

The postlaminar region of the nerve head is posterior to the lamina cribrosa and is where the axons of the retinal ganglion cells become myelinated. The axon bundles are separated by pial septae containing blood vessels and lined by astrocytes.

B. Pathological Changes in Glaucoma

The human optic nerve head has biomechanical properties, which may be altered irreversibly during the glaucomatous process. These biomechanical properties are provided at the level of the lamina cribrosa. In the glaucomatous optic nerve head, cupping of the optic disc, notching and thinning of the neuroretinal rim, disc hemorrhages, and loss of the retinal nerve fiber layer are common pathological observations by the clinician. At the tissue level, compression, stretching, and remodeling of the cribriform plates occur, in many patients in response to IOP (Quigley *et al*., 1983) (Fig. 35.1). Early glaucomatous changes in the lamina cribrosa include an outward movement and vertical compression of the cribriform plates. The bowing of the lamina cribrosa is hinged at the insertion of the lamina in the sclera and at the insertion of the lamina into the wall of the central vessels of the retina. This results in the change from U-shaped lamina in normal eyes to a W-shaped lamina in glaucomatous eyes (Varma and Minckler, 1996).

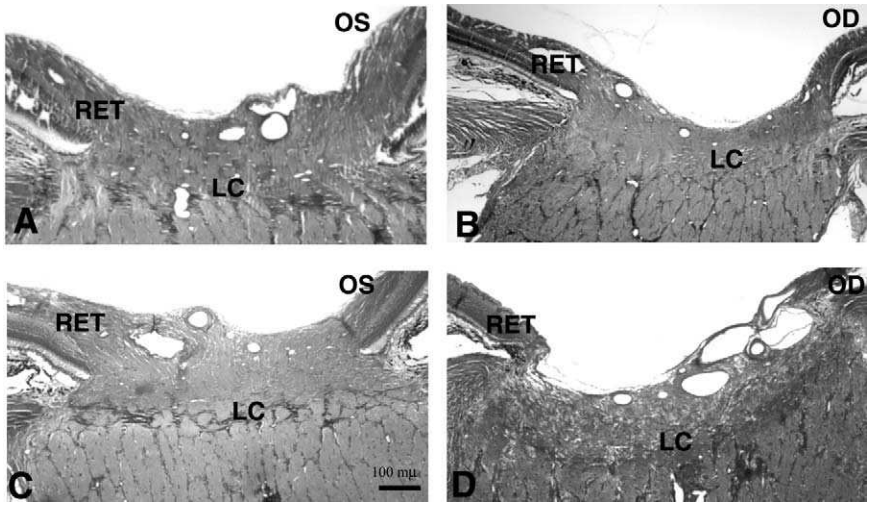

FIG. 35.1. Low magnification of sagittal views of the macaque monkey optic nerve head in experimental glaucoma. Right eyes (OD) of two Rhesus monkeys were exposed to chronic elevation of intraocular pressure at ~29.4 mm Hg for 32 weeks (B) and at ~39 mm Hg for 12 weeks. Left eyes (OS) were left untreated and used as controls; intraocular pressures were ~19 mm Hg (A) and ~11 mm Hg (C), respectively. Note in (B) a slight excavation of the optic nerve head and displacement of the central retinal vessels to the margins of the cup. Note in (D) a marked excavation of the nerve head and posterior bowing of the lamina cribrosa (LC). RET, retina. Trichromic staining.

There are changes in the quantity and composition of several matrix macromolecules, which must significantly affect the biomechanical properties of the optic nerve head. Astrocytes, the major cell population in the optic nerve head, become reactive and are probably responsible for the remodeling of the optic nerve head in glaucoma. Changes in the support component of the optic nerve head in the initial stage of glaucoma will account for irreversibility and perhaps increased susceptibility to optic nerve damage from IOP during the progression of the disease.

The remodeling of the optic nerve head in glaucoma is accomplished through the properties of ECM macromolecules, which generate a large array of structures, allow interactions between protein and nonprotein macromolecules, modulate cellular behavior and phenotypes, bind growth factors and cytokines, thus acting as a source of potent modifiers of cellular responses, and provide the scaffold for cell migration and cell–cell interactions (Raghow, 1994).

C. Role of the Extracellular Matrix in Glaucoma

Wound healing and remodeling of ECM in the brain involves several cell types. These include reactive astrocytes, microglia, and vascular endothelia, invading macrophages and oligodendrocytes, all of which contribute to the formation of a glial scar. After neural injury, reactive astrocytes synthesize ECM proteins, e.g., tenascin, laminin, and chondroitin sulfate proteoglycan, that are not expressed in adults but are expressed at high levels during development. Synthesis of these ECM macromolecules by reactive astrocytes causes remodeling of the microenvironment of the neural tissue and may provide boundaries to isolate the damaged neurons, prevent migration of inflammatory cells due to failure of the blood–brain barrier, serve as storage sites for growth factors, and deter regrowth of brain axons after injury (Fitch and Silver, 1997). Although the responses in the glaucomatous optic nerve head have components of wound healing that are similar to those seen after neural injury in the central nervous system, there is no invasion of inflammatory cells. A true glial scar does not form; nevertheless, extensive remodeling of the ECM does occur, affecting most of the major groups of ECM macromolecules.

Several laboratories, including ours, have used immunohistochemistry and molecular biological techniques to document the changes in the macromolecular components of the ECM in the human glaucomatous optic nerve head compared to age-matched normals. Previous work has demonstrated changes in different collagen types, basement membrane components, glycosaminoglycans, elastin, tenascin, and fibrillin (Morrison *et al*., 1989, 1990; Hernandez *et al*., 1990, 1994a,b; Quigley *et al*., 1991; Fukuchi *et al*., 1992, 1994; Hernandez, 1992; Pena *et al*., 1996, 1998, 1999; Gong *et al*., 1997; Varela and Hernandez, 1997).

1. Structural Extracellular Matrix

In POAG, there is enhanced expression of collagen type IV mRNA by astrocytes in the prelaminar region and *de novo* expression of elastin mRNA by laminar astrocytes (Hernandez *et al*., 1994a,b). These observations of the differential expression of ECM macromolecules, dependent on the area of the optic nerve head, strongly suggest reactivation of astrocytes and regional responses to their microenvironment.

Collagen fibers, elastic fibers, basement membranes, and proteoglycans form the ECM of the lamina cribrosa. The three-dimensional organization of the ECM in the lamina must contribute to the ability of this tissue to adapt to the diurnal changes in IOP that occur normally. However, in glaucomatous neuropathy a marked disruption of the ECM occurs. In POAG, there are marked changes in elastic fibers at the level of the lamina cribrosa, involving elastotic degeneration

(Hernandez, 1992; Netland *et al*., 1995; Pena *et al*., 1998) and upregulation of tropoelastin mRNA, indicating reactivation of synthesis of this macromolecule in glaucoma (Hernandez *et al*., 1994b). However, elastotic fibers are predominantly formed in glaucomatous optic neuropathy.

The ultrastructural morphology of elastotic fibers in the lamina cribrosa varies from large irregular masses of ribbons of elastin-labeled material to moth-eaten large fibers, with irregular distribution of elastin and microfibrils (Fig. 35.2). In the monkey model of glaucoma in use in our laboratory, elastotic fibers are also present in the ECM of the lamina cribrosa in the eye with elevated pressure but not in the contralateral eye. However, in the lamina cribrosa of the monkey model of optic nerve transection, elastotic fibers are absent in the ECM of both the transected and the contralateral eyes. These studies strongly suggest that the enhanced elastin synthesis and assembly of elastotic fibers in glaucoma represent a specific response to elevated IOP and not a response to the mere loss of axons from the optic nerve head.

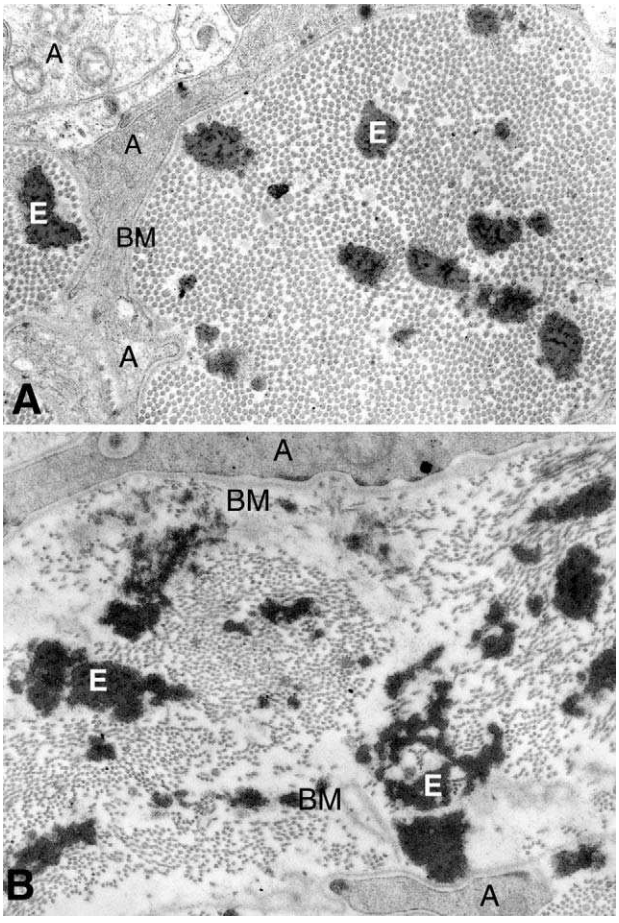

FIG. 35.2. Electron micrographs of the human lamina cribrosa of a normal eye (A) and an age matched glaucomatous eye (B). (A) In the normal eye, the fibroelastic matrix of the laminar plates consists in tightly packed collagen fibers surrounding elastic fibers (E). A well-defined basement membrane (BM) separates the astrocytes and their process (A) from the underlying matrix. (B) In glaucoma, elastic fibers (E) appear fragmented and strongly electron dense. The density of collagen fibers is markedly reduced around the elastic fibers. Note the presence of basement membrane material (BM) in the matrix. Original magnification, $\times 26,000$.

The increased area of elastin-labeled material in glaucoma (Pena *et al*., 1998) suggests that elastotic fibers may consist of newly synthesized elastin as demonstrated by the increased tropoelastin mRNA detected in the lamina cribrosa of glaucomatous eyes (Hernandez *et al*., 1994b; Pena *et al*., 1996). There is no apparent defect in the primary sequence or in the alternative splicing of tropoelastin mRNA in glaucomatous optic nerves (Pena *et al*., 1996), suggesting that the derangement of elastic fibers in the glaucomatous lamina cribrosa results from extracellular processing of tropoelastin and/or assembly into the microfibrillar scaffold.

The changes in elastic fibers are accompanied by changes in the collagen network. Qualitative changes in density and distribution of collagen fibers in the laminar plates and loss of collagen fibers around elastic fibers, as detected by morphometry, have been described (Quigley *et al*., 1991; Hernandez, 1992). Recently, a three-dimensional study using the scanning electron microscope further demonstrated the disorganization of the collagen network in the lamina cribrosa of glaucomatous monkeys. In normal eyes, a thin cushion of collagen fibers covers the surface of the laminar plates and a smooth layer of well-packed collagen fibers covers the inner surface of the laminar pores. In contrast, in glaucomatous eyes the inner surface of the pores is irregular and the pores are narrower (Sawaguchi *et al*., 1999).

In most connective tissues, elastic fibers and collagen fibers act as parallel mechanical elements to applied stress or strain. At low levels of strain, collagen fibers are easily extended with most of the strain borne by the elastic fibers. At high levels of strain, the collagen fibers limit the distension, providing strength and support to the tissue (Mercer and Crapo, 1990).

In conclusion, the changes in the ECM of the glaucomatous optic nerve head are extensive and may be caused by reactive astrocytes. As these cells alter the microenvironment, some of the changes in ECM may not be supportive of normal axons in the immediate area. Furthermore, changes in the elastic and collagen components will have an impact on the tissue's ability to respond normally to pressure, resulting in a less compliant optic nerve head. These changes in the biomechanical properties will produce a remodeled tissue with a tendency to collapse under elevated pressure, as suggested by changes in the compliance of the optic disc related to elevated IOP in human and monkey glaucomatous eyes.

2. Reactive Extracellular Matrix in Glaucoma

Reactive astrocytes synthesize new ECM proteins in areas of neural degeneration that provide boundaries to isolate damaged axons from healthy axons, that prevent hematogenous cells from invading the injured neural tissue, that affect survival of remaining axons, and that prevent axonal regrowth. Recently, we demonstrated gene expression and synthesis of tenascin, an ECM protein, synthesized by optic nerve head astrocytes (Pena *et al*., 1999). Tenascin is synthesized in development and by reactive astrocytes in central nervous system injury (Bartsch *et al*., 1992, 1994). Other ECM proteins that may be induced in glaucoma are laminin, sulfated glycoprotein 2 (SPG2), and chondroitin sulfate proteoglycans (Fitch and Silver, 1997).

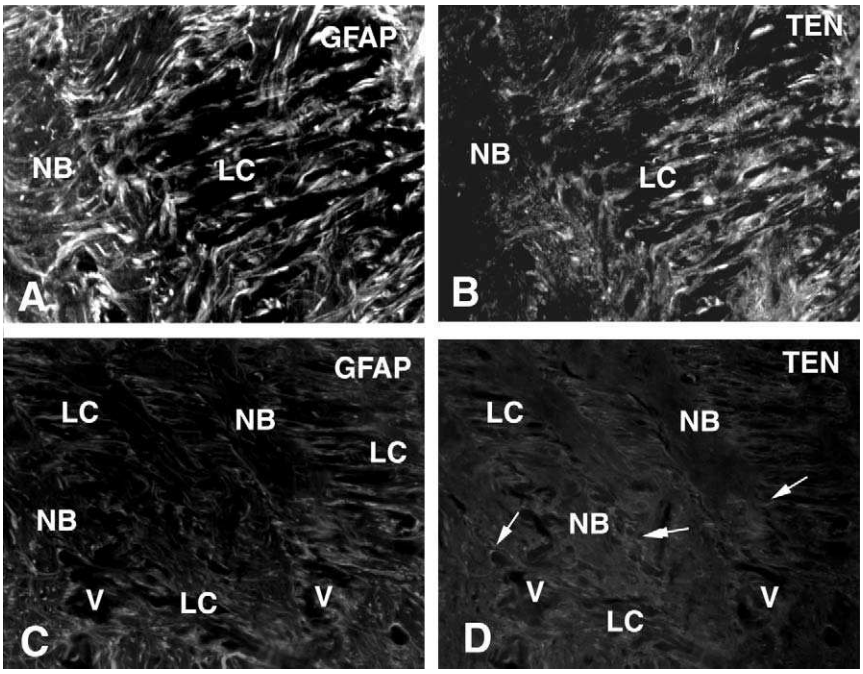

FIG. 35.3. Sagittal sections of the lamina cribrosa (LC) immunostained with antibodies against GFAP and tenascin. (A) In the normal eye, astrocytes stained with GFAP form lamellae oriented perpendicular to the nerve bundles (NB). Tenascin staining is present in the cribriform plates and around blood vessels but not in the nerve bundles. (B) In the glaucomatous eye, astrocytes bearing thick processes stained intensely with GFAP occupy the nerve bundle area. Tenascin staining is present inside the nerve bundles. Scale bar, 50 mμ.

Tenascin synthesis, a minor component of the ECM in normal optic nerve head, is upregulated in human and monkey glaucomatous optic nerve head (Pena *et al*., 1999) (Fig. 35.3). In the lamina cribrosa of human glaucomatous eyes, tenascin immunoreactivity and gene expression were localized to astrocytes in the cribriform plates and inside the nerve bundles. It is interesting to note that the promoter region of the tenascin gene contains an enhancer element responsive to mechanical stress (Chiquet-Ehrismann *et al*., 1994; Chiquet *et al*., 1996). Glaucoma is characterized by IOP, and it has been proposed that elevated IOP-related stress in the optic nerve head affects astrocytes within the tissue (Hernandez and Pena, 1997). Although, the role of tenascin in the nerve head is unknown, one can speculate that upregulation of tenascin synthesis by astrocytes in glaucoma may be partially due to IOP-related mechanical stress.

3. Extracellular Matrix Degradation

Proteolysis cleaves extracellular domains of ECM macromolecules, regulates assembly and remodeling of the tissue and releases growth factors. Through these functions, ECM-degrading enzymes participate in development, morphogenesis, growth of tissues and organs, tissue repair, and pathological processes (Parks and Sires, 1996; Werb, 1997). The major families of ECM-degrading enzymes are the matrix metalloproteinases (MMPs), the tissue inhibitors of MMP, the tissue serine proteinases, e.g., as thrombin, tissue plasminogen activator, urokinase-type tissue plasminogen activator, and plasmin. Certain ECM-degrading enzymes have been identified in brain injury, tumor invasion, and neurodegenerative disease in the brain (Romanic and Madri, 1994; Rosenberg, 1995; Pagenstecher *et al*., 1998; Uhm *et al*., 1998).

A recent study in the normal and inflamed mouse brain documented differential expression of several MMP and tissue inhibitors of MP genes. MMPs are expressed constitutively at very low levels, whereas tissue inhibitors of MPs 2 and 3 are expressed at high levels in the central nervous system of normal animals. In the inflamed brain tissue, microglia and leukocytes invading the lesions expressed high levels of MMPs. Reactive astrocytes surrounding the lesions expressed tissue inhibitors of MPl (Pagenstecher *et al*., 1998). This study indicates that in the normal central nervous system, astrocytes maintain low MMP activity by high constitutive expression of tissue inhibitors of MPs and that in response to inflammation, reactive astrocytes isolate lesions to protect healthy neurons (Pagenstecher *et al*., 1998).

MMPs may also be a component of astrocyte reactivation, which requires a change in cell shape and migration through the ECM. MMP activities are likely to be involved in the transition of quiescent astrocytes to the reactive phenotype by degrading cell surface adhesion molecules and, through disruption of the extracellular domains, by altering the cytoskeleton (Parks and Sires, 1996; Werb, 1997). Activation of growth factors, such as TGF-β, can also be mediated by proteolysis of ECM-binding proteins. The extensive remodeling of the ECM of the optic nerve head that is characteristic of glaucoma strongly suggests the presence of ECM-degrading enzymes contributed by reactive astrocytes. Studies are underway in several laboratories, including ours, to determine which MMPs

may be involved in the remodeling of the ECM in glaucoma. A preliminary screening of MMPs indicate that there are several such enzymes associated with reactive astrocytes that may account for ECM remodeling. For example, a cell membrane-associated MMP, MT-MMPl, is localized to hypertrophic astrocytes in the glaucomatous lamina cribrosa suggesting a role for these enzymes in the disease.

D. Glial Cells in Glaucoma

1. Astrocytes in the Optic Nerve Head

Astrocytes are the major glial cell type in the nonmyelinated optic nerve head in most mammalian species. In the lamina cribrosa and prelaminar region of the optic nerve head, astrocytes provide cellular support functions to the axons form the interface between connective tissue surfaces and surround blood vessels (Anderson, 1969).

In the prelaminar, lamina cribrosa, and retrolaminar axons, there are regional and functional heterogeneities of astrocytes that provide cellular support to the axons and synthesize ECM macromolecules. In the human optic nerve head, two subpopulations of astrocyte type 1 are distinguished. Type 1 A astrocytes are interpersed in the glial columns and at the edges of the cribriform plates. They express glial fibrillary acidic protein (GFAP), a cytoskeletal marker of astrocytes, but do not express neural cell adhesion molecule (NCAM). Type 1B astrocytes express both GFAP and NCAM. They are the major glial cell population in the region and are the primary sources for the synthesis of ECM in the optic nerve head during development and throughout life (Hernandez *et al*., 1991; Hernandez, 1992; Ye *et al*., 1994; Pena *et al*., 1996). Type 1B astrocytes line the vitreal surface of the optic disc, form the glial columns, surround blood vessels in the prelaminar region, form the cribriform plates in the lamina cribrosa, and separate the sclera from the optic nerve in the insertion area.

In the lamina cribrosa, astrocytes form lamellae oriented perpendicular to the axons. A well-defined basement membrane separates the astrocytes from the underlying collagen and elastic matrix. Astrocytes extend cell processes into the ECM core and establish contacts with processes of other astrocytes and lamina cribrosa cells (Fig. 35.3).

2. Reactive Astrocytes in Glaucoma

Astrocytes are a significant component of the response to injury in the central nervous system (Hatten *et al*., 1991; Eddleston and Mucke, 1993; Faden, 1993; Norenberg, 1994, 1996; Ridet *et al*., 1997; Aschner, 1998). In the brain, mature, quiescent astrocytes become ''reactive'' after injury and participate in formation of a glial scar, which does not support axonal regrowth (Ridet *et al*., 1997). The glial scar represents the scar tissue in the central nervous system. The major hallmark of the glial scar is the increased expression of GFAP by accumulation of hypertrophic astrocyte cell bodies and a thick network of processes (Hatten *et al*., 1991). Depending on the type of injury and the region of the central nervous system, glial scars also contain microglia, macrophages and other blood-borne cells, meningeal cells, fibroblasts, and oligodendrocytes. Newly formed blood vessels may also be present in the scars (Bartsch *et al*., 1992, 1994; Ajemian *et al*., 1994). Abundant ECM proteins are synthesized in the glial scars by reactive astrocytes or by invading meningeal cells (Hirsch and Bahr, 1999). Reactive astrocytes form a barrier around the injured neural area, isolating intact neural tissues from secondary lesions, which may also lead to inhibition of axonal growth.

By light microscopy, the cardinal morphologic features of reactive astrocytes are cellular hypertrophy, hyperplasia, and increased expression of GFAP and vimentin (Hatten *et al*., 1991; Eddleston and Mucke, 1993; Ridet *et al*., 1997). Studies on activity indicate that reactive astrocytes increase the synthesis of ECM macromolecules, cell adhesion molecules, and recognition molecules, a variety of growth factors and cytokines, and cell mediators and receptors. In humans, reactive astrocytes contribute to the pathogenesis of several neurodegenerative disorders including Alzheimer's disease (Tomimoto *et al*., 1997), schizophrenia (Arnold *et al*., 1995), AIDS dementia (Aschner, 1998), and acute injury (Brodkey *et al*., 1995). Reactive astrocytes exhibit regional variability of activities depending on the nature of the injury, the microenvironment at the injured site, and the distance to the injured site (Malhotra and Shnitka, 1994).

Recent studies in human glaucomatous optic neuropathy and in a rat model of elevated IOP have demonstrated synthesis of inducible nitric oxide synthase, NOS-2, by reactive astrocytes in the optic nerve head (Neufeld *et al*., 1997, 1999). NOS-2 is a powerful enzyme that generates excessive amounts of nitric oxide, which may be neurotoxic to the axons of the retinal ganglion cells (Neufeld, 1999b). These observations underscore the importance of the reactive astrocytes in the glaucomatous process.

3. Reactive Astrocyte Cytoskeleton

Changes in expression of intermediate filaments in the astrocyte cytoskeleton are characteristic of the transition of a quiescent astrocyte to the reactive phenotype Ridet. GFAP and vimentin are type III cytoplasmic intermediate filaments characteristic of astrocytes (Galou *et al*., 1997). Both proteins are developmentally regulated; e.g., in rodents, vimentin is expressed early in development in radial glia. In most astrocytes in the central nervous system, expression of vimentin ceases and GFAP expression begins at birth (Oblinger and Singh, 1993; Galou *et al*., 1996). Vimentin is expressed in immature and in reactive astrocytes, both of which are mobile cells (Galou *et al*., 1997; Takamiya *et al*., 1988). However, astrocytes in the optic nerve retain expression of both GFAP and vimentin in adulthood (Calvo *et al*., 1990). Nestin is a type IV intermediate filament that is expressed in multipotent neural progenitor cells and in reactive astrocytes rapidly after central nervous system injury, where it lasts for a prolonged period (Frisen *et al*., 1995). In addition to the transcriptional regulation of the expression of GFAP (Brenner, 1994) and vimentin in reactive astrocytes, posttranslational modifications of intermediate filaments, such as phosphorylation by protein kinases, are also part of the reorganization in reactive astrocytes (Harrison and Mobley, 1992).

Increased expression of GFAP by reactive astrocytes after neural injury is an early response that can occur in the absence

of astrocyte proliferation and is related to cell hypertrophy (Hatten *et al*., 1991). Once a glial scar is formed in the site of injury, GFAP levels decrease at the site of injury and astrocytes become quiescent. In moderate and advanced POAG in humans and in monkey eyes with chronically elevated IOP, increased immunoreactivity of GFAP was evident in astrocytes of the prelaminar region and lamina cribrosa, suggesting astrocyte reactivation. The tissue remodeling of the optic nerve head in glaucoma is associated with changes in distribution and cellular characteristics of astrocytes (Hernandez and Pena, 1997). The changes in distribution included loss of the glial columns in the prelaminar optic nerve and apparent migration of astrocytes into the nerve bundles in the lamina cribrosa (Fig. 35.4).

In addition to increased GFAP immunoreactivity, the cellular changes included hypertrophy of the cell body, thicker and longer cellular processes in the prelaminar region, and round-shape cell body and loss of processes in the lamina cribrosa (Fig 35.4) (Varela and Hernandez, 1997). Recently, specific markers for reactive astrocytes have been reported (Malhotra and Shnitka, 1994; Ridet *et al*., 1997). The monoclonal antibody J1-31 recognizes a protein associated with glial intermediate filaments in reactive astrocytes, which are adjacent to areas of neural tissue damage (Malhotra and Shnitka, 1994; Ridet *et al*., 1997). In addition, the cytoskeleton-associated stress protein, Hsp-27 (Renkawek *et al*., 1994; Kato *et al*., 1995), is also upregulated in reactive astrocytes proximal to a lesion. In human glaucoma, Hsp27 is upregulated in astrocytes in the optic nerve head (Tezel *et al*., 2000).

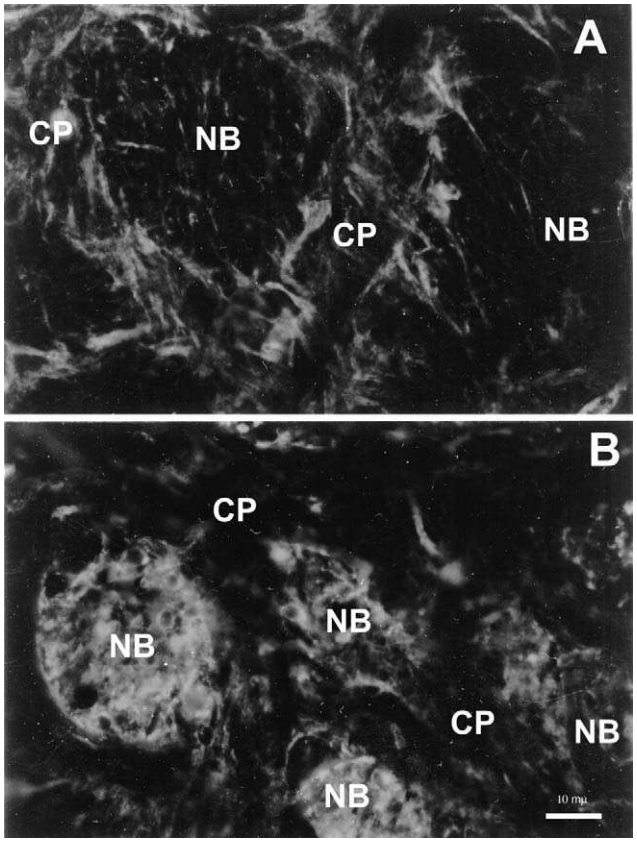

FIG. 35.4. Cross sections of the human lamina cribrosa immunostained with an antibody against GFAP. (A) In the normal lamina cribrosa, astrocytes stained with GFAP are located in the cribriform plates (CP) and extend processes into the nerve bundles (NB). (B) In the glaucomatous lamina cribrosa, astrocytes appear rounded and are located inside the nerve bundles (NB). Note that the cribriform plates are devoid of astrocytes and their processes.

4. Expression of Neural Cell Adhesion Molecule in Reactive Astrocytes

NCAMs are a family of cell surface glycoproteins that accomplish Ca^{2+}-independent adhesion, either through homophilic or heterophilic binding, between molecules on neighboring cells or between cell and ECM proteins. Changes in NCAM expression are considered a sign of reactive astrocytes (Le Gal La Salle *et al*., 1992; Lehmann *et al*., 1993; Jucker *et al*., 1995; Wolf *et al*., 1995; Roche *et al*., 1997; Cotman *et al*., 1998).

NCAMs are abundantly expressed in the central nervous system during development (Edelman, 1986a,b; Walsh and Doherty, 1997). A single gene encodes NCAMs but alternative splicing of the pre-mRNA produces at least three major isoforms, $NCAM_{180}$, $NCAM_{140}$, and $NCAM_{120}$ (Hemperly *et al*., 1990; Walsh and Doherty, 1996). These isoforms differ in the size of their cytoplasmic domains and in the mode of association to the membrane. $NCAM_{120}$ lacks a cytoplasmic domain and is attached to the membrane by a glycosylphosphatidylinositol moiety. $NCAM_{140}$ and $NCAM_{180}$ possess transmembrane and intracytoplasmic domains (Hemperly *et al*., 1990; Pan *et al*.,1992).

Previous studies have demonstrated that type 1B astrocytes constitutively express NCAM in the optic nerve head and also in culture (Ye and Hernandez, 1995; Kobayashi *et al*., 1997). In glaucoma, increased NCAM immunoreactivity is observed in most astrocytes in the lamina cribrosa and prelaminar regions, consistent with astrocyte reactivation (Varela and Hernandez, 1997). Recent studies in our laboratory indicate that $NCAM_{140}$ mRNA is constitutively expressed in normal optic nerve head, whereas $NCAM_{180}$ mRNA is the predominant isoform expressed in glaucomatous optic nerve head. *In situ* hybridization experiments localized $NCAM_{180}$ mRNA to reactive astrocytes in the glaucomatous tissue. (Ricard *et al*., 1999).

Dynamic changes in astrocytes are associated with changes in NCAM expression. $NCAM_{180}$ is more adhesive and associates with cytoskeletal molecules thus influencing cell anchoring and shape. $NCAM_{180}$ physically couples with the cytoskeleton, using actin-associated proteins such as vinculin and actinin, at the long cytoplasmic domain (Pollerburg *et al*., 1986, 1987). This may be relevant to differentiation of neural cells during central nervous system development and in adult central nervous system remodeling in response to injury (Hortsch *et al*., 1998). Furthermore, deletion of $NCAM_{180}$ exon 18 in mouse produces defects in cell migration in certain regions of the central nervous system including the retina (Tomasiewicz *et al*., 1993). Such interactions between NCAM and the cytoskeleton may be required for the migration of reactive astrocytes during optic nerve remodeling. Movement of astrocytes requires initial separation from neighboring cells and ECM substrate and subsequent adhesion and readhesion to the substrate during locomotion. We hypothesize that new, selective expression of the $NCAM_{180}$ isoform that permits

rapid changes in adhesive interactions is a necessary part of the transition of quiescent to reactive astrocyte.

5. Microglia

Microglia are a subtype of glia in the central nervous system that are activated in response to neuronal injury (Perry *et al*., 1995; Raivich *et al*., 1999; Streit *et al*., 1999). In normal tissue, microglia are quiescent and have a stellar shape with a small nucleus and a cell body with several ramified processes. Microglia are distributed in a regularly spaced array throughout neural tissue and are found within the perivascular space, surrounded by the glia limitans, and paravascular or encircling the glia limitans. There is currently some debate about whether the perivascular cells are truly microglia or monocytes. Microglia are also found in the central nervous system, not associated with blood vessels.

Recent studies in the human optic nerve head have identified the presence of quiescent microglia in normal optic nerve heads and of activated microglia in the glaucomatous nerve (Neufeld, 1999a). Stellate cells with thin, ramified processes, positive for HLA-DR and CD-45 but negative for GFAP, were identified as quiescent microglia in the human optic nerve head. These cells were found throughout the normal optic nerve head in the walls of large blood vessels and surrounding capillaries in the glial columns and the cribriform plates (Fig. 35.5). In glaucomatous eyes with moderate and severe optic nerve head damage, microglia were present as clusters of large ameboid, activated cells in the compressed lamina cribrosa and as formations of concentric circles surrounding blood vessels. In the parapapillary chorioretinal region of glaucomatous optic nerve heads, large, activated microglia were present as single cells or clusters on the termination of Bruch's membrane (Fig 35.5). Activated microglia, as clusters of cells in the parapapillary chorioretinal region, appear to form a discontinuous, linear barrier in the parenchyma near the vassels of the choriocapillaris bordering the neural tissue. The parapapillary chorioretinal region is considered a potential defect in the blood–retinal barrier. In addition, along the optic nerve/choriocapillaris–scleral interface, activated microglia appeared to form liner arrays near the choriocapillaris vessels. These cells were parenchymal and not in close association with the vasculature.

In glaucomatous tissue, activated microglia appear to be strategically positioned in relationship to blood vessels and the blood–retina barrier. In medium-sized vessels in the compressed prelaminar region, activated microglia form concentric rings in the parenchyma around the vasculature. As the glaucomatous optic nerve becomes compressed, disorganized, and remodeled, blood vessels may become leaky and compromise the blood–retinal barrier. Also, the distribution and activation of microglia in the parapapillary chlorioretinal region of glaucomatous eyes may provide, at least in part, a cellular explanation for the progressive parapapillary chlorioretinal atrophy that has been seen biomicroscopically, described clinically, and associated, by many authors, with glaucoma (Fantes and Anderson, 1989; Jonas and Grundler, 1996; Nicolela and Drance, 1996).

III. Mechanisms of Optic Nerve Damage

A. Role of Elevated Intraocular Pressure

Elevated intraocular pressure remains the most prominent risk factor in the development and progression of glaucoma. In normal individuals *in vivo*, tissues of the lamina cribrosa of the optic nerve head are exposed to a hydrostatic pressure gradient between the intraocular compartment IOP and the retrolaminar tissue pressure (Morgan *et al*., 1998). This hydrostatic pressure gradient fluctuates normally, a small amount, due to the ocular pulse and the diurnal changes in IOP (Zeimer *et al*., 1991). In glaucoma, there are elevated IOP as well as daily fluctuations and spikes of IOP. Under such conditions, the lamina cribrosa undergoes significant deformation in response to changes in IOP, which generates biomechanical stress on astrocytes and other cell types of the lamina cribrosa (Zeimer and Chen, 1987; Zeimer and Ogura, 1989; Burgoyne *et al*., 1995). *In vivo*, the nature of the IOP-related biomechanical stress that affects astrocytes or retinal ganglion cell axons is unknown but shear, tensile, or compressive forces may be involved. Similarly, the mechanisms by which IOP-related

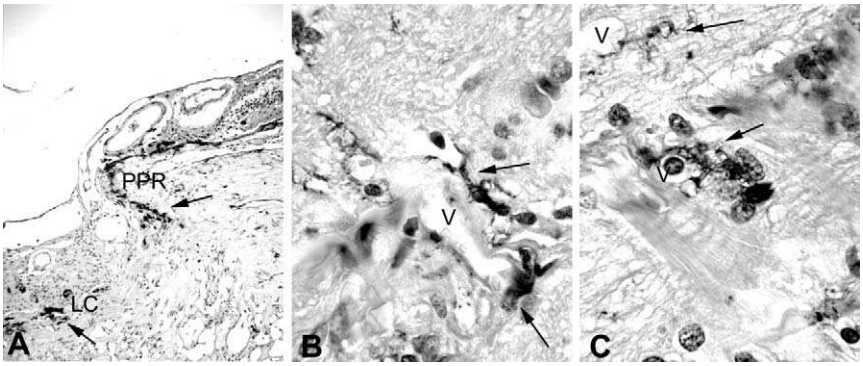

FIG. 35.5. Microglial cells in the human optic nerve head. Microglia cells were stained with anti-human HLA-DR antibody and detected with HRP. (A) Glaucomatous optic nerve head showing clusters of microglia in areas of degeneration in the lamina cribrosa (LC) and in the peripapillary region (PPR). Note the accumulation of microglia along Bruch's membrane (BM) (×150). (B) Perivascular microglia, shown at higher magnification, in an optic nerve head with glaucoma (arrows). Note the characteristic "spiny" processes of microglial cells (×1200). V, blood vessel. (C) Perivascular microglia in an age-matched normal optic nerve head (arrows). Note that the perivascular microglia are similar to perivascular microglia shown in B (×1200). From Hernandez (2000).

stress affects optic nerve head astrocytes leading to the reactive astrocyte phenotype, optic nerve head remodeling, and loss of retinal ganglion cell axons in glaucoma remain elusive.

Many other cellular functions may be affected by IOP-related physical stress including cell adhesion, transmembrane transport, and RNA processing (Wright *et al*., 1992; Maniotis *et al*., 1997; Kaarniranta *et al*., 1998). *In vitro* studies on cells of the optic nerve head may contribute to the understanding of the impact of abnormally elevated IOP in the pathophysiology of glaucomatous neuropathy.

B. Role of the Vasculature in Glaucoma

Although there is overwhelming clinical and experimental evidence that elevated IOP is a critical player in the structural changes and in retinal cell ganglion cell loss in glaucomatous optic neuropathy, a subset of patients develop glaucomatous changes at normal levels of IOP, a condition called normal-tension glaucoma (Werner, 1996). Investigators in the field point out several examples of glaucomatous damage that cannot be explained by the IOP theory: In Japan, the average IOP tends to decrease with age, in contrast in the West, where IOP levels tend to be higher in the aging population. However, the frequency of glaucomatous optic neuropathy in the Japanese population is similar to that seen in the West. There are more females than males affected by normal-tension glaucoma despite having similar IOP levels. Between blacks and whites, the levels of IOP are similar; however, the prevalence of glaucomatous optic neuropathy is four times higher in blacks (Flammer, 1994). To account for these differences, hemodynamic factors, such as ocular blood flow, decreased blood pressure, and increased prevalence in individuals suffering from cardiac ischemia have been investigated. Blood flow in the optic nerve head may be altered as a consequence of optic nerve tissue loss secondary to chronic elevation of IOP.

The nature and basis for physiological control and pathological mechanisms in the optic nerve head and related retinal circulation are unknown. However, the vasculature in the retina and optic nerve head appears to autoregulate to maintain blood flow, despite changes in perfusion pressure (Anderson, 1996). A deficit in blood flow autoregulation leading to transient ischemia may cause increased susceptibility to the damaging effects of IOP (Cioffi and Van Buskirk, 1998). Microvascular factors such as the endothelins, a family of potent long-acting vasoconstrictor peptides, are produced by the vascular endothelium to control vascular tone and regulate blood flow. Endothelin-1 may play a role in the autoregulation of blood flow of the retina and the optic nerve head (Cioffi *et al*., 1995; Oku *et al*., 1999). Endothelin-1 may cause hypoxia due to local ischemia and thereby contribute to tissue damage in glaucoma.

Hypoxia due to ischemia and decreased vascular perfusion has been proposed as a factor leading to axonal damage, retinal ganglion cell loss, and remodeling of the optic nerve head in glaucoma. Due to the clinical relevance of ischemic brain damage the effects of hypoxia on central nervous system neurons and astrocytes *in vivo* and in culture have been studied extensively (Hori *et al*., 1994; Silver *et al*., 1997; Krasney, 1999). Recent studies have demonstrated that cells respond to hypoxia via cellular oxygen sensors that regulate glycolytic genes and the cascades that regulate vascular perfusion (Semenza *et al*., 1997). To date, the role of hypoxia in the optic nerve head in glaucoma has not been defined on a cellular level. Hypoxia causes increases in ECM synthesis, gene expression of growth factors and cytokines, release of mediators, cellular activity, and neovascularization in many systems including normal and diseased ocular tissues (Shima *et al*., 1995). Hypoxia is thought to be a key regulator of angiogenesis and subsequent neovascularization (D'Amore, 1994; Aiello *et al*., 1995). Because neovascularization does not occur in glaucomatous optic neuropathy, astrocytes and vascular cells in the optic nerve head may be inhibited from making certain responses to hypoxia.

IV. Experimental Studies Relevant to Glaucomatous Optic Neuropathy

A. Animal Models

1. Primate Models of Glaucoma

Work in primates has provided most of the experimental data in glaucomatous optic neuropathy. The monkey model of laser-induced elevated IOP is probably the animal model most closely reflecting POAG, which is unique to humans. The model is based on raising IOP by argon or diode laser photocoagulation of the trabecular meshwork, resulting in decreased outflow of aqueous humor (Quigley and Hohman, 1983; Pederson and Gaasterland, 1984; Wang *et al*., 1998). The relationship between elevated IOP and cupping of the optic disc was demonstrated in a primate model of glaucoma in the early 1980s in Quigley's laboratory. Recently, two more laboratories have reported extensive studies on the histopathological changes in the optic nerve head in the monkey model of experimental glaucoma, confirming the validity of the model (Hayreh *et al*., 1999; Jonas and Hayreh, 1999; Yucel *et al*., 1999). Clinical evaluation of the model has shown a linear correlation between axonal loss and increase in cup/disc ratio (Varma *et al*., 1992). However, measurement of finer parameters of visual functions using perimetry using either a monochromatic or white stimuli indicated that the model does not provide information on retinal ganglion cell loss until a large number have died (Harwerth *et al*., 1999).

Experimental glaucoma in monkeys with chronically elevated IOP demonstrated the axonal transport was slowed down at the level of the lamina cribrosa. The retinal ganglion cells projecting to the magnocellular layer of the lateral geniculate nucleus appeared to be affected earlier in the disease (Dandona *et al*., 1991). Recently, it was shown that the pattern of degeneration of midget and parasol retinal ganglion cell in glaucoma was initiated with the reduction of the dendritic fields and ended with the decrease in size of the soma, suggesting that early functional deficits and retinal ganglion cell atrophy (Weber *et al*., 1998). The challenge of this work is to develop early detection systems that will identify patients in early stages of the disease in which neuroprotection is beneficial.

Glutamate excitotoxicity has been postulated as one of the possible factors leading to retinal ganglion cell loss in glaucoma. Elevated glutamate levels were found in the vitreous of monkeys with experimental glaucoma, suggesting that excitatory glutamate excitotoxicity may play a role on retinal

ganglion cell loss in glaucoma (Dreyer *et al*., 1996). Additional studies using the monkey model of glaucoma have shown that retinal ganglion cell populations labeled with antibodies to the neurofilament triplet were more affected by elevated IOP in glaucoma whereas amacrine, horizontal, and bipolar neurons were not affected. Large retinal ganglion cells located in the periphery of the nasal and temporal retina were lost preferentially in this study (Vickers *et al*., 1995). More recently, loss of retinal ganglion cells expressing GluR2 and NMDAR1 glutamate receptors was reported in monkey eyes with elevated IOP; however, there was no difference between retinal ganglion cells in labeling between different populations of retinal ganglion cells (Hof *et al*., 1998). The authors concluded that loss of neurofilaments by a particular population of retinal ganglion cells was a better marker of vulnerability to glaucomatous damage than expression of glutamate receptor subtype.

2. Rat Models of Glaucoma

Recently, two laboratories (Garcia-Valenzuela *et al*., 1995; Moore *et al*., 1995) reported methods for chronically increasing IOP in rat eyes to moderately elevated levels and have observed optic nerve degeneration and retinal ganglion cell loss. In one method, hyperosmotic solution is injected into the limbal venous plexus (Moore *et al*., 1995) in the other method, limbal derived veins are cauterized (Garcia-Valenzuela *et al*., 1995). These methods produce similar elevations of IOP and have demonstrated loss of nerve fibers from the optic nerve, changes in the extracellular matrix of the optic nerve head, and degeneration of retinal ganglion cells at moderately elevated IOP.

In a series of more than 130 animals, some observed over a 6-months period, we have found that an approximately 1.6-fold elevation of IOP causes a rate of retinal ganglion cell degeneration in the peripheral retina of approximately 1.4%/week. On the other hand, we have found less change of retinal ganglion cell density in central retinal areas over the 6-month period. Cupping of the optic disk was observable by ophthalmoscopy in these animals (Neufeld *et al*., 1999).

3. Inherited Glaucoma in Mouse

The use of mice as a glaucoma model is being tested (John *et al*., 1991). Measuring IOP in mice has been a major obstacle in the use of the model (Chang *et al*., 1999). John *et al*. identified mice strains that exhibit elevated IOP. The DBA/2J (D2) mice develop spontaneous age-related secondary glaucoma with elevated IOP associated with severe iries atrophy and anterior synechiae formation (Hawes *et al*., 1999). Optic nerve atrophy and retinal ganglion cell death occur in a substrain of DBA/2 mice. The difficulty of these experiments is that the IOP has to be measured invasively by cannulation of the anterior chamber of the eye. Ideally a noninvasive IOP measurement would allow performing longitudinal sudies in these animals.

B. Cell Culture Models

1. Retinal Ganglion Cell Culture

Studies on the mechanisms necessary for retinal ganglion cell survival are critical to developing neuroprotective therapies in glaucomatous optic neuropathy. Several laboratories have successfully accomplished purification and maintenance in culture of rat retinal ganglion cells isolated from developing postnatal retinas (Barres *et al*., 1988; Jo *et al*., 1999; Morgan *et al*., 1999). Recently, an *in vitro* study using cultured retinal ganglion cells confirmed *in vivo* observations that nitric oxide production from astrocytes was involved in retinal ganglion cell axonal loss in a rat model of glaucoma (see below). Anoxia-induced retinal ganglion cell death was significantly blocked by *N*-nitro-L-arginine, an inhibitor of NOS, in cocultures of rat retinal ganglion cells and astrocytes (Morgan *et al*., 1999).

Potentially relevant studies to glaucoma are those on retinal ganglion cell survival and responsiveness to trophic factors. Recent studies reported that retinal ganglion cell responsiveness to trophic factors such as BDNF, CNTF, and IGF-1 was enhanced by increasing the levels of cAMP in cultured purified retinal ganglion cells and subsequently in retinal ganglion cells *in vivo*. These studies demonstrated that retinal ganglion cells die after axotomy not only because of deprivation of trophic factor stimulation but because of concomitant loss of responsiveness to trophic factors due to loss of trophic receptors (Shen *et al*., 1999). The implication is that neuroprotective therapies for retinal ganglion cells using trophic factors must take in account that retinal ganglion cell death in glaucoma is probably due to a combination of events, which may or may not include deprivation of neurotrophic factors.

2. Optic Nerve Head Astrocyte Culture

Cultured astrocytes from the rodent optic nerve are used in many laboratories to study many aspects of astrocyte cell biology and physiology that may impact optic nerve regeneration, myelination, astrocyte axon interactions, etc. Astrocytes in the central nervous system and in the optic nerve mediate responses to injury and to chronic neurodegenerative disease. Glaucomatous optic neuropathy is not an exception and different aspects of astrocyte behavior need to be characterized. *In vitro* models using optic nerve head astrocytes from humans provide such a model system. Well-characterized astrocyte culture methods from humnan optic nerve head have become available and cultured astrocytes can be generated in sufficient numbers to perform experiments. The optic nerve head astrocyte cultures express cellular markers present *in situ* such as GFAP and NCAM (Kobayashi *et al*., 1997).

Researchers who study stress forces on cells have postulated that because of the interconnections among the ECM, cell attachments and the cytoskeleton, a physical deformation in the ECM triggers cellular responses by activating signaling cascades and gene expression (Banes *et al*., 1995; Chiquet *et al*., 1996; Galou *et al*., 1997; Ingber, 1997). For example, hydrostatic pressure, when applied as a mechanical stress to cells in culture results in deformation of the cell membrane and cytoskeleton due to the fixed cellular adhesions to the matrix, causing activation of mechanosensitive ion channels and signaling enzymes sequestered in the membrane (Bray *et al*., 1991; Zucca *et al*., 1991; Acevedo *et al*., 1993; Yang *et al*., 1993; Klein-Nulend *et al*., 1995, 1997; Schwartz *et al*., 1999). Thus, a mechanical signal at the cell membrane is transduced into an intracellular event (Maniotis *et al*., 1997). Biomechanical stress may alter the kinases associated with the

ECM–integrin–cytoskeleton system to trigger responses that change cell behavior and phenotype. In a variety of cell types, cell surface ion channels are activated or inactivated in response to mechanical tension on the membrane (Banes *et al*., 1995; Verkhratsky and Kettenmann, 1996; Sachs, 1997). At least five mechanically responsive channels have been described in astrocytes by patch clamp experiments (Bowman *et al*., 1992).

Studies indicate that increased production of prostaglandin E_2, prostacyclin, or cAMP may mediate the effects of compressive pressure on cells in culture (He and Grinnell, 1994; Klein-Nulend *et al*., 1995; Yousefian *et al*., 1995; Glantschinig *et al*., 1996). Increased synthesis of cell adhesion molecules, such as I-CAM and integrins, has been reported in response to a variety of mechanical stimuli including hydrostatic pressure (Haskin and Cameron, 1993; Davies, 1995; Resnick and Gimbrone, 1995; Salwen *et al*., 1998). Cell surface adhesion molecules have been proposed to participate in the coupling of cells to the ECM or between neighboring cells, with the cell cytoskeleton allowing mechanical stress to be transmitted intracellularly (Banes *et al*., 1995; Ingber, 1997), thus activating signaling cascades and turning on new gene expression (Bowman *et al*., 1992; Verkhratsky and Kettenmann, 1996; Oishi *et al*., 1998). The gene for ICAM-1 has a specific sequence in the promoter region, which responds to mechanical stimulation. Similar sequences have been found in the promoter region of other genes relevant to vascular pathophysiology, such as TGF-β, PDGF, and tissue plasminogen activator (Resnick *et al*., 1993).

Astrocytes in the lamina cribrosa are attached to the fibroelastic ECM and to neighboring astrocytes by cell surface adhesion molecules, which, through their transmembrane domains, are connected to the cytoskeleton. Thus, ECM changes in the microenvironment can be transmitted into the intracellular compartment. Because the cortical cytoskeleton is coupled to cell adhesion molecules, to membrane-bound enzymes, and to ion channels, biomechanical stress will cause intracellular responses in astrocytes.

Rearrangement of the actin cytoskeleton in response to hydrostatic pressure or other forms of mechanical stress occurs in many cell types (Acevedo *et al*., 1993; Haskin and Cameron, 1993; Sumpio *et al*., 1994; Crenshaw *et al*., 1996; Smith *et al*., 1996; Oluwole *et al*., 1997). The effect of pressure on actin stress fibers suggests depolymerization of filamentous actin, which has been described in cultured chondrocytes exposed to hydrostatic pressure and in cultured astrocytes in response to cyclic AMP (Parkkinen *et al*., 1995; Yousefian *et al*., 1995; Wright *et al*., 1996). In addition, we have recently demonstrated increased adenyl cyclase activity and disorganization of the actin cytoskeleton in optic nerve head astrocytes exposed to hydrostatic pressure (Wax *et al*., 2000).

V. Retinal Ganglion Cell Degeneration in Glaucoma

A. Neuronal Cell Death by Apoptosis

Various animal models, particularly those with rats and mice, have been used to study the degeneration of retinal ganglion cells. A large number of studies using optic nerve transection, optic nerve crush, and chronic, moderately elevated intraocular pressure, a glaucoma model, all indicate that retinal ganglion cells die by apoptotic mechanisms (Garcia-Valenzuela *et al*., 1995; Quigley *et al*., 1995). By examining human retinas, Quigley observed a relatively small, but nevertheless greater, number of TUNEL-positive retinal ganglion cells in glaucomatous compared to normal eyes (Kerrigan *et al*., 1997). The small number of positive cells is not surprising considering the slow progression of the disease. In tissue sections taken for pathology, there is a notable absence of inflammatory cells in glaucomatous optic nerves and retinas. Most investigators in the field believe that it is reasonable to hypothesize that the death of retinal ganglion cells in glaucoma is by apoptosis.

B. Glutamate Excitotoxicity

Glutamate is a neurotransmitter throughout the CNS and is the major neurotransmitter used by the neurons of the retina. In a variety of *in vitro* and animal models of neurodegeneration, excessive glutamate leads to apoptosis and necrosis of neurons. Similar results using *in vitro* and animal models of ischemia and optic nerve transection have been observed in the retina. Indeed, the first demonstration of glutamate neurotoxicity by Olney (1969), which led to the coining of the term ''excitotoxicity,'' was based on observations of retinal tissue, particularly the retinal ganglion cells.

Over the past few years, glutamate excitotoxicity has been implicated as a component cause of the loss of retinal ganglion cells in glaucoma. Dreyer originally demonstrated that glutamate was elevated in the vitreous humor of glaucoma patients collected at the time of surgery compared to similarly collected samples from patients that did not have glaucoma (Dreyer *et al*., 1996). Although the work in humans has not been confirmed, animal models (monkey, cat, rabbit, dog, and rat) of elevated IOP in which there is loss of retinal ganglion cells are associated with elevated glutamate in the vitreous humor (Vorwerk *et al*., 1999).

The origin of increased levels of glutamate in glaucomatous eyes is unknown. Glutamate could be released from dying retinal ganglion cells or there may be, in glaucomatous eyes, a malfunction of one of the glutamate uptake systems that normally keeps extracellular glutamate levels low. Whether such elevated glutamate could contribute in a primary or secondary manner to the death of retinal ganglion cells over several decades as glaucomatous damage progresses remains to be determined. Nevertheless, there is an ongoing pharmaceutical clinical trial with memantine, a glutamate receptor antagonist, to determine whether this drug can accomplish neuroprotection in the treatment of glaucoma.

C. Nitric Oxide Synthase

Nitric oxide is formed from L-arginine by nitric oxide synthase (NOS). Molecular cloning has identified three distinct genes expressing NOS isoforms: neuronal NOS (nNOS or NOS-1), endothelial NOS (eNOS or NOS-3), and inducible NOS (iNOS or NOS-2). The nomenclature was derived from the tissue in which they were first studied and the numbering was derived from the order in which they were cloned (Bredt and Snyder, 1994). NOS-1 and NOS-3 are constitutive, present

physiologically and calcium dependent. Under conditions of degeneration and inflammation, both isoforms may be upregulated. In contrast, NOS-2 is not constitutive, is Ca^{2+} independent, and is induced after immunological challenge and neuronal injury (Dawson *et al*., 1993; Yun *et al*., 1996).

The constitutive isoforms of NOS, NOS-1, and NOS-3 are present in the normal and glaucomatous human optic nerve heads. In contrast, the inducible isoform, NOS-2, appears in the optic nerve head of patients with primary open-angle glaucoma (Neufeld *et al*., 1997). The NOS-2 found in glaucomatous optic nerve tissue is predominantly in reactive astrocytes (Liu and Neufeld, 2000). These findings suggest induction of NOS-2 in glaucoma. Excessive NO, synthesized by NOS-2, may be neurodestructive through a peroxynitrite mechanism in the optic nerve heads of patients with glaucoma (Neufeld, 1999b). The presence of NOS-2 in reactive astrocytes in glaucomatous tissue is consistent with the optic nerve head being an early site of focal neuronal degeneration of the axons of the retinal ganglion cells in glaucoma.

The NOS-2 isoform is also induced in the rat optic nerve head in an animal model of chronic, moderately elevated IOP and is not present at all in normal tissue (Shareef *et al*., 1999). In a 6-month pharmacological experiment using this rat model of glaucoma, aminoguanidine, a relatively specific inhibitor of NOS-2 which had no effect on the elevated IOP, significantly blocked the loss of retinal ganglion cells, the cupping of the optic disk, and the degeneration of axons in the optic nerve (Neufeld *et al*., 1999).

D. Neurotrophic Factors

The survival of any nerve cell is believed to be dependent on neurotrophic factors, such as BDNF and CNTF. This is certainly true for embryonic, neonatal, and cultured nerve cells and has been postulated, and in some cases demonstrated, for adult neurons *in vivo* (Barde, 1989). Neurotrophic factors are released by postsynaptic neurons, bind to specific trk receptors in the nerve terminals, and are carried by their receptors, via retrograde axoplasmic transport, to the cell soma. There is also anterograde axoplasmic transport of neurotrophic factors and other important cellular components from cell soma to nerve terminals.

The axons of the retinal ganglion cells form synapses with neurons in the superior colliculus, the lateral geniculate nucleus, suprachiasmatic nucleus, and the pretectal nuclei and, therefore, may receive neurotrophic factors from neurons in these regions of the CNS. In glaucoma, compression of the axons of the retinal ganglion cells at the level of the lamina cribrosa may cause a blockade of axoplasmic transport and thus deprive these neurons of their target derived neurotrophic factors. Retinal ganglion cell degeneration in glaucoma may occur by apoptosis in response to deprivation of neurotrophic factors (Quigley *et al*., 1995). In support of this hypothesis, there is a large body of work demonstrating enhanced survival of retinal ganglion cells following axotomy in the rat when exogenous neurotrophic factors are administered (Mansour-Robaey *et al*., 1994). Nevertheless, there is no supporting evidence for deprivation of neurotrophic factors as a causative factor in human glaucoma. Recently, loss of NT4/5 and BDNF immunostaining was reported in the optic nerve head and retina in a rat model of glaucoma, suggesting interruption of axonal transport at the level of the nerve head or loss of endogenous production of neurotrophins (Johnson *et al*., 2000).

References

Acevedo, A., Bowser, S., Gerritsen, M., and Bizios, R. (1993). Morphological and profliferative responses of endothelial cells to hydrostatic pressure: Role of fibroblast growth factor. *J. Cell Physiol.* **157**, 603–614.

Aiello, L. P., Northrup, J. M., Keyt, B. A., Takagi, H., and Iwamoto, M. A. (1995). Hypoxic regulation of vascular endothelial growth factor in retinal cells. *Arch. Ophthalmol. (Chicago)* **113**, 1538–1544.

Ajemian, A., Ness, R., and David, S. (1994). Tenascin in the injured rat optic nerve and in non-neuronal cells in vitro: Potential role in neural rapair. *J. Comp. Neurol.* **340**, 233–242.

Alward, W. L., Johnson, A. T., Nishimura, D. Y., Sheffield, V. C., and Stone, E. M. (1996). Molecular genetics of glaucoma: Current status. *J. Glaucoma* **5**, 276–284.

Alward, W. L., Fingert, J. H., Coote, M. A., Johnson, A. T., Lerner, S. F., Junqua, D., Durcan, F. J., McCartney, P. J., Mackey, D. A., Sheffield, V. C., and Stone, E. M. (1998). Clinical features associated with mutations in the chromosome 1 open-angle glaucoma gene (GLC1A). *N. Engl. J. Med.* **338**, 1022–1027.

Anderson, D. A. (1969). Ultrastructure of human and monkey lamina cribrosa and optic nerve head. *Arch. Ophthalmol. (Chicago)* **82**, 800–814.

Anderson, D. A. (1996). Glaucoma, capillaries and pericytes. 1. Blood flow regulation. *Ophthalmologica* **210**, 257–262.

Arnold, S., Franz, B., Trojanowski, J., Moberg, P., and Gur, R. (1995). Glial fibrillary acidic protein-immunoreactive astrocytosis in elderly patients with schizophrenia and dementia. *Acta Neuropathol.* **91**, 269–277.

Aschner, M. (1998). Astrocytic functions and physiological reactions to injury: The potential to induce and/or exacerbate neuronal dysfunction—a forum position paper. *NeuroToxicology* **19**, 7–18.

Banes, A. J., Tsuzaki, M., and Yamamoto, J. (1995). Mechanoreception at the cellular level: The detection intrpretation, and diversity of responses to mechanical signals. *Biochem. Cell Biol.* **73**, 349–365.

Barde, Y. A. (1989). Trophic factors and neuronal survival. *Neuron* **2**, 1525–1534.

Barres, B. A., Silverstein, B. E., Corey, D. P., and Chun, L. L. (1988). Immunological, morphological, and electrophysiological variation among retinal ganglion cells purified by panning. *Neuron* **1**, 791–803.

Bartsch, U., Bartsch, S., Dorries, U., and Schachner, M. (1992). Immunohistological localization of tenascin in the developing and lesioned adult mouse optic nerve. *Eur. J. Neurosci.* **4**, 338–352.

Bartsch, U., Faissner, A., Trotter, J., Dörries, U., Bartsch, S., Mohajeri, H., and Schachner, M. (1994). Tenascin demarcates the boundary between the myelinated and nonmyelinated part of retinal ganglion cell axons in the developing and adult mouse. *J. Neurosci.* **14**, 4756–4768.

Bowman, C. L., Ding, J. P., Sachs, F., and Sokabe, M. (1992). Mechanotransducing ion channels in astrocytes. *Brain Res.* **584**, 272–286.

Bray, D., Money, N. P., Harold, F., and Bamburg, J. R. (1991). Responses of growth cones to changes in osmolality of the surrounding medium. *J. Cell Physiol.* **98**, 507–515.

Bredt, D. S., and Snyder, S. H. (1994). Nitric oxide: A physiologic messenger molecule. *Annu. Rev. Biochem.* **63**, 175–195.

Brenner, M. (1994). Structure and transcriptional regulation of the GFAP gene. *Brain Pathol.* **4**, 245–257.

Brodkey, J. A., Laywell, E. D., OBrien, T. F., Faissner, A., Stefansson, K., Dorries, H. U., Schachner, M., and Steindler, D. A. (1995). Focal brain injury and upregulation of a developmentally regulated extracellular matrix protein. *J. Neurosurg.* **82**, 106–112.

Burgoyne, C. F., Quigley, H. A., Thompson, H. W., Vitale, S., and Varma, R. (1995). Early changes in optic disc compliance and surface position in experimental glaucoma. *Ophthalmology* **102**, 1800–1809.

Calvo, J. L., Carbonell, A. L., and Boya, J. (1990). Coexpression of vimentin and glial fibrillary acidic protein in astrocytes of the adult rat optic nerve. *Brain Res.* **532**, 355–357.

Chang, B., Smith, R. S., Hawes, N. L., Anderson, M. G., Zabaleta, A., Savinova, O., Roderick, T. H., Heckenlively, J. R., Davisson, M. T., and John, S. W. (1999). Interacting loci cause severe iris atrophy and glaucoma in DBA/2J mice. *Nat. Genet.* **21**, 405–409.

Chiquet-Ehrismann, R., Tannheimer, M., Koch, M., Brunner, A., Spring, J., Martin, D., Baumgartner, S., and Chiquet, M. (1994). Tenascin-C expression by fibroblasts is elevated in stressed collagen gels. *J. Cell Biol.* **127**, 2093–2101.

Chiquet, M., Matthisson, M., Koch, M., Tannheimer, M., and Chiquet, E. R. (1996). Regulation of extracellular matrix synthesis by mechanical stress. *Biochem. Cell Biol.* **74**, 737–744.

Cioffi, G. A. and Van Buskirk, E. M. (1998). Microvasculature of the anterior optic nerve. *Surv. Ophthalmol.* **38**, S107–S116.

Cioffi, G. A., Orgul, S., Onda, E., Bacon, D. R., and Van Buskirk, E. M. (1995). An in vivo model of chronic optic nerve ischemia: The dose-dependent effects of endothelin-1 on the optic nerve microvasculature. *Curr. Eye Res.* **14**, 1147–1153.

Cotman, C. W., Hailer, N. P., Pfister, K. K., Soltesz, I., and Schachner, M. (1998). Cell adhesion molecules in neural plasticity and pathology: Similar mechanisms, distinct organizations? *Prog. Neurobiol.* **55**, 659–669.

Crenshaw, H. C., Allen, J. A., and Skeen, V. (1996). Hydrostatic pressure has different effects on the assembly of tubulin, actin, myosin II, vinculin, talin vimentin, and cytokeratin in mammalian tissue cells. *Exp. Cell Res.* **227**, 285–297.

D'Amore, P. A. (1994). Mechanisms of retinal and choroidal neovascularization. *Invest. Ophthalmol. Visual Sci.* **35**, 3974–3979.

Dandona, L., Hendrickson, A., and Quigley, H. A. (1991). Selective effects of experimental glaucoma on axonal transport by retinal ganglion cells to the dorsal lateral geniculate nucleus. *Invest. Ophthalmol. Visual Sci.* **32**, 1593–1599.

Davies, P. F. (1995). Flow-mediated endothelial mechanotransduction. *Physiol. Rev.* **75**, 519–560.

Dawson, V. L., Dawson, T. M., Bartley, D. A., Uhl, G. R., and Snyder, S. H. (1993). Mechanisms of nitric oxide-mediated neurotoxicity in primary brain cultures. *J. Neurosci.* **13**, 2651–2661.

Dreyer, E. B., Zurakowski, D., Schumer, R. A., Podos, S. M., and Lipton, S. A. (1996). Elevated glutamate levels in the vitreous body of humans and monkeys with glaucoma. *Arch. Ophthalmol. (Chicago)* **114**, 299–305.

Eddleston, M., and Mücke, L. (1993). Molecular profile of reactive astrocytes—implications for their role in neurologic disease. *Neuroscience* **54**, 15–36.

Edelman, G. M. (1986a). Cell adhesion molecules in the regulation of animal form and tissue pattern. *Annu. Rev. Cell Biol.* **2**, 81–116.

Edelman, G. M. (1986b). Cell adhesion molecules in neural histogenesis. *Annu. Rev. Physiol.* **48**, 417–430.

Elkington, A. R., Inman, C. B., Steart, P. V., and Weller, R. O. (1990). The structure of the lamina cribrosa of the human eye: An immunocytochemical and electron microscopical study. *Eye* **4**, 42–57.

Faden, A. I. (1993). Experimental neurobiology of central nervous system trauma. *Crit. Rev. Neurobiol.* **7**, 175–186.

Fantes, F. E. and Anderson, D. R. (1989). Clinical histologic correlation of human peripapillary anatomy. *Ophthalmology* **96**, 20–25.

Fingert, J. H., Heon, E., Liebmann, J. M., Yamamoto, T., Craig, J. E., Rait, J., Kawase, K., Hoh, S. T., Buys, Y. M., Dickinson, J., Hockey, R. R., Williams-Lyn, D., Trope, G., Kitazawa, Y., Ritch, R., Mackey, D. A., Alward, W. L., Sheffield, V. C., and Stone, E. M. (1999). Analysis of myocilin mutations in 1703 glaucoma patients from five different populations. *Hum. Mol. Genet.* **8**, 899–905.

Fitch, M. and Silver, J. (1997). Glial cell extracellular matrix: Boundaries for axon growth in development and regeneration. *Cell Tissue Res.* **290**, 379–384.

Flammer, J. (1994). The vascular concept of glaucoma. *Surv. Ophthalmol.* **38** (Suppl.), S3–S6.

Frisen, J., Johansson, C., Torok, C., Risling, M., and Lendahl, U. (1995). Rapid, widespread, and longlasting induction of nestin contributes to the generation. *J. Cell Biol.* **131**, 453–464.

Fukuchi, T., Sawaguchi, S., Hara, H., Shirakashi, M., and Iwata, K. (1992). Extracellular matrix changes of the optic nerve lamina cribrosa in monkey eyes with experimental chronic glaucoma. *Albrecht von Graefe's Arch. Klin. Exp. Ophthalmol.* **230**, 421–427.

Fukuchi, T., Sawaguchi, S., Yue, J. T., Iwata, K., Hara, H., and Kaiya, T. (1994). Sulfated proteoglycans in the lamina cribrosa of normal monkey eyes and monkey eyes with laser-induced glaucoma. *Exp. Eye Res.* **58**, 231–244.

Galou, M., Colucci, G. E., Ensergueix, D., Ridet, J. L., Gimenez Y Ribotta, M., Privat, A., Babinet, C., and Dupouey, P. (1996). Disrupted glial fibrillary acidic protein network in astrocytes from vimentin knockout mice. *J. Cell Biol.* **133**, 853–863.

Galou, M., Gao, J., Humbert, J., Mericskay, M., Li, Z., Paulin, D., and Vicart, P. (1997). The importance of intermediate filaments in the adaptation of tissues to mechanical stress: Evidence from gene knockout studies. *Biol. Cell* **89**, 85–97.

Garcia-Valenzuela, E., Shareef, S., Walsh, J., and Sharma, S. C. (1995). Programmed cell death of retinal ganglion cells during experimental glaucoma. *Exp. Eye Res.* **61**, 33–44.

Glantschinig, H., Varga, F., Rumpler, M., and Klaushofer, K. (1996). Prostacyclin (PGI_2): A potential mediator of c-fos expression induced by hydrostatic pressure in osteoblastic cells. *Eur. J. Clin. Invest.* **26**, 544–548.

Gong, H., Ye, W., Freddo, T. F., and Hernandez, M. R. (1997). Hyaluronic acid in the normal and glaucomatous optic nerve. *Exp. Eye Res.* **64**, 587–595.

Harrison, B. and Mobley, P. (1992). Phosphorylation of glial fibrillary acidic protein and vimentin by cytoskeletal-associated intermediate filament protein kinase activity in astrocytes. *J. Neurochem.* **58**, 320–327.

Harwerth, R. S., Carter-Dawson, L., Shen, F., Smith, E. L., and Crawford, M. L. (1999). Ganglion cell losses underlying visual field defects from experimental glaucoma. *Invest. Ophthalmol. Visual Sci.* **40**, 2242–2250.

Haskin, C. and Cameron, I. (1993). Physiological levels of hydrostatic pressure alter morphology and organization of cytoskeletal and adhesion proteins in MG-63 osteosarcoma cells. *Biochem. Cell Biol.* **71**, 27–35.

Hatten, M. E., Liem, R. K., Shelanski, M. L., and Mason, C. A. (1991). Astroglia in CNS injury. *Glia* **4**, 233–243.

Hawes, N. L., Smith, R. S., Chang, B., Davisson, M., Heckenlively, J. R., and John, S. W. (1999). Mouse fundus photography and angiography: A catalogue of normal and mutant phenotypes. *Mol. Vision* **5**, 22.

Hayreh, S. S. (1978). *In* "Glaucoma, Conceptions of a Disease" (K. Heilman, and K. T. Richardson, eds.), pp. 104–180. Saunders, Philadelphia.

Hayreh, S. S., Pe'er, J., and Zimmerman, M. B. (1999). Morphologic changes in chronic high-pressure experimental glaucoma in rhesus monkeys. *J. Glaucoma* **8**, 56–71.

He, Y., and Grinnell, F. (1994). Stress relaxation of fibroblasts activates a cyclic AMP signaling pathway. *J. Cell Biol.* **126**, 457–464.

Hemperly, J. J., DeGuglielmo, J. K., and Reid, R. A. (1990). Characterization of cDNA clones defining variant forms of human neural cell adhesion molecule N-CAM. *J. Mol. Neurosci.* **2**, 71–78.

Hernandez, M. R. (1992). Ultrastructural immunocytochemical analysis of elastin in the human lamina cribrosa. Changes in elastic fibers in primary open-angle glaucoma. *Invest. Ophthalmol. Visual Sci.* **33**, 2891–2903.

Hernandez, M. R. (2000). The optic nerve head in glaucoma: Role of astrocytes. *Prog. Retinal Eye Res.* **19**(3), 297–321.

Hernandez, M. R., and Gong, H. (1996). *In* "The Glaucomas: Basic Sciences" (R., Ritch, M. B., Shields, and T., Krupin, eds.), 2nd ed., pp. 213–249. Mosby-Year Book, St. Louis, MO.

Hernandez, M. R., and Pena, J. D. (1997). The optic nerve head in glaucomatous optic neuropathy. *Arch. Ophthalmol. (Chicago)* **115**, 389–395.

Hernandez, M. R., Andrzejewska, W. M., and Neufeld, A. H. (1990). Changes in the extracellular matrix of the human optic nerve head in primary open-angle glaucoma. *Am. J. Ophthalmol.* **109**, 180–188.

Hernandez, M. R., Wang, N., Hanley, N. M., and Neufeld, A. H. (1991). Localization of collagen types I and IV mRNAs in human optic nerve head by in situ hybridization. *Invest. Ophthalmol. Visual Sci.* **32**, 2169–2177.

Hernandez, M. R., Ye, H., and Roy, S. (1994a). Collagen type IV gene expression in human optic nerve heads with primary open angle glaucoma. *Exp. Eye Res.* **59**, 41–52.

Hernandes, M. R., Yang, Y., and Ye, H. (1994b). Activation of elastin mRNA expression in human optic nerve heads with primary open angle glaucoma. *J. Glaucoma* **3**, 214–225.

Hirsch, S., and Bahr, M. (1999). Immunocytochemical characterization of reactive optic nerve astrocytes and meningeal cells. *Glia* **26**, 36–46.

Hof, P. R., Lee, P. Y., Yeung, G., Wang, R. F., Podos, S. M., and Morrison, J. H. (1998). Glutamate receptor subunit GluR2 and NMDAR1 immunoreactivity in the retina of macaque monkeys with experimental glaucoma does not identify vulnerable neurons. *Exp. Neurol.* **153**, 234–241.

Hori, O., Matsumoto, M., Maeda, Y., Ueda, H., Ohtsuki, T., Stern, D. M., Kinoshita, T., Ogawa, S., and Kamada, T. (1994). Metabolic and biosynthetic alterations in cultured astrocytes exposed to hypoxia/reoxygenation. *J. Neurochem.* **62**, 1489–1495.

Hortsch, M., O'Shea, K. S., Zhao, G., Kim, F., Vallejo, Y., and Dubreuil, R. R. (1998). A conserved role for L1 as a transmembrane link between neuronal adhesion and membrane cytoskeleton assembly. *Cell Adhes. Commun.* **5**, 61–73.

Ingber, D. E. (1997). Tensegrity: The architectural basis of cellular mechanotransduction. *Annu. Rev. Physiol.* **59**, 575–599.

Jo, S. A., Wang, E., and Benowitz, L. I. (1999). Ciliary neurotrophic factor is and axogenesis factor for retinal ganglion cells. *Neuroscience* **89**, 579–591.

John, S. W., Anderson, M. G., and Smith R. S. (1999). Mouse genetics: A tool to help unlock the mechanisms of glaucoma. *J. Glaucoma* **8**, 400–412.

Johnson, A., Alward, W., Sheffield, V., and Stone, E. (1996). *In* "The Glaucomas. Basic sciences" (R., Ritch, M., Shields, and T., Krupin, eds.), 2nd ed., pp. 39–54. Mosby, St. Louis, Mo.

Johnson, E. C., Deppmeier, L. M., Wentzien, S. K., Hsu, I., and Morrison, J. C. (2000). Chronology of optic nerve head and retinal responses to elevated intraocular pressure. *Invest. Ophthalmol. Visual Sci.* **41**, 431–442.

Jonas, J. B. and Grundler, A. (1996). Optic disc morphology in "age-related atrophic glaucoma." *Albrecht von Graefes Arch. Klin. Exp. Ophthalmol.* **234**, 744–749.

Jonas, J. B., and Hayreh, S. S. (1999). Localized retinal nerve fibre layer defects in chronic experimental high pressure glaucoma in rhesus monkeys. *Br. J. Ophthalmol.* **83**, 1291–1295.

Jucker, M., Mondadori, C., Mohajeri, H., Bartsch, U., and Schachner, M. (1995). Transient upregulation of NCAM mRNA in astrocytes in response to entorhinal cortex lesions and ischemia. *Brain Res. Mol. Brain Res.* **28**, 149–156.

Kaarniranta, K., Elo, M., Sironen, R., Lammi, M. J., Goldring, M. B., Eriksson, J. E., Sistonen, L., and Helminen, H. J. (1998). Hsp70 accumulation in chondrocytic cells exposed to high continuous hydrostatic pressure coincides with mRNA stabilization rather than transcriptional activation. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 2319–2324.

Kato, H., Araki, T., Itoyama, Y., Kogure, K., and Kato, K. (1995). An immunohistochemical study of heat shock protein-27 in the hippocampus in a gerbil model of cerebral ischemia and ischemic tolerance. *Neuroscience* **68**, 65–71.

Kerrigan, L. A., Zack, D. J., Quigley, H. A., Smith, S. D., and Pease, M. E. (1997). TUNEL-positive ganglion cells in human primary open-angle glaucoma. *Arch. Ophthalmol. (Chicago)* **115**, 1031–1035.

Klein-Nulend, J., Van Der Plas, A., Semeins, C. M., Ajubi, N. E., Frangos, J. A., Nijeweide, P. J., and Burger, E. H. (1995). Sensitivity of osteocytes to biochemical stress in vitro. *FASEB J.* **9**, 441– 445.

Klein-Nulend, J., Roelofsen, J., and Semeins, C. M. (1997). Mechanical stimulation of osteopontin mRNA expression and synthesis in bone cell cultures. *J. Cell. Physiol.* **170**, 174–181.

Kobayashi, S., Vidal, I., Pena, J. D., and Hernandez, M. R. (1997). Expression of neural cell adhesion molecule (NCAM) characterizes a subpopulation of type 1 astrocytes in human optic nerve head. *Glia* **20**, 262–273.

Kolker, A. E., and Hetherington, J. R. (1983). *In* "Becker and Shaffer's Diagnosis and Therapy of the Glaucomas" A. E. Kolker, and J. R., Hetherington, eds.), 5th ed., pp. 55–63. Mosby, St. Louis, MO.

Krasney, J. A. (1999). Hypoxia and the blood-brain barrier: introduction. *Adv. Exp. Med. Biol.* **474**, 109–110.

Le Gal La Salle, G., Rougon, G., and Valin, A. (1992). The embryonic form of neural cell surface molecule (E-NCAM) in the rat hippocampus and its reexpression on glial cells following kainic acid-induced status epilepticus. *J. Neurosci.* **12**, 872–882.

Lehmann, S., Kuchler, S., and Gobaille, S. (1993). Lesion-induced re-expression of neonatal recongnition molecules in adult rat cerebellum. *Brain Res. Bull.* **30**, 515–521.

Leske, M. C., Connell, A. M., Wu, S. Y., Hyman, L. G., and Schachat, A. P. (1995). Risk factors for open-angle glaucoma. The Barbados Eye Study. *Arch. Ophthalmol. (Chicago)* **113**, 918–924.

Liu, B., and Neufeld, A. H. (2000). Expression of nitric oxide synthesa-2 (Nos-2) in reactive astrocytes of the human glaucomatous optic nerve head. *Glia* **30**, 178–186.

Malhotra, S., and Shnitka, T. (1994). Adaptive plasticity and diversity among reactive astrocytes in central nervous system lesions. *Biol.* **49**, 273–302.

Maniotis, A. J., Chen, C. S., and Ingber, D. E. (1997). Demonstration of mechanical connections between integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 849–854.

Mansour-Robaey, S., Clarke, D. B., Wang, Y. C., Bray, G. M., and Aguayo, A. J. (1994). Effects of ocular injury and administration of brain-derived neurotrophic factor on survival and regrowth of axotomized retinal ganglion cells. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 1632–1636.

Mercer, R. R., and Crapo, J. D. (1990). Spatial distribution of collagen and elastin fibers in the lungs. *J. Appl. Physiol.* **69**, 756– 765.

Mertts, M., Garfield, S., Tanemoto, K., and Tomarev, S. I. (1999). Identification of the region in the N-terminal domain responsible for the cytoplasmic localization of Myoc/Tigr and its association with microtubules. *Lab. Invest.* **79**, 1237–1245.

Moore, C. G., Epley, D., Milne, S. T., and Morrison, J. C. (1995). Long-term non-invasive measurement of intraocular pressure in the rat eye. *Curr. Eye Res.* **14**, 711–717.

Morgan, J., Caprioli, J., and Koseki, Y. (1999). Nitric oxide mediates excitotoxic and anoxic damage in rat retinal ganglion cells cocultured with astroglia. *Arch. Ophthalmol. (Chicago)* **117**, 1524–1529.

Morgan, W. H., Yu, D. Y., Alder, V. A., Cringle, S. J., Cooper, R. L., House, P. H., and Constable, I. J. (1998). The correlation between cerebrospinal fluid pressure and retrolaminar tissue pressure. *Invest. Ophthalmol. Visual Sci.* **39**, 1419–1428.

Morrison, J. C., L'Hernault, N. L., Jerdan, J. A., and Quigley, H. A. (1989). Ultrastructural location of extracellular matrix components in the optic nerve head. *Arch. Ophthalmol. (Chicago)* **107**, 123–129.

Morrison, J. C., Dorman-Pease, M. E., Dunkelberger, G. R., and Quigley, H. A. (1990). Optic nerve head extracellular matrix in primary optic

atrophy and experimental glaucoma. *Arch. Ophthalmol. (Chicago)* **108**, 1020–1024.

Netland, P. A., Ye, H., Streeten, B. W., and Hernandez, M. R. (1995). Elastosis of the lamina cribrosa in pseudoexfoliation syndrome with glaucoma. *Ophthalmology* **102**, 878–886.

Neufeld, A. H. (1999a). Microglia in the optic nerve head and the region of parapapillary chorioretinal atrophy in glaucoma. *Arch. Ophthalmol. (Chicago)* **117**, 1050–1056.

Neufeld, A. H. (1999b). Nitric oxide: A potential mediator of retinal ganglion cell damage in glaucoma. *Surv. Ophthalmol.* **43**(Suppl. 1), S129–S135.

Neufeld, A. H., Hernandez, M. R., and Gonzalez, M. (1997). Nitric oxide synthase in the human glaucomatous optic nerve head. *Arch. Ophthalmol. (Chicago)* **115**, 497–503.

Neufeld, A. H., Sawada, A., and Becker, B. (1999). Inhibition of nitric oxide synthase 2 by aminoguanidine provides neuroprotection of retinal ganglion cells in a rat model of chronic glaucoma. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 9944–9948.

Nicolela, M. T. and Drance, S. M. (1996). Various glaucomatous optic nerve appearances: Clinical correlations. *Ophthalmology* **103**, 640–649.

Norenberg, M. D. (1994). Astrocyte responses to CNS injury. *J. Neuropathol. Exp. Neurol.* **53**, 213–220.

Norenberg, M. D. (1996). *In* "The Role of Glia in Neurotoxicity" (M. Aschner, and H. K. Kimelberg, eds.), Ist ed. pp. 93–107. CRC Press, Boca Raton, FL.

Oblinger, M. M., and Singh, L. D. (1993). Reactive astrocytes in neonate brain upregulate intermediate filament gene expression in response to axonal injury. *J. Dev. Neurosci.* **11**, 149–156.

Oishi, Y., Uezono, Y., Yanagihara, N., Izumi, F., Nakamura, T., and Suzuki, K. (1998). Transmural compression-induced proliferation and DNA synthesis through activation of a tyrosine kinase pathway in rat astrocytoma RCR-1 cells. *Brain Res.* **781**, 159–166.

Oku, H., Sugiyama, T., Kojima, S., Watanabe, T., and Azuma, I. (1999). Experimental optic cup enlargement caused by endothelin-1-induced chronic optic nerve head ischemia. *Surv. Ophthalmol.* **44**(Suppl. 1), S74–S84.

Olney, J. W. (1969). Glutaate-induced retinal degeneration in neonatal mice. Electron microscopy of the acutely evolving lesion. *J. Neuropathol. Exp. Neurol.* **28**, 455–474.

Oluwole, B. O., Mili, W., and Sumpio, B. E. (1997). Gene regulation by mechanical forces. *Endothelium* **5**, 85–93.

Pagenstecher, A., Stalder, A., Kincaid, C., Shapiro, S., and Campbell, I. (1998). Differential expression of matrix metalloproteinase and tissue inhibitor of matrix metalloproteinase genes in the mouse central nervous system in normal and inflammatory states. *J. Pathol.* **152**.

Pan, L. C., Margolskee, R. F., and Blau, H. M. (1992). Cloning muscle isoforms of neural adhesion molecule using an episomal shuttle vector. *Somatic Cell Mol. Genet.* **2**, 163–177.

Parkkinen, J. J., Lammi, M. J., Inkinen, R., Jortikka, M., Tammi, M., Virtanen, I., and Helminen, H. J. (1995). Influence of short-term hydrostatic pressure on organization of stress fibers in cultured chondrocytes. *J. Orthop. Res.* **13**, 495–502.

Parks, W. C., and Sires, U. I. (1996). Matrix metalloproteinases in skin biology and disease. *Curr. Opin. Dermatol.* **3**, 240–247.

Pederson, J. E., and Gaasterland, D. E. (1984). Laser-induced primate glaucoma. I. Progression of cupping. *Arch. Ophthalmol. (Chicago)* **102**, 1689–1692.

Pena, J. D., Roy, S., and Hernandez, M. R. (1996). Tropoelastin gene expression in optic nerve heads of normal and glaucomatous subjects. *Matrix Biol.* **15**, 323–330.

Pena, J. D., Netland, P. A., Vidal, I., Dorr, D. A., Rasky, A., and Hernandez, M. R. (1998). Elastosis of the lamina cribrosa in glaucomatous optic neuropathy. *Exp. Eye Res.* **67**, 517–524.

Pena, J. D., Varela, H. J., Ricard, C. S., and Hernandez, M. R. (1999). Enhanced tenascin expression associated with reactive astrocytes in human optic nerve heads with primary open angle glaucoma. *Exp. Eye Res.* **68**, 29–40.

Perry, V. H., Bell, M. D., Brown, H. C., and Matyszak, M. K. (1995). Inflammation in the nervous system. *Curr. Opin. Neurobiol.* **5**, 636–641.

Polansky, J. R., Fauss, D. J., Chen, P., Chen, H., Lutjen-Drecoll, E., Johnson, D., Kurtz, R. M., Ma, Z. D., Bloom, E., and Nguyen, T. D. (1997). Cellular pharmacology and molecular biology of the trabecular meshwork inducible glucocorticoid response gene product. *Ophthalmologica* **211**, 126–139.

Pollerburg, G. E., Schanchner, M., and Davoust, J. (1986). Differentiation state-dependent surface mobilities of two forms of the neural cell adhesion molecule. *Nature (London)* **324**, 227–236.

Pollerburg, G. E., Burridge, K., Krebs, K. E., Goodman, S. R., and Schachner, M. (1987). The 180-kD component of the neural cell adhesion molecule N-CAM is involved in cell-cell contacts and cytoskeleton-membrane interactions. *Cell Tissue Res.* **250**, 227–236.

Quigley, H. A. (1992). *In* "Pharmacology of Glaucoma" (S. M., Drance, E. M., Van Burskik, and A. H., Neufeld, eds.), pp. 265–272. Williams & Williams, Baltimore.

Quigley, H. A., and Hohman, R. M. (1983). Laser energy levels for trabecular meshwork damage in the primate eye. *Invest. Ophthalmol. Visual Sci.* **24**, 1305–1307.

Quigley, H. A., and Vitale, S. (1997). Models of open-angle glaucoma prevalence and incidence in the United States. *Invest. Ophthalmol. Visual Sci.* **38**. 83–91.

Quigley, H. A., Hohman, R. M., Addicks, E. M., Massof, R. W., and Green, W. R. (1983). Morphologic changes in the lamina cribrosa correlated with neural loss in open-angle glaucoma. *Am. J. Ophthalmol.* **95**, 673–691.

Quigley, H. A., Dorman-Pease, M. E., and Brown, A. E. (1991). Quantitative study of collagen and elastin of the optic nerve head and sclera in human and experimental glaucoma. *Curr. Eye Res.* **10**, 877–888.

Quigley, H. A., Nickells, R. W., Kerrigan, L. A., Pease, M. E., Thibault, D. J., and Zack, D. J. (1995). Retinal ganglion cell death in experimental glaucoma and after axotomy occurs by apoptosis. *Invest. Ophthalmol. Visual Sci.* **36**, 774–786.

Raghow, R. (1994). The role of extracellular matrix in postinflammatory wound healing and fibrosis. *FASEB J.* **8**, 823–831.

Raivich, G., Bohatschek, M., Kloss, C. U., Werner, A., Jones, L. L., and Kreutzberg, G. W. (1999). Neuroglial activation repertoire in the injured brain: Graded response, molecular mechanisms and cues to physiological function. *Brain Res. Rev.* **30**, 77–105.

Renkawek, K., Bosman, G. J. C. G. M., and de Jong, W. W. (1994). Expression of small heat-shock protein hsp27 in reactive gliosis in alzheimer disease and other types of dementia. *Acta Neuropathol.* **87**, 511–519.

Resnick, N., and Gimbrone, M. A. (1995). Hemodynamic forces are complex regulators of endothelial gene expression. *FASEB J.* **9**, 874–882.

Resnick, N., Collins, D. T., and Bonthron, D. T. (1993). Platelet-derived growth factor b chain promoter contains a cis-acting fluid shear stress responsive element. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 4591–4595.

Ricard C. S. (1999). Differential expression of neural cell adhesion molecule isoforms in normal and glaucomatous human optic nerve heads. *Mol. Brain Res.* **74**, 69–82.

Ridet, J. L., Malhotra, S. K., Privat, A., and Gage, F. H. (1997). Reactive astrocytes-Cellular and molecular cues to biological function. *Trends Neurosci.* **20**, 570–577.

Roche, P. H., Figarella-Branger, D., Daniel, L., Bianco, N., Pellet, W., and Pellissier, J. F. (1997). Expression of cell adhesion molecules in normal nerves, chronic axonal neuropathies and Schwann cell tumors. *J. Neurol. Sci.* **151**, 127–133.

Romanic, A. and Madri, J. (1994). Extracellular matrix-degrading proteinases in the nervous system. *Brain Pathol.* **4**, 145–156.

Rosenberg, G. (1995). Matrix metalloproteinases in brain injury. *J. Neurotrauma* **12**, 833–842.

Sachs, F. (1997). Mechanical transduction by ion channels: How forces reach the channel. *Soc. Gen. Physiol. Ser.* **52**, 209–218.

Salwen, S. A., Szarowski, D. H., Turner, J. N., and Bizios, R. (1998). Three-dimensional changes of the cytoskeleton of vascular endothelial cells exposed to sustained hydrostatic pressure. *Med. Biol. Eng. Comput.* **36**, 520–527.

Sawaguchi, S., Yue, B. Y., Fukuchi, T., Abe, H., Suda, K., Kaiya, T., and Iwata, K. (1999). Collagen fibrillar network in the optic nerve head of normal monkey eyes and monkey eyes with laser-induced glaucoma—a scanning electron microscopic study. *Curr. Eye Res.* **18**, 143–149.

Schwartz, E. A., Bizios, R., Medow, M. S., and Gerritsen, M. E. (1999). Exposure of human vascular endothelial cells to sustained hydrostatic pressure stimulates proliferation. Involvement of the alpha V integrins. *Circ. Res.* **84**, 315–322.

Semenza, G. L., Agani, F., Booth, G., Forsythe, J., Iyer, N., Jiang, B. H., Leung, S., Roe, R., Wiener, C., and Yu, A. (1997). Structural and functional analysis of hypoxiainducible factor 1. *Kidney Int.* **51**, 553–555.

Shareef, S., Sawada, A., and Neufeld, A. H. (1999). Isoforms of nitric oxide synthase in the optic nerves of rat eyes with chronic moderately elevated intraocular pressure. *Invest. Ophthalmol. Visual Sci.* **40**, 2884–2891.

Shen, S., Wiemelt, A. P., McMorris, F. A., and Barres, B. A. (1999). Retinal ganglion cells lose trophic responsiveness after axotomy. *Neuron* **23**, 285–295.

Shima, D. T., Adamis, A. P., Ferrara, N., Yeo, K. T., Yeo, T. K., Allende, R., Folkman, J., and D'Amore, P. A. (1995). Hypoxic induction of endothelial cell growth factors in retinal cells: Identification and characterization of vascular endothelial growth factor (VEGF) as the mitogen. *Mol. Med.* **1**, 182–193.

Silver, I. A., Deas, J., and Erecinska, M. (1997). Ion homeostasis in brain cells: Differences in intracellular ion responses to energy limitation between cultured neurons and glial cells. *Neuroscience* **78**, 589–601.

Smith, R. L., Rusk, S. F., and Ellison, B. E. (1996). In vitro stimulation of articular chondrocyte mRNA and extracellular matrix synthesis by hydrostatic pressure. *J. Orthop. Res.* **14**, 53– 60.

Stoilova, D., Child, A., Trifan, O. C., Crick, R. P., Coakes, R. L., and Sarfarazi, M. (1996). Localization of a locus (GLC1B) for adult-onset primary open angle glaucoma to the 2cen-q13 region. *Genomics* **36**, 142–150.

Stone, E. M., Fingert, J. H., Alward, W. L. M., Nguyen, T. D., Polansky, J. R., Sunden, S. L. F., Nishimura, D., Clark, A. F., Nystuen, A., Nichols, B. E., Mackey, D. A., Ritch, R., Kalenak, J. W., Craven, E. R., and Sheffield, V. C. (1997). Identification of a gene that causes primary open angle glaucoma. *Science* **275**, 668–670.

Streit, W. J., Walter, S. A., and Pennell, N. A. (1999). Reactive microgliosis. *Prog. Neurobiol.* **57**, 563–581.

Sumpio, B. E., Widmann, M. D., Ricotta, J., Awolesi, M. A., and Watase, M. (1994). Increased ambient pressure stimulates proliferation and morphologic changes in cultured endothelial cells. *J. Cell. Physiol.* **158**, 133–139.

Takahashi, H., Noda, S., Imamura, Y., Nagasawa, A., Kubota, R., Mashima, Y., Kudoh, J., Oguchi, Y., and Shimizu, N. (1998). Mouse myocilin (Myoc) gene expression in ocular tissues. *Biochem. Biophys. Res. Commun.* **248**. 104–109.

Takahashi, H., Noda, S., Mashima, Y., Kubota, R., Ohtake, Y., Tanino, T., Kudoh, J., Minoshima, S., Oguchi, Y., and Shimizu, N. (2000). The Myocilin (MYOC) gene expression in the human trabecular meshwork. *Curr. Eye Res.* **20**, 81–84.

Takamiya, Y., Kohsaka, S., Toya, S., Otani, M., and Tsukada, Y. (1988). Immunohistochemical studies on the proliferation of reactive astrocytes and the expression of cytoskeletal proteins following brain injury in rats. *Brain Res.* **466**, 201–210.

Tezel G., Hernandez M. R., and Wax M. (2000). Immunostaining of heat shock proteins in the retina and optic nerve head of human glaucomatous eyes. *Arch. Ophthalmol. (Chicago)* (in press).

Tielsch, J. M., Sommer, A., Katz, J., Royall, R. M., Quigley, H. A., and Javitt, J. (1991). Racial variations in the prevalence of primary open-angle glaucoma. The Baltimore Eye Survey. *JAMA, J. Am. Med. Assoc.* **266**, 369–374.

Tomasiewicz, H., Ono, K., Yee, D., Thompson, C., Goridis, C., Rutishauser, U., and Magnuson, T. (1993). Genetic deletion of a neural cell adhesion molecule variant (N-CAM-180) produces distinct defects in the central nervous system. *Neuron* **11**, 1163–1174.

Tomimoto, H., Akiguchi, I., Wakita, Suenaga, T., Nakamura, S. I., and Kimura, J. (1997). Regressive changes of astroglia in white matter lesions in cerebrovascular disease and Alzheimer's disease patients. *Acta Neuropathol.* **94**, 146–152.

Uhm, J., Dooley, N., Oh, L., and Yong, V. (1998). Olidgodendrocytes utilize a matrix metalloprteinase, MMP-9, to extend processes along an astrocyte extracellular matrix. *Glia* **22**, 53–63.

Varela, H. J., and Hernandez, M. R. (1997). Astrocyte responses in human optic nerve head with primary open-angle glaucoma. *J. Glaucoma* **6**, 303–313.

Varma, R and Minckler, D. S. (1996). *In* ''The Glaucomas. Basic Sciences'' (R. Ritch, M. B. Chields, and T. Krupin, eds.), 2nd ed., pp. 139–175. Mosby, St. Louis, MO.

Varma, R., Quigley, H. A., and Pease, M. E. (1992). Changes in optic disk characteristics and number of nerve fibers in experimental glaucoma. *Am. J. Ophthalmol.* **114**, 554–559.

Verkhratsky, A., and Kettenmann, H. (1996). Calcium signalling in glial cells. *Trends Neurosci.* **19**, 346–352.

Vickers, J. C., Schumer, R. A., Podos, S. M., Wang, R. F., Riederer, B. M., and Morrison, J. H. (1995). Differential vulnerability of neurochemically identified subpopulations of retinal neurons in a monkey model of glaucoma. *Brain Res.* **680**, 23–35.

Vorwerk, C. K., Gorla, M. S., and Dreyer, E. B. (1999). An experimental basis for implicating excitotoxicity in glaucomatous optic neuropathy. *Surv. Ophthalmol.* **43**(Suppl. 1), S142–S150.

Walsh, F. S., and Doherty, P. (1996). Cell adhesion and neuronal regeneration. *Curr. Opin. Cell Biol.* **8**, 707–713.

Walsh, F. S., and Doherty, P. (1997). Neural cell adhesion molecule of the immunoglobulin superfamily: Role in axon growth and guidance. *Annu. Rev. Dev. Biol.* **13**, 425–456.

Wang, R. F., Schumer, R. A., Serle, J. B., and Podos, S. M. (1998). A comparison of argon laser and diode laser photocoagulation of the trabecular meshwork to produce the glaucoma monkey model. *J. Glaucoma* **7**, 45–49.

Wax, M. B., Tezel, G., Kobayashi, S., and Hernandez, M. R. (2000). Responses of different cell lines from ocular tissues to elevated hydrostatic pressure. *B. J. Ophthalmol.* (in press).

Weber, A. J., Kaufman, P. L., and Hubbard, W. C. (1998). Morphology of single ganglion cells in the glaucomatous primate retina. *Invest. Ophthalmol. Visual Sci.* **39**, 2304–2320.

Werb, Z. (1997). ECM and cell surface proteolysis: Regulating cellular ecology. *Cell (Cambridge, Mass)* **91**, 439–442.

Werner, E. (1996). *In* ''the Glaucomas. Clinical Sciences'' (R. Ritch, M. B. Shields, and T. Krupin, eds.), 2nd ed., pp. 769–797. Mosby, Louis, Mo.

Wirtz, M. K., Samples, J. R., Rust, K., Lie, J., Nordling, L., Schilling, K., Acott, T. S., and Kramer, P. L. (1999). GLC1F, a new primary open-angle glaucoma locus, maps to 7q35-q36. *Arch. Ophthalmol. (Chicago)* **117**, 237–241.

Wolf, H. K., Wellmer, J., Muller, M. B., Wiestler, O. D., Hufnagel, A., and Pietsch, T. (1995). Glioneuronal malformative lesions and dysembryoplastic neuroepithelial tumors in patients with chronic pharmacoresistant epilepsies. *J. Neuropathol. Exp. Neurol.* **54**, 245–254.

Wright, M., Jobanputra, P., Bavington, C., Salter, D. M., and Nuki, G. (1996). Effects of intermittent pressure-induced strain on the electrophysiology of cultured human chondrocytes: Evidence for the presence of stretch-activated membrane ion channels. *Clin. Sci.* **90**, 61–71.

Wright, M. O., Stickwell, R. A., and Nuki, G. (1992). Response of plasma membrane to applied hydrostatic pressure in chondrocytes and fibroblasts. *Connect. Tissue Res.* **28**, 49–70.

Yang, J. L., Neufeld, A. H., Zorn, M. B., and Hernandez, M. R. (1993). Collagen type I mRNA levels in cultured human lamina cribrosa cells: Effects of elevated hydrostatic pressure. *Exp. Eye Res.* **56**, 567–574.

Ye, H., and Hernandez, M. R. (1995). Heterogeneity of astrocytes in human optic nerve head. *J. Comp. Neurol.* **362**, 441–452.

Ye, H., Yang, J., and Hernandez, M. R. (1994). Localization of collagen type III mRNA in normal human optic nerve heads. *Exp. Eye Res.* **58**, 53–63.

Yousefian, J., Firouzian, F., Shanfeld, J., Ngan, P., Lanese, R., and Davidovitch, Z. (1995). A new experimental model for studying the response of periodontal ligament cells to hydrostatic pressure. *Am. J. Orthod. Dentofacial Orthop.* **108**, 402–409.

Yucel, Y. H., Kalichman, M. W., Mizisin, A. P., Powell, H. C., and Weinreb, R. N. (1999). Histomorphometric analysis of optic nerve changes in experimental glaucoma. *J. Glaucoma* **8**, 38–45.

Yun, H. Y., Dawson, V. L., and Dawson, T. M. (1996). Neurobiology of nitric oxide. *Crit. Rev. Neurobiol.* **10**, 291–316.

Zeimer, R. C., and Chen, K. (1987). Comparison of a noninvasive measurement of optic nervehead mechanical compliance with an invasive method. *Invest. Ophthalmol. Visual Sci.* **28**, 1735–1739.

Zeimer, R. C., and Ogura, Y. (1989). The relation between glaucomatous damage and optic nerve head mechanical compliance. *Arch. Ophthalmol. (Chicago)* **107**, 1232–1234.

Zeimer, R. C., Wilensky, J. T., Gieser, D. K., and Viana, M. A. (1991). Association between intraocular pressure peaks and progression of visual field loss. *Ophthalmology* **98**, 64–69.

Zucca, G., Botta, L., Mira, E., Manfrin, M., Poletti, A., Buizza, A., and Valli, P. (1991). Effects of hydrostatic pressure on sensory discharge in frog semicircular canals. *Acta Otolaryngol.* **111**, 820–826.

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Alice Cronin-Golomb

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Color Vision, Object Recognition, and Spatial Localization in Aging and Alzheimer's Disease

Alzheimer's disease affects multiple brain areas that are devoted to the processing of basic visual capacities and to higher-order visual cognition. This chapter describes the effect of this disorder on the basic visual capacity of color discrimination and on the visuocognitive domains of object recognition and spatial localization. For each function—color discrimination, object recognition, and spatial localization—the review begins with documentation and a discussion of the behavioral evidence for deficits in Alzheimer's disease relative to normal aging. The next topics are the relation of basic visual deficits to cognitive impairment and their brain bases, focusing on the occipitotemporal and occipitoparietal visual processing streams. The finding that basic visual deficits predict visuocognitive performance has implications for determining the brain substrates of cognitive dysfunction. The next section evaluates the relative dysfunction of object recognition and spatial localization in Alzheimer's disease, including subgroups defined by specific behavioral and neuropathological profiles. It is clear that both visual processing streams are affected in this disease. In many patients, deficits in object recognition may be more pronounced than deficits in spatial localization, whereas in subgroups such as patients exhibiting the symptoms of Bálint's syndrome, this pattern may be reversed. Finally, consideration is given to the clinical relevance of impaired vision and visual cognition in normal aging and Alzheimer's disease. Understanding fundamental disorders in visual processing may lead to interventions that restore visual capacities with the goal of enhancing cognitive ability.

I. Introduction

Alzheimer's disease (AD), the most common cause of dementia in elderly adults, is viewed as a disorder primarily of memory by patients and their caregivers. Although the memory deficit in AD is usually predominant, researchers have long known that the disease is characterized by impairments in multiple additional domains, including visual cognition. In his original case study, Alois Alzheimer described abnormalities in object discrimination and recognition and in spatial localization:

> (S)he could not find her way about her home ...She was disoriented as to time and place... She suffered from serious perceptual disorders ...While reading she would omit sentences... She did not remember the use of particular objects. (Alzheimer, 1907)

More recently, researchers have attained a consensus that even lower-level visual functions are impaired in a large number of patients with AD, although these findings have not yet made the leap to the diagnostic manuals. For example, the most recent edition of the Diagnostic and Statistical Manual of Mental Disorders states that few sensory signs occur early in AD (American Psychiatric Association, 1994).

In accordance with their own observations, and expectations, of the prominence of the memory impairment, as well as those of the clinical staffs with whom they come into contact, patients with AD report vision problems to their physicians less frequently than do healthy elderly individuals (McCormack *et al*., 1994). Nevertheless, visual dysfunction is prevalent in AD (Mendola *et al*., 1995). The neuropathology of this disorder affects several brain areas that are devoted to visual processing of low-level visual functions as well as higher-order visual cognition.

The goal of this chapter is to summarize what is known about one relatively low-level visual function in AD, color discrimination, and the two principal domains of visual cognition, object recognition and spatial localization. This summary includes discussion of the behavioral evidence for deficits, the relation of visual dysfunction to cognitive impairment, the brain bases of the deficits, and the clinical relevance of

impaired vision and higher-order visual cognition in this disorder.

II. Color Discrimination

A. Evidence for Color Discrimination Deficits

Color discrimination deficits occur in a significant proportion of individuals with AD. These deficits are usually subtle enough that patients rarely complain in the clinic about losses of color vision. Use of clinical tests of color discrimination or psychophysical assessments reveals the impairments, principally along the tritan (blue-yellow) color axis. We have reported that patients with AD, but not healthy elderly adults, show a disproportionate deficit in the discrimination of short wavelengths relative to medium and long wavelengths on three clinical tests of color vision: the City University Colour Vision Test (Cronin-Golomb *et al*., 1991, 1993a) and the Farnsworth D-15 and Lanthony New Color Tests (Cronin-Golomb *et al*., 1993a).

The City University Colour Vision Test (Fletcher, 1980) consists of 10 trials, each depicting a central color circle surrounded by four comparison colors on a black background. Observers point to the surrounding circle that is most similar in hue to the target, though none is in fact identical to the target. The test is short and has low cognitive demands, making it possible to assess color discrimination in even severely demented patients. There are four response types: correct, protanomalous-like error, deuteranomalous-like error, and tritanomalous-like error. The terms protan, deutan, and tritan refer to errors in red, green, and blue hue discrimination, respectively, and are used commonly in discussions of dysfunctions of retinal photoreceptors. Because the optic media and the retinocalcarine pathway are usually normal in AD (Rizzo *et al*., 1992; Davies *et al*., 1995), we use terms such as tritanomalous-like in order to avoid implicating the retina and to raise the possibility that color discrimination deficits in AD have a cortical basis. None of the participants in our studies reported a history of color blindness or other historical difficulty in perceiving colors. The AD patients' self-report was corroborated by their accompanying caregivers. All had normal binocular acuity ranging from 16/20 to 20/40 Snellen.

In our initial study (Cronin-Golomb *et al*., 1991), 54% of the 37 patients with AD made one or more errors, whereas the 12 healthy elderly adults performed at or near ceiling level. There was an unequal distribution of error types in the AD group, with tritanomalous-like errors exceeding other types. In the replication study (Cronin-Golomb *et al*., 1993a), hue discrimination errors were made by 66% of the 32 AD patients, with the 32 control participants performing at or near ceiling. Again, tritanomalous-like errors exceeded other error types. In both studies, there was no significant correlation between test performance and dementia severity as measured by mental status exam.

Our finding of a tritanomalous-like deficit in AD has been replicated in another laboratory using this same test (Gilmore *et al*., 1993). We have observed a similar pattern of tritanomalous-like color discrimination deficit in Down syndrome (Rocco *et al*., 1997), a disorder that shares with AD numerous neuropathological (Hof *et al*., 1995) and metabolic (Pietrini *et al*., 1997) characteristics in visual association cortex. Of 21 adults with Down syndrome, 48% made one or more errors on the test, with tritanomalous-like errors exceeding others. Their performance contrasted with the near-ceiling level attained by mentally retarded adults without Down syndrome, even when subgroups were matched for IQ. The similarity in pattern of performance of patients with AD and adults with Down syndrome is depicted in Fig. 36.1.

Because our findings of a selective color discrimination deficit in AD were based on a single clinical color discrimination test, the City University, we broadened our investigation to include other clinical measures in an effort to assess how general the findings might be. The same 32 AD patients described above for the City University replication study (Cronin-Golomb *et al*., 1993a) were also administered two tests that required the arrangement of color caps in a smooth, uninterrupted hue sequence. For the Farnsworth D-15 test (Farnsworth, 1947), there are 16 caps of desaturated hues including a blue starting cap. For the Lanthony New Color Test, chroma 8 (Lanthony, 1978), there are 25 caps, 10 of which represent the gray scale and the other 15 of various saturated hues, with no designated starting cap. Observers pick out and sequence the gray scale caps and then sequence the color caps as per the Farnsworth test. Error analysis for both tests provides the number of each hue-discrimination error type. The Lanthony test additionally provides information on brightness discrimination through use of the gray scale. Such information is valuable because deficient brightness discrimination alone may lead to poor performance on color vision tests even in the absence of a genuine impairment in the ability to discriminate hues.

The tritanomalous-like pattern of performance that we observed for the City University Test was repeated, strikingly, for the sequencing tests. Major hue sequencing errors, defined

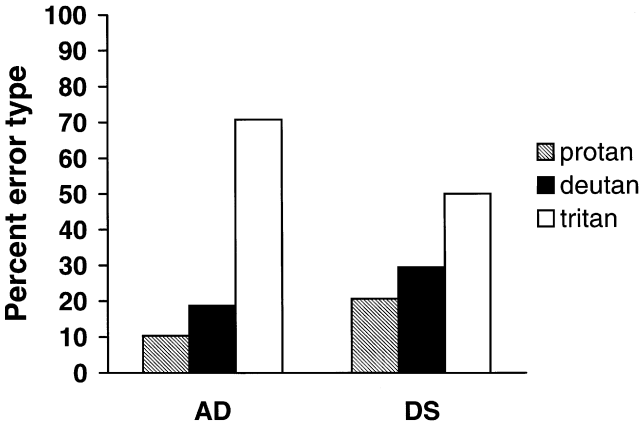

FIG. 36.1. Percentage of each hue discrimination error type on the City University Colour Vision Test for patients with Alzheimer's disease (AD) and Down syndrome (DS). The AD group ($n = 32$, of whom 21 made a total of 34 errors) and DS group ($n = 21$, of whom 10 made a total of 48 errors) each committed a disproportionately large number of tritanomalous-like errors relative to protanomalous- or deuteranomalous-like errors. The control groups made relatively few errors. Results for the AD and elderly control groups are described in Cronin-Golomb *et al*. (1993a). Results for the DS and mentally retarded (non-DS) control group are described in Rocco *et al*. (1997).

as errors falling along a specific axis (protan, deutan, or tritan) that went beyond transpositions of adjacent caps, were made by 50% of the 32 AD patients but less than 16% of the 32 control participants on the Farnsworth test and by 52% of the 29 AD patients and 25% of the control participants on the Lanthony hue test. Only tritanomalous-like errors significantly differentiated the groups for both tests. Across tests, tritanomalous-like errors by the AD patients exceeded protoanomalous- and deuteranomalous-like errors, combined, by a ratio of 2:1, whereas the distribution of error types was relatively even across hue axes for the control participants. Importantly, the AD and control groups did not differ in their ability to sequence the Lanthony gray caps, indicating that the AD patients had normal brightness discrimination and therefore implying that poor performance on the hue sequencing tests was due to poor color discrimination rather than to problems with luminance sensitivity or the cognitive demands of the tests. In accordance with the latter assertion, there was no significant correlation between hue-discrimination performance and dementia severity in the AD group for either test.

Our reports of a tritanomalous-like deficit in color discrimination in AD have been supported by the results of detailed psychophysical assessments (Kurylo *et al*., 1994a), which showed that a significantly larger proportion of AD patients than age-matched healthy adults were impaired at discriminating between blue and violet hues, whereas there was no group difference in discriminating between yellow and orange hues.

The behavioral findings indicate that AD is not simply an exaggeration of the normal aging process. Although age-related yellowing of the lens can result in mild color discrimination deficits along the tritan axis in healthy elderly relative to younger adults (Matjucha and Katz, 1994), the AD patients in the studies described above were matched for age to the control group, and neuro-ophthalmological examination did not reveal obvious group differences in the appearance of the ocular media, including the lens. The disproportionate tritanomalous-like impairment seen in AD was mirrored in adults with Down syndrome, a disorder that shares with AD numerous behavioral and neuropathological characteristics.

B. Relation of Color Discrimination Dysfunction to Cognitive and Functional Deficits

Poor performance of AD patients on the Stroop Color-Word Test is well established (e.g., (Koss *et al*., 1984; Binetti *et al*., 1996; Spieler *et al*., 1996) and exceeds normal age-related decrements (reviewed in Spieler *et al*., 1996). This test is widely used as a measure of executive function and attention and is thought to reflect the integrity of the frontal lobes (Lezak, 1995). The goal of the test is to assess the ability to inhibit one response in favor of another. First, on the word reading subtest, an observer reads as quickly as possible color words (''red,'' ''green,'' ''blue'') printed in black ink on a white background. Second, on the color identification subtest, the observer reads aloud the ink color (red, green, or blue) of ''X'' symbols on a white background. Finally, on the interference subtest, the observer reads aloud the ink color (red, green, or blue) for color words that are incongruent with the ink color (for example, read ''green'' when the ink color is green for the printed word ''blue''). Two studies have reported poor performance by individuals with AD on the color identification subtest specifically (Cohen *et al*., 1988; Fisher *et al*., 1990). In a third study using somewhat different methodology, it was noted that that color confusion errors, though few in number, may have complicated comparisons of error rates across the groups of AD and healthy young and old participants (Spieler *et al*., 1996). None of these studies examined basic color vision in the same participants. The findings raised the question of whether a color discrimination impairment in AD was influencing performance on the color identification subtest, which might then contribute to compromised performance on the critical interference subtest.

We examined the relation between a wide variety of basic visual capacities and cognitive capacities in participants with probable AD in order to test the general hypothesis that visual dysfunction predicts cognitive performance (Cronin-Golomb *et al*., 1995). In regard to the question of sources of poor cognitive performance on the Stroop Color-Word Test, we found for 38 AD patients who had received both the City University Colour Vision Test and the Stroop test that tritanomalous-like errors on the City University test accounted for 7% of the variance in performance on the color-naming subtest of the Stroop and for 2% of the variance on the interference subtest. Although the predictive value is low overall, the prevalence of the color discrimination deficit in AD is significant, and it is therefore likely in individual patients that this deficit may contribute to poor performance on various cognitive tests that coincidentally require normal color discrimination. Our AD prevalence estimate for the City University test is 28%, based on the performance of 74 AD patients and 54 age-matched control participants, and using a strict cutoff score such that the probability of a healthy elderly control participant obtaining that score was 1% or less (Mendola *et al*., 1995).

Knowledge about abnormalities in color perception in AD may lead not only to an understanding of certain cognitive impairments, but may also be employed to enhance cognition and daily function in individuals with AD. In a study of ingestive behavior in severely demented inpatients with probable AD, Dunne and Neargarder (1999) reported that enhanced color contrast of tableware (bright red plates and cups substituted for institutional white) resulted in significant increases in the volume of food and liquid ingested relative to baseline. The volume ingested declined again in the postintervention (white) phase. This pattern held for each of the nine tested participants. Dunne and Neargarder based their study on the premise that enhancement of color contrast in AD may enhance behavioral performance, and that the color selected for manipulation should be one outside of the compromised blue-yellow axis. Koss and Gilmore (1998) likewise reported that enhanced contrast of dinnertime tableware affected behavior, specifically resulting in increased food intake and decreases in the frequency of night-time agitation (''sundowning'') in 13 residents of an inpatient dementia unit. The amount of food ingested at dinner was significantly different across intervention conditions, with more food consumed during the dinner intervention, whereas there was no change in amount of food ingested at lunchtime, when no intervention was effected. The frequency of agitated nighttime behaviors decreased dramatically during the intervention condition.

C. Brain Bases of Color Discrimination Impairment

The color discrimination deficits observed in individuals with AD are likely to be of cortical origin. Neuroophthalmological examination including electrophysiological testing yielded age-appropriate results in a large sample of the AD participants in our studies. These same patients showed significant impairments on vision tests, including color discrimination (Rizzo *et al*., 1992). The findings indicated that defects in the anterior visual structures up to and including the optic radiations did not account for the observed visual impairments. In normal aging, optical changes account for about 40% of the decline in sensitivity of short-wavelength-sensitive (blue) cones. The rest of the decline has been attributed to receptor and postreceptor neural changes that do not affect blue cones preferentially (reviewed in Matjucha and Katz, 1994).

Examination of autopsy tissue suggests that, in the very late stages of AD, some cases may show degeneration of retinal ganglion cells (Hinton *et al*., 1986; Blanks *et al*., 1991, 1996a,b). Other investigators have found no evidence of retinal ganglion cell or optic nerve degeneration (Curcio and Drucker, 1993; Davies *et al*., 1995). In living patients, it is reportedly difficult to obtain and to evaluate reliably photographs of the retinal nerve fiber layer, especially in cases with severe dementia (Hedges *et al*., 1996). Tsai and colleagues (1991) reported no significant difference in retinal nerve fiber layer abnormalities, nor in optic disc pallor, in AD relative to control eyes, though they found more AD abnormalities on topographic measures of the optic nerve head.

Whereas the status of the retina and optic nerve is inconclusive, it is clear that there is significant AD pathology in subcortical and cortical brain regions devoted to color processing. As described in the next sections on Object Recognition and Spatial Localization, behavioral studies suggest that AD affects both the ventral (occipitotemporal) and the dorsal (occipitoparietal) visual processing streams. The ventral stream receives input from the parvocellular, or color-opponent and magnocellular, or broadband pathways that extend from the retina to visual association cortex. Color processing is dependent upon the parvocellular pathway. In AD, neuritic plaques are found in the parvocellular regions of the lateral geniculate nucleus (Leuba and Saini, 1995). Of note, pathological changes occur in the specific sublayer of the primary visual center that receives color information from the lateral geniculate nucleus (Beach and McGeer, 1988) as well as in the pulvinar and superior colliculus (Leuba and Saini, 1995), subcortical regions that along with the lateral geniculate nucleus may be involved in chromatic processing (Barbur *et al*., 1998).

AD neuropathological changes are more widespread in visual association cortices than in the retinocalcarine pathway, based on histological examination (Pearson *et al*., 1985; Lewis *et al*., 1987; Arnold *et al*., 1991) and electrophysiological assessment (Wright *et al*., 1984, 1987; Aguglia *et al*., 1991; Rizzo *et al*., 1992; Celesia *et al*., 1993; Martinelli *et al*., 1996). Extensive pathology occurs in occipital and inferotemporal cortex (Lewis *et al*., 1987), and it is this pathology, particularly in the fusiform gyrus, that presumably underlies the observed changes in color discrimination (Cronin-Golomb *et al*., 1993a; McKeefry and Zeki, 1997; Zeki and Marini, 1998). In type and extent, the abnormalities seen in AD go well beyond the loss of neurons in striate cortex described in normal aging (Polidori *et al*., 1993). Some investigators even dispute whether there is any age-related reduction of neuronal density in striate cortex (Spear, 1993).

III. Object Discrimination and Recognition

A. Evidence for Deficits in Object Discrimination and Recognition

The ability to discriminate and recognize objects is disrupted in patients with AD relative to healthy elderly adults. By ''objects,'' we mean letters, words, faces, and patterns, as well as common objects such as animals and manufactured items. Difficulties in reading letters and words are well documented in the AD literature (Cogan, 1979, 1985; Appel *et al*., 1982; Cummings *et al*., 1986; Butter *et al*., 1996; Furey-Kurkjian *et al*., 1996; Gilmore *et al*., 1996).

Object and pattern discrimination deficits are common in AD (Mendez *et al*., 1990a,b). They include difficulties in identifying line drawings of overlapping or masked objects (Mendez *et al*., 1990a,b; Butter *et al*., 1996; Giannakopoulos *et al*., 1999), fragmented objects (Corkin, 1982; Mendez *et al*., 1990a,b; Mendola *et al*., 1995; Mielke *et al*., 1995; Butter *et al*., 1996), pattern contours (Kurylo *et al*., 1996), figures embedded in noise (Kurylo *et al*., 1994b; Kurylo, 1999), or complete objects (Huff, 1990; Mendez *et al*., 1990a,b; Jacobs *et al*., 1995; Furey-Kurkjian *et al*., 1996; Giannakopoulos *et al*., 1999).

The prevalence of object discrimination deficits may be high in individuals with AD, and the degree of impairment substantially exceeds normal age-related decrements in performance on tasks such as visual closure (Read, 1988), word discrimination (Lawrence *et al*., 1998), and pattern completion (Esposito *et al*., 1999). In our study, the prevalence rate for dysfunction was 30% for identification of incomplete pictures alone (14/46 AD patients) (Mendola *et al*., 1995). Even more striking were the findings of Mendez and colleagues (1990a), who reported that 57% of patients (17/30) were unable to visually recognize actual common objects, and 100% were impaired on figure–ground analysis, including those with the mildest dementia severity. Classic visual agnosia occurs in a subgroup of patients (Cogan, 1979, 1985; Hof and Bouras, 1991; Mendez *et al*., 1990b). Curiously, a history of visual agnosia may be associated with visual hallucinations in AD patients, possibly suggesting common cortical substrates (Cogan, 1985; Holroyd and Sheldon-Keller, 1995). Alternatively, the combination of impaired object recognition and paranoid thinking may predispose some patients to the experience of hallucinations (Mendez *et al*., 1990b).

Face discrimination deficits in AD presumably arise at multiple stages of information processing, including levels such as memory (Wilson *et al*., 1982; Bäckman and Herlitz, 1990; Ricker *et al*., 1994), semantic knowledge (Hodges *et al*., 1993; Greene and Hodges, 1996), naming (Becker *et al*., 1995), or cognitive interpretation (Mendez *et al*., 1992). Mendez and colleagues (1992) evaluated the records of 217 patients with AD and found that 25% showed cognitive interpretation deficits. Of these 55 patients, 34 exhibited transient misidentifications of familiar persons; 11 showed Capgras syndrome, in

which they denied the identity of their caregiver and substituted an imposter; five misidentified themselves in mirrors; three showed overt prosopagnosia; and two had other unusual deficits in person identification. Cogan (1985) described multiple face recognition impairments within a single case. Difficulties with both famous faces (Mendez *et al*., 1990a) and unfamiliar faces (Kurylo *et al*., 1996) have been reported. In accordance with theories that distinct pathways are involved in the processing of familiar and unfamiliar faces (Bruce and Young, 1986), double dissociations occur in AD, with some patients more impaired on the recognition of familiar faces and others on the discrimination of unfamiliar faces (Della Sala *et al*., 1995). It is clear, however, that a large percentage of patients are impaired at processing both types of faces (Becker *et al*., 1995). Age-related decrements in face discrimination and recognition occur in the healthy population (Eslinger and Benton, 1983; Koss *et al*., 1991), to a lesser degree than in AD.

Perceptual organization may be another stage of information processing contributing to performance on tasks of face discrimination and recognition. It has been reported that facial processing in AD is related to the ability to identify objects from a complex array (Ricker *et al*., 1994), a capacity that is dependent on figure–ground discrimination and featural synthesis, both of which are impaired in AD (Mendez *et al*., 1990a; Kurylo *et al*., 1994b). Patients also have difficulty with perceptual grouping that uses the cues of proximity and global organization. The perceptual grouping deficit along with the impaired ability to identify degraded forms are correlated with deficits in face discrimination in AD (Kurylo *et al*., 1996; Kurylo 1999).

B. Relation of Visual Dysfunction to Deficits in Object Discrimination and Recognition

There is evidence that basic-level visual deficits, specifically in low spatial frequency contrast sensitivity, may contribute significantly to the difficulty that healthy elderly as well as demented individuals have with face discrimination. Contrast sensitivity reflects the minimum amount of contrast that an individual needs to resolve a stimulus of a given size. Standard stimuli are sinusoidally modulated gratings, the number (cycles) of which varies within a set overall width, conventionally one degree of visual angle. The measure of this spatial frequency is cycles per degree. Stimuli of different spatial frequencies have different contrast thresholds. For most human observers, contrast sensitivity is less acute at low and high relative to mid-range frequencies, producing the characteristic contrast sensitivity curve. When the stimulus is a face, it is conventional to use a face width as the measure of overall size. The unit of measure is cycles per face rather than cycles per degree. Cycles per face is defined as the number of sinusoidal repetitions that can be placed within the eye-level width of the face. In order to distinguish between spatial frequency as measured in cycles per degree and face measurements in cycles per face, we refer to the latter as the facial frequency. Faces contain low frequency information in their general shape, shading, and contours, as well as higher frequency information in fine details. In normal observers, contrast sensitivity at low spatial frequencies is related to the ability to detect and discriminate faces. Healthy older adults require higher contrast than young adults to detect and discriminate photographed faces (Owsley *et al*., 1981).

Contrast sensitivity at low spatial frequencies is salient to face discrimination in patients with AD (Nissen *et al*., 1985). We have reported that contrast sensitivity is impaired in AD, especially at low spatial frequencies (Cronin-Golomb *et al*., 1991, 1995; Mendola *et al*., 1995). Because changes in face size result in shifts in the sensitivity curve, we hypothesized that by adjusting the size of face stimuli presented, we could use the natural frequency response of the visual system to enhance the perception of the low facial frequency content and thereby enhance discrimination of face stimuli in AD patients. We found that reducing face size in order to enhance relatively the contrast sensitivity at low facial frequencies resulted in normal face discrimination in AD. Relative enhancement of middle or high facial frequencies, however, through use of medium- and large-sized faces, respectively, was associated with impaired performance (Cronin-Golomb *et al*., 2000).

It is counterintuitive that a reduction in face size should lead to better performance, because in normal older individuals, small angular character size for stimuli such as letters and words (under 0.3 degrees) often leads to impaired performance, such as on tests of reading speed (Akutsu *et al*., 1991). For faces, however, the important information shifts into a lower spatial frequency range as the size of the face increases. The control participants had good sensitivity for the lower spatial frequencies, as shown by their scores on the contrast sensitivity test, and may have been able to use that information for face discrimination. By contrast, the patients with AD had relatively poor sensitivity in that range. They would therefore have been less able to use that critical band of information and consequently performed the face discrimination task more poorly than did the control group.

For the group of 18 control participants reported in our study, age was strongly correlated with the ability to discriminate faces of any size. No such correlation was present for our sample of 18 AD patients matched to the control group for age, education, binocular central acuity, and ratio of men to women (8 men and 10 women in each group), indicating that in this sample, contrast sensitivity status was much more salient to face discrimination than were age effects. It is worth commenting on a different pattern of results in an additional 10 AD patients who were not included in the final study because they were not as well matched to the control group for the demographics of age, education, and ratio of men to women. In this excluded sample, the AD patients were somewhat older, less educated, and most were men (8 of 10). They were more demented than the final study group, though not so demented that their ability to understand task instructions was significantly compromised (mean score of 19.7 on Mini-Mental State Exam, compared to 21.6 in the final study group). Their contrast sensitivity at the high spatial frequency of 18 cycles per degree was relatively depressed (mean log sensitivity 0.54, compared to 0.75 in the final study group), as was Snellen acuity (median 20/30, compared to a median of 20/20 in the final study group). They did not show the effect of low-frequency enhancement on face discrimination, performing equally poorly with all three face sizes. Though their performance with large (mean score 11.3 correct of 17) and medium

sizes (mean score 10.1) was comparable to that of the final study sample (mean scores of 11.0 and 10.6 respectively), they were substantially impaired with the small faces (mean score 9.3), whereas the final study sample attained scores that were not significantly different from those of the control group (mean AD score 12.1). Although these excluded patients were few in number, their failure to demonstrate the pattern of performance shown by the control-matched study sample suggests that some constellations of demographic and visual features permit while others may prevent the visually mediated enhancement of cognitive performance. It will be important to investigate further these feature constellations.

Whereas contrast sensitivity appears to be relevant to understanding face discrimination deficits in AD, low-level visual symptoms associated with dorsal-stream dysfunction, such as optic ataxia, oculomotor apraxia, and simultanagnosia, do not seem related to poor face discrimination (Furey-Kurkjian *et al*., 1996).

In regard to the discrimination and recognition of nonfacial stimuli, we have reported that performance on a pattern masking task accounted for at least 25% of the variance on tests of object identification (Gollin Incomplete Pictures, 50%), word identification (Stroop Color-Word Test, 46%), and pattern completion (Raven's Coloured Progressive Matrices, 25%). Low spatial frequency contrast sensitivity was also a good predictor of performance on object-based cognitive tasks (Cronin-Golomb *et al*., 1995). Mendez and colleagues (1990a) likewise found strong correlations of visual symptoms with performance on tests of figure–ground discrimination and object recognition.

C. Brain Bases of the Impairment in Object Discrimination and Recognition

Object discrimination and recognition are subserved by the temporal lobes, as shown in humans by lesion studies (Milner, 1958; Meier and French, 1965; Lansdell, 1968; Newcombe and Russell, 1969; Newcombe *et al*., 1987) and by studies of regional cerebral blood flow (Haxby *et al*., 1991, 1994; Esposito *et al*., 1999). The temporal lobes undergo a loss of volume as a concomitant of normal aging (Coffey *et al*., 1992), which may be related to some of the mild age-related declines in performance described above (section III,A). Brain activation as measured by cerebral blood flow may be extended with increased age. For example, like young adults, elderly adults recruit temporal regions during a pattern-completion task, but unlike young adults, they fail to suppress prefrontal regions (Esposito *et al*., 1999).

The primary source of dysfunction in object discrimination and recognition in AD is the direct disruption of the object recognition pathway from the occipital to the temporal lobes, including extensive neuropathological damage to the temporal lobe itself (Brun and Englund, 1981; Pearson *et al*., 1985; Lewis *et al*., 1987; Braak *et al*., 1989; Arnold *et al*., 1991; Hof and Bouras, 1991). Relative to primary visual cortex, the density of neurofibrillary tangles increases 20-fold in parastriate cortex (Brodmann's area 18) and doubles again in inferotemporal cortex (Brodmann's area 20). The tangle distribution correlates positively with the regional distribution of pyramidal neurons that give rise to long corticocortical projections, which appear to be disrupted in AD (Lewis *et al*., 1987). Tangle density in occipital and occipitotemporal cortices (Brodmann's areas 18, 19, and 37) also correlates with a history of associative visual agnosia (Giannakopoulos *et al*., 1999). Within the temporal lobes, AD patients with a variety of visual complaints show reduced glucose metabolism relative to normal individuals (Pietrini *et al*., 1996). Although AD patients exhibit increased regional cerebral blood flow in the temporal lobes that is comparable to that seen in healthy elderly adults on a face-matching task, there may be abnormal functional connectivity in the AD group, who, compared to the control group, show additional activation in occipital and frontal cortices (Grady *et al*., 1993). Right temporal and hippocampal volume reduction in AD, as shown on structural imaging, are associated with impaired performance on a test of face recognition, whereas left hippocampal reduction of volume is related to word recognition memory (Cahn *et al*., 1998).

The occipitotemporal visual processing stream receives input from the parvocellular and magnocellular pathways that extend from the retina to visual association cortex. Functions associated mainly with the magnocellular pathway, the principal input to the occipitoparietal stream, are described in the section on spatial localization below. Those functions dependent upon the parvocellular pathway, such as color and texture discrimination, are impaired in many individuals with AD (Cronin-Golomb *et al*., 1991, 1993a; Kurylo *et al*., 1994a; Mendola *et al*., 1995). Parvocellular pathway dysfunction is supported by findings of a greater density of senile plaques in parvocellular than in magnocellular regions of the lateral geniculate nucleus (Leuba and Saini, 1995).

IV. SPATIAL LOCALIZATION

A. Evidence for Deficits in Spatial Localization

Spatial localization, broadly defined, includes the ability to orient oneself to aspects of the environment and to relate spatially those aspects of the environment external to the self. Clinical observations and research findings indicate that AD leads to severe impairments in numerous aspects of spatial localization (Mendez *et al*., 1990a,b; Ogden, 1990; Cronin-Golomb *et al*., 1993b; Kurylo *et al*., 1996), many of which are preserved in normal aging. The various aspects of spatial function may be categorized as constructional abilities, personal (egocentric) localization, extrapersonal (allocentric) localization, and spatial cognition.

Constructional apraxia includes deficiencies in copying or drawing two- or three-dimensional designs, and in assembling whole forms from elements. It occurs in the early as well as later stages of AD (Henderson *et al*., 1989; Mendez *et al*., 1990b; Ogden, 1990; Ricker *et al*., 1994; Cahn-Weiner *et al*., 1999). Complex form copying often is preserved in normal elderly adults (Koss *et al*., 1991; Janowsky and Thomas-Thrapp, 1993). By contrast, three-dimensional construction such as measured with the Block Design Test may be less accurate for old than for young adults (Plude *et al*., 1986; Koss *et al*., 1991) and may furthermore become less accurate with age within an elderly sample (Wahlin *et al*., 1993; Libon *et al*., 1994). An understudied possible concomitant of constructional difficulty in some patients with AD is unilateral spatial neglect, which may arise early (Ishiai *et al*., 1996) or late in the disease course (Venneri *et al*., 1998) and may affect visual search of objects in space (Mendez *et al*., 1997).

Personal (egocentric) orientation refers to the judgment of the spatial relation of one's own body to objects external to it, whereas extrapersonal (allocentric) orientation refers to the assessment of the spatial relation between objects external to the body. Patients with AD show impairments in both types of orientation. The disruption of personal orientation in AD is demonstrated by patients' poor use of a road map that requires them to negotiate directions in relation to their own body position (Mendola *et al*., 1995; Armstrong and Cloud, 1998). Although normal elderly adults significantly outperform patients with AD, they tend to be slower and less accurate at some types of map reading and perspective-taking than are young adults (Ohta *et al*., 1981; Aubrey and Dobbs, 1990; Aubrey *et al*., 1994). In healthy adults ranging in age from 50 to 85, age correlated significantly with performance. It is noteworthy that healthy women are slower at making map-position decisions than are age-matched men, though they are not less accurate (Aubrey *et al*., 1994). Gender differences often are not examined in the literature for either normal aging or AD, although they have been documented even in basic visual capacities such as motion perception (Gilmore *et al*., 1994). Environmental familiarity effects also are salient in normal aging (Kirasic, 1989) and remain mostly unexplored.

Extrapersonal orientation deficits are shown by difficulties in manipulating objects in space and in relating environmental landmarks to each other spatially. A common sign in AD is the tendency to become lost in familiar surroundings (Cogan, 1979, 1985; Henderson *et al*., 1989) or to follow unfamiliar routes. This problem may arise from an inability to judge one's position or self-movement in relation to the environment (personal orientation), or to judge spatial landmarks in the environment in relation to each other (extrapersonal orientation), or both (Mendez *et al*., 1990b; Tetewsky and Duffy, 1999).

Spatial cognition comprises such abilities as form matching, mental rotation, judgment of line orientation, and object location. Form matching is impaired in very early (Kaskie and Storandt, 1995) and midstage AD (Rosen and Mohs, 1982). AD deficits in mental rotation include the inability to rotate objects in order to effect form matching (Mendola *et al*., 1995; Kurylo *et al*., 1996) as well as the failure to detect object rotations (Mendez *et al*., 1990a; Kaskie and Storandt, 1995). Normal older adults tend to be slower than young adults on tests of mental rotation (Dollinger, 1995), but the age groups may be equally accurate if the tests are untimed (Cerella *et al*., 1981; Sharps and Gollin, 1987; Grady *et al*., 1994). Judgment of at least some aspects of line orientation is impaired in AD (Ska *et al*., 1990; Ricker *et al*., 1994; Finton *et al*., 1998), whereas findings in the normal aging literature are mixed. Equivalent performance has been reported across the wide age range of 55 to 84 years (Ska *et al*., 1990) and in groups ages 64–74 *versus* 75–94 (Libon *et al*., 1994). Others find age-related decrements in performance over a span of 65 to 94 years (Eslinger and Benton, 1983). AD patients also show a reduced ability to scan fields in order to locate objects and to reach for them (Mendez *et al*., 1990a).

The prevalence of spatial dysfunction may be high. We reported prevalence rates of 39% for a test of mental rotation (14/36 AD patients) and 29% for a test of map reading (10/35 AD patients) (Mendola *et al*., 1995). Spatial localization may be especially impaired in a subgroup of patients (Ross *et al*., 1996), including those with symptoms of Bálint's syndrome or other such prominent visual symptoms (Kiyosawa *et al*., 1989; Trick *et al*., 1989; Mendez *et al*., 1990a,b,c; Pietrini *et al*., 1996; see Chapters 10 and 18). Common types of deficits in these patients include difficulties in driving, following lines of text when reading, telling time from a watch, writing, and drawing (Pietrini *et al*., 1996).

B. Relation of Visual Dysfunction to Deficits in Spatial Localization

Relatively low-level visual deficits may contribute to the difficulty that AD patients have with some aspects of spatial localization. The perception of optic flow, which is related to motion perception, was found to be impaired in some individuals with AD and was correlated both with dementia severity and with memory for a real-world spatial navigation task (Tetewsky and Duffy, 1999). Some patients with AD plus Bálint's syndrome show deficient contrast sensitivity at low spatial frequencies relative to AD patients without this visuospatial syndrome (Mendez *et al*., 1990c).

The presence of visual symptoms has been reported to correlate with performance on tests of eye–hand coordination, a spatial function (Mendez *et al*., 1990a). We have reported rather weak correlations of visual abilities with performance on higher-order spatial localization tests (Cronin-Golomb *et al*., 1995). Specifically, the best visual predictor, low spatial frequency contrast sensitivity, accounted for only 11% of the variance on a mental rotation task, and for only 2% of the variance on a test of road-map reading.

C. Brain Bases of the Impairment in Spatial Localization

Spatial localization is subserved by the parietal lobes, as shown by human lesion studies (Butters and Barton, 1970; Butters *et al*., 1970, 1972; Newcombe *et al*., 1987) and functional imaging (Cohen *et al*., 1996; Richter *et al*., 1997). The same tasks supported by the parietal lobes often are not supported by the temporal lobes (Butters *et al*., 1972; Newcombe *et al*., 1987; Cohen *et al*., 1996), with the exception of tasks that require semantic knowledge as well as visuospatial abilities, such as clock drawing (Cahn-Weiner *et al*., 1999). Temporal areas appear to be important in drawing of complex figures (Mega *et al*., 1998). In normal individuals, performance of a localization task resulted in increased regional cerebral blood flow in superior parietal cortex (Haxby *et al*., 1991, 1994). On this type of task, brain activation patterns change with age, with cerebral blood flow increased in temporal as well as parietal regions in healthy elderly adults. By contrast, young adults recruit parietal regions relatively exclusively. The fact that the older adults perform the task as well as younger adults indicates that changes in activation patterns with age do not translate directly into changes in cognitive performance (Grady *et al*., 1994).

The main source of dysfunction in spatial localization in AD is the disruption of the dorsal pathway from the occipital to the parietal lobes (Grady *et al*., 1993), including extensive neuropathology in the parietal lobe itself (Brun and Englund, 1981; Pearson *et al*., 1985; Lewis *et al*., 1987; Braak *et al*., 1989; Arnold *et al*., 1991). In regard to metabolic function,

AD patients with a variety of visual complaints showed reduced glucose metabolism relative to normal individuals in some occipital and parietal regions (Kiyosawa *et al*., 1989; Pietrini *et al*., 1996). In the case of unilateral spatial neglect, imaging scans provide evidence of asymmetric parietal atrophy in AD (Kaida *et al*., 1998; Venneri *et al*., 1998).

The main input of the dorsal stream is the magnocellular pathway. Some visual capacities that are dependent upon the magnocellular pathway and dorsal stream may be performed normally by AD patients. For example, normal performance is seen for motion perception at some speeds (Kurylo *et al*., 1994a; Mendola *et al*., 1995) and for flicker perception (Cronin-Golomb *et al*., 1991; Mendola *et al*., 1995). Poor performance by patients with AD on some tasks of motion perception (Trick and Silverman, 1991; Gilmore *et al*., 1994) may reflect relatively high cognitive demands of those tasks (Cronin-Golomb, 1995).

Even with minimal cognitive demands, however, tests of dorsal-stream dysfunction may reveal impairments in AD. Mentis and colleagues (1996), using passive visual stimulation, described abnormal AD response to high-frequency pattern flash and to apparent motion, findings that are suggestive of dysfunction of striate cortex and the middle temporal area, MT, respectively. An understanding of whether or not AD patients are impaired on motion tasks will need to take into account not only task demands but also the possibility of dissociations. For example, one study reported normal motion detection (unconscious) and abnormal motion perception (conscious) in AD (Silverman *et al*., 1994), a dissociation that supports the proposition that the source of most visual dysfunction in AD occurs beyond striate cortex. The medial superior temporal area of the dorsal stream, MST, is the likely substrate of the processing of optic flow, which in turn is associated with memory for spatial navigation (Tetewsky and Duffy, 1999).

Disproportionate compromise of the occipitoparietal stream and its input, the magnocellular pathway, appears to occur in AD patients who show visual symptoms characteristic of Bálint's syndrome (Hof *et al*., 1989, 1990, 1997; Mendez *et al*., 1990c), as well as in some AD patients who exhibit suggestive electrophysiological abnormalities (Trick *et al*., 1989). The neuropsychological profile of such patients is distinguishable from that of AD patients without the symptoms of extensive occipitoparietal dysfunction. For example, constructional difficulties, such as in complex figure drawing and block construction, may be significantly worse in the Bálint's subgroup than in other AD patients (Furey-Kurkjian *et al*., 1996). Biparietal atrophy may be prominent in the subgroup of patients with visuospatial difficulties that in the early stages of the disease are out of proportion to deficits in other domains, such as memory and language (Ross *et al*., 1996).

V. Comparison of Object and Spatial Function

A. Evidence for Deficits in Object and Spatial Function

On spatial tasks, normal aging, speed or accuracy of performance often is reduced relative to performance on tasks of object discrimination and recognition (Koss *et al*., 1991; Grady *et al*., 1994; Lawrence *et al*., 1998). In one study, the degree of dissociation in performance between object discrimination (assessed with the Benton Facial Recognition Test) and spatial localization (assessed with Judgment of Line Orientation) increased with age. Specifically, spatial localization was worse in 9 of the 12 healthy elderly adults who showed a dissociation of 2 or more standard deviations. It was noteworthy, however, that dissociations occurred in quite few of the 178 normal individuals tested (Eslinger and Benton, 1983).

Most patients with AD perform poorly on tests of object discrimination and recognition and also on tests of spatial localization (Mendez *et al*., 1990a,b; Coslett *et al*., 1995; Butter *et al*., 1996; Kurylo *et al*., 1996). Some comparison studies suggest that within and across patients, object recognition is more impaired than spatial localization in AD patients without specific visual symptomatology (Butter *et al*., 1996; Kurylo *et al*., 1996), though others report more severe impairment in spatial localization (constructional abilities) than in object discrimination (face perception) (Haxby *et al*., 1990). It often is difficult to match object and spatial tests for difficulty, as pointed out by Mentis and colleagues (1996).

Because of the heterogeneity of cognitive performance from patient to patient, different AD samples may well give different results, making it difficult to infer relative preservation and impairment of performance in various domains of visual cognition. Some AD patients appear to be unimpaired on a test of face perception but unable to perform a dot location test (Mentis *et al*., 1996). By contrast, Kurylo and colleagues (1996) reported that the same tests yielded the opposite pattern of results in their AD sample: abnormal face discrimination and normal dot location. These investigators attempted to compare AD performance on tests of object recognition and spatial localization (four of each) by conducting separate discriminant analyses for each test type. They found that even though the patients were significantly impaired on both sets of tests, the object recognition tests were better at discriminating AD patients from healthy elderly adults than were the spatial tests (Fig. 36.2).

B. Relation of Visual Dysfunction to Deficits in Object and Spatial Function

Lower-level visual dysfunction may contribute differentially to downstream dysfunction in object recognition and spatial localization, suggesting that AD may affect the two cortical visual processing streams in different ways. Our correlative study of vision and cognition in AD (Cronin-Golomb *et al*., 1995) indicated that deficits in pattern masking and contrast sensitivity accounted for substantially more performance variance on tests of object completion, pattern completion, and word identification (25–50%) than on tests of mental rotation or map reading (2–11%). A similar finding was reported by Mendez and colleagues (1990a), who found the strongest correlation of some visual symptoms to be with performance on tests of figure–ground discrimination ($r = 0.72$) rather than on tests of spatial localization. These investigators did report, however, that eye–hand coordination, a spatial function, was as strongly correlated with visual symptoms as was performance on tests of object recognition ($r = 0.63$ for both).

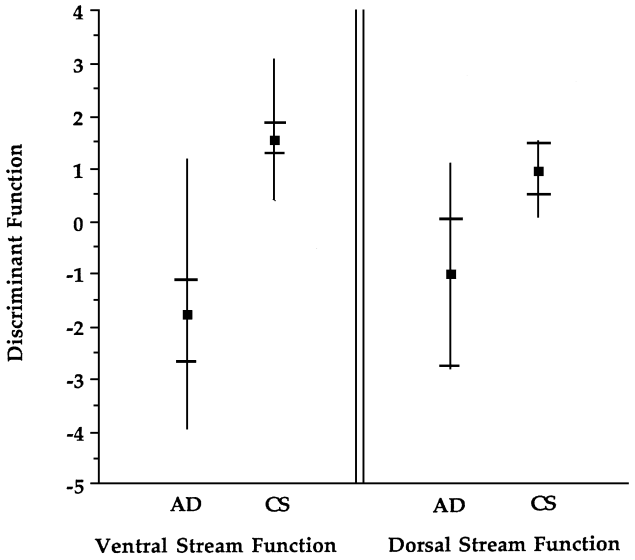

FIG. 36.2. Distribution of discriminant function scores for each of two test categories (ventral stream, object recognition *versus* dorsal stream, spatial localization) from the standardized discriminant function that best differentiated patients with Alzheimer's disease (AD) and healthy elderly control participants (CS). Distribution mean (filled square), 25th and 75th percentiles (short horizontal lines) and range (vertical lines) are indicated for each group. From Kurylo *et al.* (1996). Copyright © 1996 by the American Psychological Association. Reprinted with permission.

C. Brain Bases of the Impairment in Object and Spatial Function

Comparison studies such as those of Butter and colleagues (1996) and Kurylo and colleagues (1996), which suggest that object recognition is more impaired than spatial localization in AD patients unselected for visual symptomatology, are in accordance with studies showing greater density of AD neuropathology in inferotemporal relative to posterior parietal cortex (Arnold *et al.*, 1991; Bouras *et al.*, 1994) and the greater density of senile plaques in parvocellular than in magnocellular regions of the lateral geniculate nucleus (Leuba and Saini, 1995). By contrast, in normal aging there is some suggestion that age-related differences in brain activation were more widespread for a spatial task (mental rotation) than for an object (face) discrimination task (Grady *et al.*, 1994).

Subgroups of AD patients showing "visuoperceptual" or "visuospatial" impairments out of proportion to deficits in memory, language, and other domains may represent two different clinical syndromes, depending on the specific locus of bilateral posterior atrophy. Whereas the biparietal subgroup may be characterized by impairments of spatial localization and visually guided movement with or without Bálint's syndrome, the occipitotemporal subgroup may be characterized by visual agnosia, alexia, and difficulties in the processing of color and faces (Ross *et al.*, 1996).

Although the evidence points to disruption of both the dorsal and ventral visual processing streams across the AD population as a whole, the relative and possibly differential vulnerability of the respective streams to the AD process in individuals and subgroups remains mostly unknown. One single-case study reported no difference in the density of neurofibrillary tangles in the dorsal versus the ventral regions of parastriate and peristriate cortex (Levine *et al.*, 1993). To date, more extensive pathology in one stream relative to the other in extrastriate cortex has been documented only in cases of AD with Bálint's syndrome, with the dorsal stream more affected than the ventral (Hof *et al.*, 1990).

On the basis of the available histological, metabolic, and behavioral evidence, we conclude that in AD there is dysfunction at multiple sites along the ventral and dorsal visual processing streams. We interpret the behavioral results as implicating the posterior parietal cortex as the primary locus of the spatial localization deficit in AD, and the inferior temporal lobe as the primary locus of the object recognition deficit in AD. Pathological changes in these cortical areas are presumed to disrupt cognitive capacities directly. The convergence of evidence further suggests that an additional source of cognitive dysfunction in AD is upstream in the extrastriate occipital cortex as well as the magno- and parvocellular pathways that provide input to the dorsal and ventral cortical visual streams.

VI. Clinical Relevance of Impaired Vision and Visual Cognition

Information on vision and visual cognition may be useful in functional assessment and intervention for the individual patient with AD as well as for relatively normal elderly adults. Clinicians and family members need to be alerted to the possibility that an older adult may experience difficulties in activities of daily living that are attributable to specific visual dysfunctions, especially in light of our findings that prevalent visual deficits can strongly predict cognitive impairment. For example, evidence of impaired color discrimination in AD may contraindicate the use of color coding to help a patient distinguish between different room doors, pill bottles, or other objects important to everyday activities.

Poor object recognition resulting from impaired inputs to temporal cortex and degeneration of that cortex itself leads to the expectation of deficient functional interaction with everyday objects. Deficits in spatial localization, arising from pathological change in parietal cortices and their inputs, may render an individual unable to orient effectively to the environment. This difficulty may manifest itself as decreased locomotion in some patients and wandering in others, and is especially likely to occur after a change from processing a long-known spatial layout (such as a home) to a new, unfamiliar layout (a relative's home, or an institution). Even after months or years in the relatively new environment, there may be little accommodation by the patient because of the impaired ability to encode as well as retrieve spatial information.

Recognizing that the source of difficulties in daily function may be at least in part visual can provide clinicians and researchers with new ways to develop and apply interventions. The goal would be to enhance cognitive performance through interventions aimed at restoring visual capacities. For example, as described above, enhancing color contrast may improve aspects of daily function in institutionalized AD patients, including increases in the amounts of food and liquids ingested, and decreases in the frequency of night-time agitated

behaviors (Koss and Gilmore, 1998; Dunne and Neargarder, 1999). Designers of living spaces for patients with AD have made great strides in recent years in attempting to minimize visuocognitive deficits (Brawley, 1997; Warner, 1998). Strategies include use of living areas that are highly differentiated (to employ residual object discrimination and recognition) and abolishment of long corridors and maze-like walking paths (to minimize difficulties in spatial localization). The application of basic findings on vision and visual cognition in AD to everyday life is relatively recent and encourages further research to aid in this effort.

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References

Aguglia, U., Gambarelli, D., Farnarier, G., and Quattrone, A. (1991). Different susceptibilities of the geniculate and extrageniculate visual pathways to human Creutzfeldt-Jakob disease (a combined neurophysiological and neuropathological study). *Electroencephalogr. Clin. Neurophysiol.* **78**, 413–423.

Akutsu, H., Legge, G. E., Ross, J. A., and Schuebel, K. J. (1991). Psychophysics of reading. X. Effects of age-related changes in vision. *J. Gerontol. Psychol. Sci.* **46**, P325–P331.

Alzheimer, A. (1907). A characteristic disease of the cerebral cortex *In* "Allgemeine zeitschrift für Psychiatrie und Psychisch-Gerichtliche Medizin," (E. Schultze and O. Snell, eds.), Vol. 64, pp. 146–148. Georg Relmer, Berlin.

American Psychiatric Association (APA). (1994). "Diagnostic and Statistical Manual of Mental Disorders." APA, Washington, DC.

Appel, J., Kertesz, A., and Fisman, M. (1982). A study of language functioning in Alzheimer patients. *Brain Lang.* **17**, 73–91.

Armstrong, C. L., and Cloud, B. (1998). The emergence of spatial rotation deficits in dementia and normal aging. *Neuropsychology* **12**, 208–217.

Arnold, S. E., Hyman, B. T., Flory, J., and Damasio, A. R. (1991). The topographical and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer's disease. *Cereb. Cortex* **1**, 103–116.

Aubrey, J. B., and Dobbs, A. R. (1990). Age and sex differences in the mental realignment of maps. *Exp. Aging Res.* **16**, 133–139.

Aubrey, J. B., Li, K. Z. H., and Dobbs, A. R. (1994). Age differences in the interpretation of misaligned "you are here" maps. *J. Gerontol. Psychol. Sci.* **49**, P29–P31.

Bäckman, L., and Herlitz, A. (1990). The relationship between prior knowledge and face recognition memory in normal aging and Alzheimer's disease. *J. Gerontol.* **45**, 94–100.

Barbur, J. L., Sahraie, A., Simmons, A., Weiskrantz, L., and Williams, S. C. R. (1998). Residual processing of chromatic signals in the absence of a geniculostriate projection. *Vision Res.* **38**, 3447–3453.

Beach, T. G., and McGeer, E. G. (1988). Lamina-specific arrangement of astrocytic gliosis and senile plaques in Alzheimer's disease. *Brain Res.* **463**, 357–361.

Becker, J., Lopez, O., and Boller, F. (1995). Understanding impaired analysis of faces by patients with probable Alzheimer's disease. *Cortex* **31**, 129–137.

Binetti, G., Magni, E., Padovani, A., Cappa, S. F., Bianchetti, A., and Trabucchi, M. (1996). Executive dysfunction in early Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* **60**, 91–93.

Blanks, J. C., Torigoe, Y., Hinton, D. R., and Blanks, R. H. I. (1991). Retinal degeneration in the macula of patients with Alzheimer's disease. *Ann. N. Y. Acad. Sci.* **640**, 44–46.

Blanks, J. C., Torigoe, Y., Hinton, D. R., and Blanks, R. H. I. (1996a). Retinal pathology in Alzheimer's disease. I. Ganglion cell loss in foveal/parafoveal retina. *Neurobiol. Aging* **17**, 377–384.

Blanks, J. C., Schmidt, S. Y., Torigoe, Y., Porrello, K. V., Hinton, D. R., and Blanks, R. H. I. (1996b). Retinal pathology in Alzheimer's disease. II. Regional neuron loss and glial changes in GCL. *Neurobiol. Aging* **17**, 385–395.

Bouras, C., Hof, P. R., Giannakopoulos, P., Michel, J.-P., and Morrison, J. H. (1994). Regional distribution of neurofibrillary tangles and senile plaques in the cerebral cortex of elderly patients: A quantitative evaluation of a one-year autopsy population from a geriatric hospital. *Cereb. Cortex* **4**, 138–150.

Braak, H., Braak, E., and Kalus, P. (1989). Alzheimer's disease: Areal and laminar pathology in the occipital isocortex. *Acta Neuropathol.* **77**, 494–506.

Brawley, E. C. (1997). "Designing for Alzheimer's Disease." Wiley, New York.

Bruce, V., and Young, A. (1986). Understanding face recognition. *B. J. Psychol.* **77**, 305–327.

Brun, A., and Englund, E. (1981). Regional pattern of degeneration in Alzheimer's disease: Neuronal loss and histopathological grading. *Histopathology* **5**, 549–564.

Butter, C. M., Trobe, J. D., Foster, L. N., and Berent, S. (1996). Visual-spatial deficits explain visual symptoms in Alzheimer's disease. *Am. J. Ophthalmol.* **122**, 97–104.

Butters, N., and Barton, M. (1970). Effect of parietal lobe damage on the performance of reversible operations in space. *Neuropsychologia* **8**, 205–214.

Butters, N., Barton, M., and Brody, B. A. (1970). Role of the right parietal lobe in the mediation of cross-modal associations and reversible operations in space. *Cortex* **6**, 174–190.

Butters, N., Soeldner, C., and Fedio, P. (1972). Comparison of parietal and frontal lobe spatial deficits in man. Extrapersonal vs personal (egocentric) space. *Percept. Mot. Skills* **34**, 27–34.

Cahn, D. A., Sullivan, E. V., Shear, P. K., Marsh, L., Fama, R., Lim, K. O., Yesavage, J. A., Tinklenberg, J. R., and Pfefferbaum, A. (1998). Structural MRI correlates of recognition memory in Alzheimer's disease. *J. Int. Neuropsychol. Soc.* **4**, 106–114.

Cahn-Weiner, D. A., Sullivan, E. V., Shear, P. K., Fama, R., Lim, K. O., Yesavage, J. A., Tinklenberg, J. R., and Pfefferbaum, A. (1999). Brain structural and cognitive correlates of clock drawing performance in Alzheimer's disease. *J. Int. Neuropsychol. Soc.* **5**, 502–509.

Celesia, G. G., Villa, A. E., Bringell, M., Rubboli, G., Bolcioni, G., and Fiori, M. G. (1993). An electrophysiological study of visual processing in Alzheimer's disease. *Electroencephalogr. Clin. Neurophysiol.* **87**, 97–104.

Cerella, J., Poon, L., and Fozard, J. L. (1981). Mental rotation and age reconsidered. *J. Gerontol.* **36**, 620–624.

Coffey, C. E., Wilkinson, W. E., Parashos, I. A., Soady, S. A. R., Sullivan, R. J., Patterson, L. J., Figiel, G. S., Webb, M. C., Spritzer, C. E., and Djang, W. T. (1992). Quantitative cerebral anatomy of the aging human brain: A cross-sectional study using magnetic resonance imaging. *Neurology* **42**, 527–536.

Cogan, D. G. (1979). Visuospatial dysgnosia. *Am. J. Ophthalmol.* **88**, 361–368.

Cogan, D. G. (1985). Visual disturbances with focal progressive dementing disease. *Am. J. Ophthalmol.* **100**, 68–72.

Cohen, J., Cronin-Golomb, A., Growdon, J. H., and Corkin, S. (1988). Color vision deficits in Alzheimer's disease. *Soc. Neurosci. Abstr.* **14**, 219.

Cohen, M. S., Kosslyn, S. M., Breiter, H. C., DiGirolamo, G. J., Thompson, W. L., Anderson, A. K., Bookheimer, S. Y., Rosen, B. R., and Belliveau, J. W., (1996). Changes in cortical activity during mental rotation: A mapping study using functional MRI. *Brain* **119**, 89–100.

Corkin, S. (1982). Some relationships between global amnesias and the memory impairments of Alzheimer's disease. *In* "Alzheimer's Disease: A Report of Progress in Research" (S. Corkin, K. L. Davis, J. H. Growdon, and E. Usdin, eds.), pp. 149–164. Raven Press, New York.

Coslett, H. B., Stark, M., Rajaram, S., and Saffran, E. M. (1995). Narrowing the spotlight: A visual attentional disorder in presumed Alzheimer's disease. *NeuroCase* **1**, 305–318.

Cronin-Golomb, A. (1995). Vision in Alzheimer's disease. *The Gerontologist* **35**, 370–376.

Cronin-Golomb, A., Corkin, S., Rizzo, J. F., Cohen, J., Growdon, J. H., and Banks, K. S. (1991). Visual dysfunction in Alzheimer's disease: Relation to normal aging. *Ann. Neurol*. **29**, 41–52.

Cronin-Golomb, A., Sugiura, R., Corkin, S., and Growdon, J. H. (1993a). Incomplete achromatopsia in Alzheimer's disease. *Neurobiol. Aging* **14**, 471–477.

Cronin-Golomb, A., Corkin, S., and Rosen, T. J. (1993b). Neuropsychological assessment of dementia. *In* "Dementia" (P. J. Whitehouse, ed.), pp. 130–164. Davis, Philadelphia.

Cronin-Golomb, A., Corkin, S., and Growdon, J. H. (1995). Visual dysfunction predicts cognitive deficits in Alzheimer's disease. *Optom. Vision Sci*. **72**, 168–176.

Cronin-Golomb, A., Cronin-Golomb, M., Dunne, T. E., Brown, A. C., Jain, K., Cipolloni, P. B., and Auerbach, S. H. (2000). Facial frequency manipulation normalizes face discrimination in Alzheimer's disease. *Neurology* **54**, 2316–2318.

Cummings, J. L., Houlihan, J. P., and Hill, M. A. (1986). The pattern of reading deterioration in dementia of the Alzheimer type: Observations and implications. *Brain Lang*. **29**, 315–323.

Curcio, C. A., and Drucker, D. N. (1993). Retinal ganglion cells in Alzheimer's disease and aging. *Ann. Neurol*. **33**, 248–257.

Davies, D. C., McCoubrie, P., McDonald, B., and Jobst, K. A. (1995). Myelinated axon number in the optic nerve is unaffected by Alzheimer's disease. *Br. J. Ophthalmol*. **79**, 596–600.

Della Sala, S., Muggia, S., Spinnler, H., and Zuffi, M. (1995). Cognitive modeling of face processing: Evidence from Alzheimer's patients. *Neuropsychologia* **33**, 675–687.

Dollinger, S. M. C. (1995). Mental rotation performance: Age, sex, and visual field differences. *Dev. Neuropsychol*. **11**, 215–222.

Dunne, T. E., and Neargarder, S. (1999). Applications of vision research to everyday activity. *Proc. Abstr. Annu. Meet. East. Psychol. Assoc*. **70**, 55.

Eslinger, P. J., and Benton, A. L. (1983). Visuoperceptual performances in aging and dementia: Clinical and theoretical implications. *J. Clin. Neuropsychol* **5**, 213–220.

Esposito, G., Kirkby, B. S., Van Horn, J. D., Ellmore, T. M., and Berman, K. F. (1999). Context-dependent, neural system-specific neurophysiological comcomitants of ageing: Mapping PET correlates during cognitive activation. *Brain* **122**, 963–979.

Farnsworth, D. (1947). "The Farnsworth Dichotomous Test for Color Blindness Panel D-15." Psychological Corporation, New York.

Finton, M. J., Lucas, J. A., Graff-Radford, N. R., and Uitti, R. J. (1998). Analysis of visuospatial errors in patients with Alzheimer's disease or Parkinson's disease. *J. Clin. Exp. Neuropsychol*. **20**, 186–193.

Fisher, L., Freed, D. M., and Corkin, S. (1990). Stroop Color-Word Test performance in patients with Alzheimer's disease. *J. Clin. Exp. Neuropsychol*. **12**, 752–765.

Fletcher, R. (1980). "The City University Colour Vision Test." Keeler, London.

Furey-Kurkjian, M. L., Pietrini, P., Graff-Radford, N. R., Alexander, G. E., Freo, U., Szczepanik, J., and Schapiro, M. B. (1996). Visual variant of Alzheimer disease: Distinctive neuropsychological features. *Neuropsychology* **10**, 294–300.

Giannakopoulos, P., Gold, G., Duc, M., Michel, J.-P., Hof, P. R., and Bouras, C. (1999). Neuroanatomic correlates of visual agnosia in Alzheimer's disease. *Neurology* **52**, 71–77.

Gilmore, G. C., Koss, E., Wenk, H. E., and Whitehouse, P. J. (1993). Visual dysfunction in Alzheimer's disease. *Neurology* **43**, A173.

Gilmore, G. C, Wenk, H. E., Naylor, L. A., and Koss, E. (1994). Motion perception and Alzheimer's disease. *J. Gerontol*. **49**, P52–P57.

Gilmore, G. C., Thomas, C. W., Klitz, T., Persanyi, M. W., and Tomsak, R. (1996). Contrast enhancement eliminates letter identification speed deficits in Alzheimer's disease. *J. Clin. Geropsychol*. **2**, 307–320.

Grady, C. L., Haxby, J. V., Horwitz, B., Gillette, J., Salerno, J. A., Gonzales-Aviles, A., Carson, R. E., Herscovitch, P., Schapiro, M. B., and Rapoport, S. I. (1993). Activation of cerebral blood flow during a visuoperceptual task in patients with Alzheimer-type dementia. *Neurobiol. Aging* **14**, 35–44.

Grady, C. L., Maisog, J. M., Horwitz, B., Ungerleider, L. G., Mentis, M. J., Salerno, J. A., Pietrini, P., Wagner, E., and Haxby, J. V. (1994). Age-related changes in cortical blood flow activation during visual processing of faces and location. *J. Neurosci*. **14**, 1450–1462.

Greene, J. D., and Hodges, J. R. (1996). Identification of famous faces and famous names in early Alzheimer's disease. Relationship to anterograde episodic and general semantic memory. *Brain* **119**, 111–128.

Haxby, J. V., Grady, C. L., Koss, E., Horwitz, B., Heston, L. L., Schapiro, M. B., Friedland, R. P., and Rapoport, S. I. (1990). Longitudinal study of cerebral metabolic asymmetries and associated neuropsychological patterns in early dementia of the Alzheimer type. *Arch. Neurol. (Chicago)* **47**, 753–760.

Haxby, J. V., Grady, C. L., Horwitz, B., Ungerleider, L. G., Mishkin, M., Carson, R. E., Herscovitch, P., Schapiro, M. B., and Rapoport, S. I. (1991). Dissociation of object and spatial visual processing pathways in human extrastriate cortex. *Proc. Natl. Acad. Sci. U.S.A*. **88**, 1621–1625.

Haxby, J. V., Horwitz, B., Ungerleider, L. G., Maisog, J. M., Pietrini, P., and Grady, C. L. (1994). The functional organization of human extrastriate cortex: A PET-rCBF study of selective attention to faces and locations. *J. Neurosci*. **14**, 6336–6353.

Hedges, T. R., Galves, R. P., Spiegelman, D., Barbas, N. R., Peli, E., and Yardley, C. J. (1996). Retinal nerve fiber layer abnormalities in Alzheimer's disease. *Acta Ophthalmol. Scand*. **74**, 271–275.

Henderson, V. W., Mack, W., and Williams, B. W. (1989). Spatial disorientation in Alzheimer's disease. *Arch. Neurol. (Chicago)* **46**, 391–394.

Hinton, D. R., Sadun, A. A., Blanks, J. C., and Miller, C. A. (1986). Optic nerve degeneration in Alzheimer's disease. *N. Engl. J. Med*. **315**, 485–487.

Hodges, J., Salmon, D., and Butters, N. (1993). Recognition and naming of famous faces in Alzheimer's disease: A cognitive analysis. *Neuropsychologia* **31**, 775–788.

Hof, P. R., and Bouras, C. (1991). Object recognition deficit in Alzheimer's disease. Possible disconnection of the occipito-temporal component of the visual system. *Neurosci. Lett*. **122**, 53–56.

Hof, P. R., Bouras, C., Constantinidis, J., and Morrison, J. H. (1989). Balint's syndrome in Alzheimer's disease: Specific disruption of the occipito-parietal visual pathway. *Brain Res*. **493**, 368–375.

Hof, P. R., Bouras, C., Constantinidis, J., and Morrison, J. H. (1990). Selective disconnection of specific visual association pathways in cases of Alzheimer's disease presenting with Balint's syndrome. *J. Neuropathol. Exp. Neurol*. **49**, 168–184.

Hof, P. R., Bouras, C., Perl, D., Sparks, L., Mehta, N., and Morrison, J. H. (1995). Age-related distribution of neuropathologic changes in the cerebral cortex of patients with Down's syndrome. *Arch. Neurol. (Chicago)* **52**, 379–391.

Hof, P. R., Vogt, B. A., Bouras, C., and Morrison, J. H. (1997). Atypical form of Alzheimer's disease with prominent posterior cortical atrophy:

A review of lesion distribution and circuit disconnection in cortical visual pathways. *Vision Res*. **37**, 3609–3625.

Holroyd, S., and Sheldon-Keller, A. (1995). A study of visual hallucinations in Alzheimer's disease. *J. Geriat. Psychiatry* **3**, 198–205.

Huff, F. L. (1990). Language in normal aging and age-related neurological diseases. *In* ''Handbook of Neuropsychology'' (F. Boller and J. Grafman, eds.), Vol. 4, pp. 251–264. Elsevier, Amsterdam.

Ishiai, S., Okiyama, R., Koyama, Y., and Seki, K. (1996). Unilateral spatial neglect in Alzheimer's disease. *Acta Neurol. Scand*. **93**, 219–224.

Jacobs, D. M., Sano, M., Dooneief, G., Marder, K., Bell, K. L., and Stern, Y. (1995). Neuropsychological detection and characterization of preclinical Alzheimer's disease. *Neurology* **45**, 957–962.

Janowsky, J. S., and Thomas-Thrapp, L. J. (1993). Complex figure recall in the elderly: A deficit in memory or constructional strategy? *J. Clin. Exp. Neuropsychol*. **15**, 159–169.

Kaida, K., Takeda, K., Nagata, N., and Kamakura, K. (1998). Alzheimer's disease with asymmetric parietal lobe atrophy: A case report. *J. Neurol. Sci*. **160**, 96–99.

Kaskie, B., and Storandt, M. (1995). Visuospatial deficit in dementia of the Alzheimer type. *Arch. Neurol. (Chicago)* **52**, 422–425.

Kirasic, K. C. (1989). The effects of age and environmental familiarity on adults' spatial problem-solving performance: Evidence of a hometown advantage. *Exp. Aging Res*. **15**, 181–187.

Kiyosawa, M., Bosley, T. M., Chawluk, J., Jamieson, D., Schatz, N. J., Savino, P. J., Sergott, R. C., Reivich, M., and Alavi, A. (1989). Alzheimer's disease with prominent visual symptoms: Clinical and metabolic evaluation. *J. Ophthalmol*. **96**, 1077–1086.

Koss, E., and Gilmore, G. C. (1998). Environmental interventions and functional ability of AD patients. *In* ''Research and Practice in Alzheimer's Disease'' (B. Vellas, J. Fitten, and G. Frisoni, eds.), pp. 185–191. Serdi/Springer, Paris/New York.

Koss, E., Ober, B. A., Delis, D. C., and Friedland, R. P. (1984). The Stroop color-word test: Indicator of dementia severity. *Int. J. Neurosci*. **24**, 53–61.

Koss, E., Haxby, J. V., DeCarli, C., Schapiro, M. B., Friedland, R. P., and Rapoport, S. I. (1991). Patterns of performance preservation and loss in healthy aging. *Dev. Neuropsychol*. **7**, 99–113.

Kurylo, D. D. (1999). Perceived spatial relationships in Alzheimer's disease. *Invest. Ophthalmol. Visual Sci*. **40**, S388.

Kurylo, D. D., Corkin, S., Dolan, R. P., Rizzo, J. F., Parker, S. W., and Growdon, J. H. (1994a). Broad-band visual capacities are not selectively impaired in Alzheimer's disease. *Neurobiol. Aging* **15**, 305–311.

Kurylo, D. D., Corkin, S., and Growdon, J. H. (1994b). Perceptual organization in Alzheimer's disease. *Psychol. Aging* **9**, 562–567.

Kurylo, D. D., Corkin, S., Rizzo, J. F., and Growdon, J. H. (1996). Greater relative impairment of object recognition than visuospatial abilities in Alzheimer's disease. *Neuropsychology* **10**, 74–81.

Lansdell, H. (1968). Effect of extent of temporal lobe ablations on two lateralized deficits. *Physiol. Behav.* **3**, 271–273.

Lanthony, P. (1978). The new color test. *Doc. Ophthalmol*. **46**, 191–199.

Lawrence, B., Myerson, J., and Hale, S. (1998). Differential decline of verbal and visuospatial processing speed across the adult life span. *Aging, Neuropsychol. Cogn*. **5**, 129–146.

Leuba, G., and Saini, K. (1995). Pathology of subcortical visual centres in relation to cortical degeneration in Alzheimer's disease. *Neuropathol Appl. Neurobiol*. **21**, 410–422.

Levine, D. N., Lee, J. M., and Fisher, C. M. (1993). The visual variant of Alzheimer's disease: A clinicopathologic case study. *Neurology* **43**, 305–313.

Lewis, D. A., Campbell, M. J., Terry, R. D., and Morrison, J. H., (1987). Laminar and regional distributions of neurofibrillary tangles and neuritic plaques in Alzheimer's disease, a quantitative study of visual and auditory cortices. *J. Neurosci*. **7**, 1799–1808.

Lezak, M. D. (1995). ''Neuropsychological Assessment.'' Oxford University Press, New York.

Libon, D. J., Glosser, G., Malamut, B. L., Kaplan, E., Goldberg, E., Swenson, R., and Sands, L. P. (1994). Age, executive functions, and visuospatial functioning in healthy older adults. *Neuropsychology* **8**, 38–43.

Martinelli, V., Locatelli, T., Comi, G., Lia, C., Alberoni, M., Bressi, S., Rovaris, M., Franceschi, M., and Canal, N. (1996). Pattern visual evoked potential mapping in Alzheimer's disease: Correlations with visuospatial impairment. *Dementia* **7**, 63–68.

Matjucha, I. C. A., and Katz, B. (1994). Neuro-ophthalmology of aging. *In* ''Clinical Neurology of Aging'' (M. L. Albert and J. E. Knoefel, eds.), pp. 421–447. Oxford University Press, New York.

McCormack, W. C., Kukull, W. A., Van Belle, G., Bowen, J. G., Teri, L., and Larson, E. B. (1994). Symptom patterns and comorbidity in the early stages of Alzheimer's disease. *J. Am. Geriatr. Soc*. **42**, 517–521.

McKeefry, D. J., and Zeki, S. (1997). The position and topography of the human colour centre as revealed by functional magnetic resonance imaging. *Brain* **120**, 2229–2242.

Mega, M. S., Thompson, P. M., Cummings, J. L., Back, C. L., Xu, M. L., Zohoori, S., Goldkorn, A., Moussai, J., Fairbanks, L., Small, G. W., and Toga, A. W. (1998). Sulcal variability in the Alzheimer's brain. *Neurology* **50**, 145–151.

Meier, M. J., and French, L. A. (1965). Lateralized deficits in complex visual discrimination and bilateral transfer of reminiscence following unilateral temporal lobectomy. *Neuropsychologia* **3**, 261–272.

Mendez, M. F., Mendez, M. A., Martin, R., Smyth, K. A., and Whitehouse, P. J. (1990a). Complex visual disturbances in Alzheimer's disease. *Neurology* **40**, 439–443.

Mendez, M. F., Tomsak, R. L., and Remler, B. (1990b). Disorders of the visual system in Alzheimer's disease. *J. Clin. Neuro-ophthalmol*. **10**, 62–69.

Mendez, M. F., Turner, J., Gilmore, G. C., Remler, B., and Tomsak, R. L. (1990c). Balint's syndrome in Alzheimer's disease: Visuospatial functions. *Int. J. Neurosci*. **54**, 339–346.

Mendez, M. F., Martin, R. J., Smyth, K. A., and Whitehouse, P. J. (1992). Disturbances of person identification in Alzheimer's disease. *J. Nerv. Ment. Dis*. **180**, 94–96.

Mendez, M. F., Cherrier, M. M., and Cymerman, J. S. (1997). Hemispatial neglect on visual search tasks in Alzheimer's disease. *Neuropsychiatry, Neuropsychol. Behav. Neurol*. **10**, 203–208.

Mendola, J. D., Cronin-Golomb, A., Corkin, S., and Growdon, J. H. (1995). Prevalence of visual deficits in Alzheimer's disease. *Optom. Vision Sci*. **72**, 155–167.

Mentis, M. J., Horwitz, B., Grady, C. L., Alexander, G. E., VanMeter, J. W., Maisog, J. M., Pietrini, P., Schapiro, M. B., and Rapoport, S. I. (1996). Visual cortical dysfunction in Alzheimer's disease evaluated with a temporally graded ''stress test'' during PET. *Am. J. Psychiatry* **153**, 32–40.

Mielke, R., Kessler, J., Fink, G., Herholz, K., and Heiss, W.-D. (1995). Dysfunction of visual cortex contributes to disturbed processing of visual information in Alzheimer's disease. *Int. J. Neurosci*. **82**, 1–9.

Milner, B. (1958). Psychological defects produced by temporal lobe excision. *Res. Publ. Assoc. Res. Nerv. Ment. Dis*. **36**, 244–257.

Newcombe, F., and Russell, W. R. (1969). Dissociated visual perceptual and spatial deficits in focal lesions of the right hemisphere. *J. Neurol. Neurosurg. Psychiatry* **32**, 73–81.

Newcombe, F., Ratcliff, G., and Damasio, H. (1987). Dissociable visual and spatial impairments following right posterior cerebral lesions: Clinical, neuropsychological and anatomical evidence. *Neuropsychologia* **25**, 149–161.

Nissen, M. J., Corkin, S., Buonanno, F. S., Growdon, J. H., Wray, S., and Bauer, J. (1985). Spatial vision in Alzheimer's disease: General findings and a case report. *Arch. Neurol. (Chicago)* **42**, 667–671.

Ogden, J. A. (1990). Spatial abilities and deficits in aging and age-related disorders. *In* ''Handbook of Neuropsychology'' (F. Boller and J. Grafman, eds.), Vol. 4, pp. 265–278. Elsevier, Amsterdam.

Ohta, R. J., Walsh, D. A., and Kraus, I. K. (1981). Spatial perspective-taking ability in young and elderly adults. *Exp. Aging Res*. **7**, 45–63.

Owsley, C., Sekuler, R., and Boldt, C. (1981). Aging and low-contrast vision: Face perception. *Invest. Ophthalmol. Vision Sci*. **21**, 362–365.

Pearson, R. C. A., Esiri, M. M., Hiorns, R. W., Wilcock, G. K., and Powell, T. P. S. (1985). Anatomical correlates of the distribution of the pathological changes in the neocortex in Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A*. **82**, 4531–4534.

Pietrini, P., Furey, M. L., Graff-Radford, N., Freo, U., Alexander, G. E., Grady, C. L., Dani, A., Mentis, M. J., and Schapiro, M. B. (1996). Preferential metabolic involvement of visual cortical areas in a subtype of Alzheimer's disease: Clinical implications. *Am. J. Psychiatry* **153**, 1261–1268.

Pietrini, P., Dani, A., Furey, M. L., Alexander, G. E., Freo, U., Grady, C. L., Mentis, M. J., Mangot, D., Simon, E. W., Horwitz, B., Haxby, J. V., and Schapiro, M. B. (1997). Low glucose metabolism during brain stimulation in older Down's syndrome subjects at risk for Alzheimer's disease prior to dementia. *Am. J. Psychiatry* **154**, 1063–1069.

Plude, D. J., Milberg, W. P., and Cerella, J. (1986). Age differences in depicting and perceiving tridimensionality in simple line drawings. *Exp. Aging Res*. **12**, 221–225.

Polidori, C., Zeng, Y. -C., Zaccheo, D., and Amenta, F. (1993). Age-related changes in the visual cortex: A review. *Arch. Gerontol. Geriatr*. **17**, 145–164.

Read, D. E. (1988). Age-related changes in performance on a visual-closure task. *J. Clin. Exp. Neuropsychol*. **10**, 451–466.

Richter, W., Ugurbil, K., Georgopoulos, A., and Kim, S.-G. (1997). Time-resolved fMRI of mental rotation. *NeuroReport* **8**, 3702.

Ricker, J., Keenan, P., and Jacobson, M. (1994). Visuoperceptual-spatial ability and visual memory in vascular dementia and dementia of the Alzheimer type. *Neuropsychologia* **32**, 1287–1296.

Rizzo, J. F., Cronin-Golomb, A., Growdon, J. H., Corkin, S., Rosen, T. J., Sandberg, M. A., Chiappa, K. H., and Lessell, S. (1992). Retino-calcarine function in Alzheimer's disease: A clinical and electrophysiological study. *Arch. Neurol. (Chicago)* **49**, 93–101.

Rocco, F. J., Cronin-Golomb, A., and Lai, F. (1997). Alzheimer-like visual profile in Down syndrome. *Alzheimer Dis. Assoc. Disord*. **11**, 88–98.

Rosen, W. G., and Mohs, R. C. (1982). Evolution of cognitive decline in dementia. *In* ''Alzheimer's Disease: A Report of Progress in Research'' (S. Corkin, K. L. Davis, and J. H. Growdon, eds.), pp. 133–139. Raven Press, New York.

Ross, S. J. M., Graham, N., Stuart-Green, L., Prins, M., Xuereb, J., Patterson, K., and Hodges, J. R. (1996). Progressive biparietal atrophy: An atypical presentation of Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* **61**, 388–395.

Sharps, M. J., and Gollin, E. S. (1987). Speed and accuracy of mental image rotation in young and elderly adults. *J. Gerontol*. **42**, 342–344.

Silverman, S. E., Tran, D. B., Zimmerman, K. M., and Feldon, S. E., (1994). Dissociation between the detection and perception of motion in Alzheimer's disease. *Neurology* **44**, 1814–1818.

Ska, B., Poissant, P., and Joanette, Y. (1990). Line orientation judgement in normal elderly and subjects with dementia of Alzheimer's type. *J. Clin. Exp. Neuropsychol*. **12**, 695–702.

Spear, P. D. (1993). Neural bases of visual deficits during aging. *Vision Res*. **33**, 2589–2609.

Spieler, D. H., Balota, D. A., and Faust, M. E. (1996). Stroop performance in healthy younger and older adults and in individuals with dementia of the Alzheimer's type. *J. Exp. Psychol. Hum. Percept. Perform*. **22**, 461–479.

Tetewsky, S. J., and Duffy, C. J. (1999). Visual loss and getting lost in Alzheimer's disease. *Neurology* **52**, 958–965.

Trick, G. L., and Silverman, S. E. (1991). Visual sensitivity to motion: Age-related changes and deficits in senile dementia of the Alzheimer type. *Neurology* **41**, 1437–1440.

Trick, G. L., Barris, M. C., and Bickler-Bluth, M. (1989). Abnormal pattern electroretinograms in patients with senile dementia of the Alzheimer type. *Ann. Neurol*. **26**, 226–231.

Tsai, C. S., Ritch, R., Schwartz, B., Lee, S. S., Miller, N. R., Chi, T., and Hsieh, F. Y. (1991). Optic-nerve head and nerve fiber layer in Alzheimer's disease. *Arch. Ophthalmol. (Chicago)* **109**, 199–204.

Venneri, A., Pentore, R., Cotticelli, B., and Della Sala, S. (1998). Unilateral spatial neglect in the late stage of Alzheimer's disease. *Cortex* **34**, 743–752.

Wahlin, T.-B. R., Bäckman, L., Wahlin, A., and Winblad, B. (1993). Visuospatial functioning and spatial orientation in a community-based sample of healthy very old persons. *Arch. Gerontol Geriatr*. **17**, 165–177.

Warner, M. (1998). ''The Complete Guide to Alzheimer's Proofing Your Home.'' Purdue University Press, West Lafayette, IN.

Wilson, R., Kasniak, A., Bacon, L., Fox, J., and Kelly, M. (1982). Facial recognition memory in dementia. *Cortex* **18**, 329–336.

Wright, C. E., Harding, G. F. A., and Orwin, A. (1984). Presenile dementia: The use of flash and pattern visual evoked potential in diagnosis. *Electroencephalogr. Clin. Neurophysiol*. **57**, 405–415.

Wright, C. E., Drasdo, N., and Harding, G. F. A. (1987). Pathology of the optic nerve and visual association areas, Information given by the flash and pattern visual evoked potential and the temporal and spatial contrast sensitivity function. *Brain* **110**, 107–120.

Zeki, S., and Marini, L. (1998). Three cortical stages of colour processing in the human brain. *Brain* **121**, 1669–1685.

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Robert D. Frisina, Jr.

37

Anatomical and Neurochemical Bases of Presbycusis

Age-related hearing loss—presbycusis—is associated with two major symptoms: loss of sensitivity to sound, especially for high pitches, and a reduced ability to understand speech in background noise. Ultimately, these functional sensory perceptual problems result from neurological changes in the ear and brain that occur as we age. This chapter focuses upon neuroanatomical and biochemical degradations in the inner ear and central auditory system that may underlie hearing problems associated with old age. The basic findings to date suggest that sensitivity deficits result from the loss of cochlear sensory cells, metabolic cells, microvasculature, and neurons of the eighth cranial nerve that occur as a result of aging and age-related environmental insults. In addition, the problem of comprehending speech in acoustically noisy environments is exacerbated by alterations in the normal number, structure, and biochemistry of neurons in the parts of the central nervous system used for hearing. The latter deficits can result from direct aging effects on the brain, or as a response to reduced inputs to the central auditory system resulting from the age-related pathologies of the inner ear.

I. Introduction

This chapter will focus on the possible etiologies of permanent, progressive age-related sensorineural hearing loss that resides in the inner ear or brain. If taken in conjunction with other chapters on presbycusis in this volume, the present chapter provides some of the possible neuroanatomical or neurochemical bases for the behavioral and physiological characterizations of age-related hearing loss described elsewhere. It is only with this knowledge of the underlying anatomy and neurochemistry that therapeutic interventions involving gene therapy or biochemical medications can be developed and implemented.

II. Inner Ear

A. High-Pitch Hearing Loss: Declines in Hair Cells and Spiral Ganglion Cells

It has been known for some time that the primary cause of the high-frequency audiometric threshold decline in sensitivity characteristic of presbycusis—age-related hearing loss—is the destruction of hair cells in the basal (high-frequency) turn of the cochlea (auditory portion of the inner ear). From an aging perspective, this decline in the number of hair cells begins with the loss of outer hair cells, which are generally more susceptible than inner hair cells to age-related insults such as hypoxia, fever, noise, antibiotics, or other internal and external environmental insults. Losses of inner hair cells also occur with age, which can decrease the number of neural channels carrying information to the brain, thus reducing or distorting information relayed to the brain about complex sounds such as speech. In advanced age groups, nearly complete loss of inner hair cells can result in profound hearing loss and can be accompanied by severe reduction of spiral ganglion cells (neural information channels to the brain).

1. Animal Models

a. Genetically Inbred Mouse Strains. Neurobiologists and hearing scientists investigating the neural bases of presbycusis have found it useful to utilize different strains of inbred mice that lose their hearing at different rates. The hearing characteristics of these strains are described in more detail in other chapters of this handbook dealing with age-related hearing loss. Briefly, two of the most commonly utilized strains are the CBA and C57 mice. The CBA mouse loses its hearing at a slow rate as a function of age and loses sensitivity at all frequencies. In contrast, the C57 strain loses its hearing very rapidly as a function of age, and tends to lose hearing first in the high frequencies due to hair cell loss in the basal turn of the cochlea. The CBA is a model of the human condition in that its slow progressive hearing loss occurs on a time scale similar to that of humans suffering from presbycusis if one corrects for the absolute difference in the average life spans of mice and men, i.e., 2 years versus seven decades, respectively. The

C57 mouse model mimics the high-frequency component of human age-related hearing loss, and has been used effectively to determine the central effects of peripheral hearing loss. Taken together, these two strains along with other mouse strains can help delineate central versus peripheral aging effects in the auditory system. For example, a 6-month-old C57 mouse has a relatively young brain but an ''old'' ear. The young brain, however, has undergone a peripherally induced reduction in physiological inputs superimposed upon it due to the death of auditory nerve fibers from the basal turn of the cochlea that normally would provide input. An old CBA mouse has an old brain and an old ear, which in many ways resembles the situation in a presbycusic patient with an old ear and brain.

Hair cell loss takes place rapidly, starting at about 2 months of age, initially in the basal turn of the cochlea in C57 mice. Specific pathologies of the organ of Corti in the age range of 3 to 12 months include significant decline, clumping, and distortion of outer hair cells; degeneration of pillar cells; but little loss of inner hair cells (Henry and Chole, 1980; Willott, 1991). At 2 years of age, very little is left of the organ of Corti in the basal turn. Spongr *et al*. (1997a,b) performed systematic counts of inner and outer hair cells for C57 mice of four different age groups. Data for the 26-month-old group are presented in Fig. 37.1A. Notice that hair cell loss is most extreme in the basal turn (furthest from the apex). Also, observe that losses of both hair cell types are 100% in the base in these old animals, whereas in the apex outer hair cell declines are about 80%, and inner hair cell reductions are at approximately 20% in the C57 cochlea.

C57 mice have reductions in spiral ganglion cells that accompany the hair cell losses (Willott, 1991). At the end of the second year of life, there is a 40% loss in the apical turns (low frequency) of the cochlea and 80% or more in the basal turns (high frequency). Degeneration of spiral ganglion neurons goes through several steps (Cohen *et al*., 1990): (1) demyelination, including loosening and unraveling of myelin sheaths; (2) pathological contact with fusion of cells that are partly demyelinated; (3) clumping of demyelinated cell bodies that become surrounded by cytoplasmic processes; (4) resorption; (5) disappearance of basal lamina at contact sites; (6) encircling of clumped ganglion cell bodies by Schwann cells; (7) extensive mitochondrial death; (8) formation of empty spaces where ganglion cell bodies used to be.

Compared to the C57, the CBA mouse strain loses hair cells and spiral ganglion cells at a much slower rate, with very little loss at even 16 months of age (Henry and Chole, 1980). Spongr *et al*. (1997a) confirmed this and demonstrated that at 18 months of age, a 30–50% loss of outer hair cells was present only in the extreme basal and apical portions of the cochlea, with no loss of inner hair cells. The old-age configuration of hair cells for CBA mice is shown in Fig. 37.1B. Notice that the functions are U-shaped, with significant losses present only in the most apical and basal portions of the cochlea. These 26-month data show that there are no hair cell losses in the middle turns of the CBA cochlea, whereas outer hair cell losses occur at 50–60% in the cochlear extremes, and inner hair cell losses reach 10–30% in the most apical and basal portions.

Loss of spiral ganglion neurons is not as severe or frequency-specific as in the C57. For example, in 2-year-old

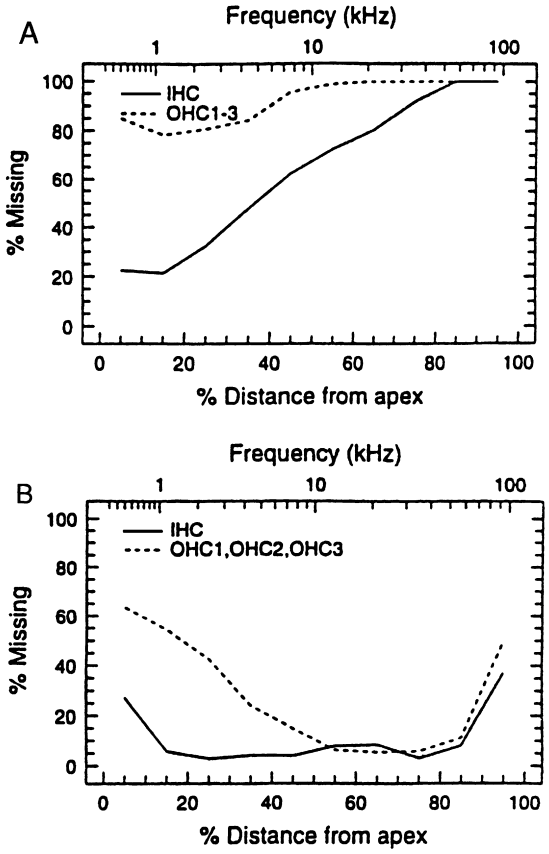

FIG. 37.1. Cytocochleogram displaying pattern of hair cell loss in the old (26-month) C57 and CBA cochlea. (A) Notice that for C57 mice, both inner and outer hair cells have the most significant declines in the cochlear base (furthest distance from the apex). (B) For CBA mice, the functions are U-shaped in that most losses are in the base and apex. For each graph, the data represent the mean of cytocochleograms from 10 animals. This format displays the percentage of missing inner and outer hair cells in 10% intervals as a function of distance from the apex. From Spongr *et al*. (1997a) with permission from the American Institute of Physics and the author.

CBA mice, the density of spiral ganglion cells in Rosenthal's canal declines by only 5–20%, and in 30-month-old mice, declines at all frequencies are only about 30% (Willott, 1991).

Willott and Erway (1998) more recently have evaluated hearing and cochlear cell losses in 25 different recombinant inbred mice derived from C57 and DBA strains. The DBA strain has an accelerated peripheral hearing loss that occurs at a rate even faster than the C57, and is also high-frequency in nature (Willott *et al*., 1984). These recombinant inbred mice, referred to as BXD strains, were derived by successive brother–sister mating from original F1 hybrids of a C57 mouse and a DBA mouse. Evidence suggests (Erway *et al*., 1993; Willott *et al*., 1995) that C57 and DBA mice share a common age-related-hearing loss gene (*Ahl*) on chromosome 10. DBAs, with the more severe hearing loss, are homozygous for two additional age-related-hearing-loss genes: *Ahl2*, also on chromosome 10, and *Ahl3*. Thus, they postulate that each BXD strain is composed of one of four possible genotypes: (a) homozygous for *Ahl* only, like C57s; (b) homozygous for all three, like DBAs; (c) homozygous for *Ahl* and *Ahl2*; (d) homo-

zygous for *Ahl* and *Ahl3*. Given a large enough sample, it was hypothesized that a quarter of the mice would be like C57s, a quarter like DBAs, and half in between. This hypothesis was supported in the Willott and Erway (1998) study, as three distinct phenotypes in appropriate proportions emerged in the BXD recombinant mice: one with an age-related hearing loss and spiral ganglion cell diminution like C57 mice, another with the more severe losses of the DBA strain, and the rest with intermediate levels of hearing and spiral ganglion cell loss as a function of age, presumed to be homozygotic for two of the three age-related-hearing-loss genes.

b. Other Rodents. Bohne *et al.* (1990) examined age-related declines in hair cells of the laboratory chinchilla, an outbred rodent with a life span of 12–20 years. A slow, progressive pattern similar to the CBA mouse strain was observed in that through middle age (8–11.5 years), declines in all turns of the cochlea were less than 10% for outer hair cells and less than 5% for inner hair cells. In old age (12–19 years), although less severe than in mice, losses were most prevalent in both the apex (17% decline) and the base (25% fall) for outer hair cells, with declines of 11–15% for the middle turns. For inner hair cells, the reductions were 6% in the base and apex of the cochlea and 4% for the middle turns. Clumping and tangled stereocilia were not common in the young animals, but increased with age. Chinchilla pillar cells also degenerated with age, starting with losses of cuticular plate substance. Degeneration of spiral ganglion cells was associated with loss of inner hair cells, but rarely occurred in regions with just outer hair cell loss.

McFadden *et al.* (1997) performed an interdisciplinary investigation of age-related hearing loss in chinchillas by comparing a young group with a "young" elderly group with an age range of 11–15 years. Their hair cell counts were similar to Bohne *et al.* (1990), as shown in Fig. 37.2, which was correlated with a modest decline in hearing sensitivity, especially in the high frequencies. They also showed increases in the prevalence of phalangeal scars and a wider distribution of pigmented granules (lipofuscin or melanin) in the organ of Corti of old animals, as displayed here in Fig. 37.3.

As demonstrated by the Medical University of South Carolina's hearing research team headed by Mills and Schmiedt, the gerbil, with a life span of about 3 years, is a useful model for peripheral studies of age-related hearing loss. Unlike most rodents, but like the chinchilla, gerbils have good low-frequency hearing. Gerbils have a slow, progressive hearing loss that reaches about 20 dB for low frequencies and 30 dB in high frequencies in old age (36 months; Schmiedt, 1993). There is almost no loss of inner hair cells in aged gerbils, and interestingly, the loss of outer hair cells is most significant in the apex of the cochlea, whereas the greatest hearing loss is in the high frequencies (Tarnowski *et al.*, 1991). Keithley *et al.* (1988) report that spiral ganglion cell loss in gerbils starts at about 24 months, and in the oldest animals (36–42 months) reductions of 15–25% occurred throughout the cochlea.

Covell and Rogers (1957) and Ingham *et al.* (1999) have investigated age-related changes in the cochlea and their relationships to hearing loss in guinea pigs. The guinea pig, having a maximal life span of 6–7 years, shows very slow, minimal loss of hair cells and auditory sensitivity with age. In very old animals, the only significant loss of outer hair cells occurs in the apical turn of the cochlea. There are very slight declines in inner hair cells and spiral ganglion cells. The outer hair cell losses of the apex increased from the first to the third row: first row, 20%; second row, 25%; third row, 30%. In terms of a model for presbycusis, the rate of age-related loss in guinea pigs would be slower than that seen in most humans, and except for the oldest group of presbycusics with significant cochlear apex damage, the guinea pig has the opposite configuration of hair cell damage from classic presbycusis. Last, hearing loss in old guinea pigs is relatively minimal (30–40 dB) and constant across frequencies, suggesting, as for gerbils, that loss of outer hair cells outside the basal turn is not generally a good predictor of elevations in hearing thresholds.

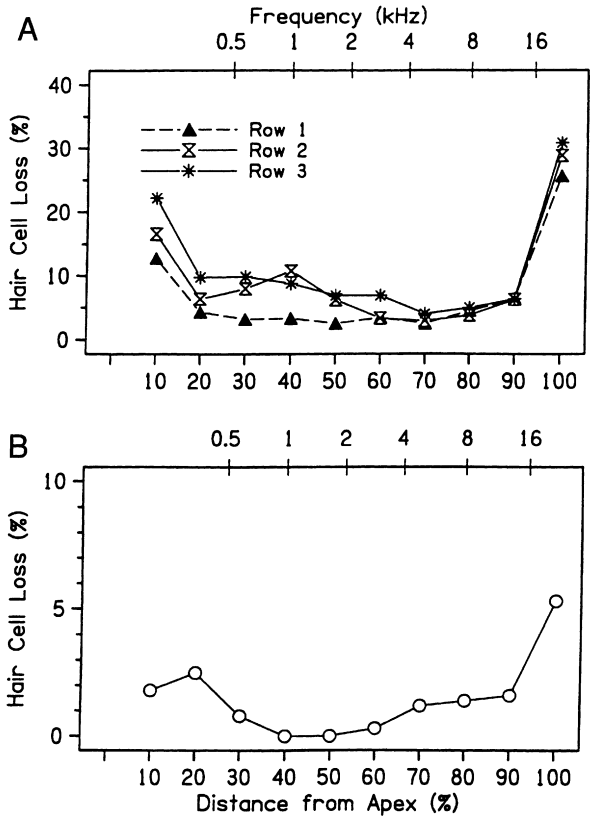

FIG. 37.2. Hair cell declines in chinchillas are U-shaped, reminiscent of findings in old humans and CBA mice. (A) Outer hair cells have their greatest losses in the extreme base, with severity of decline increasing from the 1st row to the 3rd row. (B) Inner hair cells also show a U-shaped function, although it is less concave (flatter loss than the outer hair cells). Cytocochleogram format same as the previous figure. From McFadden *et al.* (1997) with permission from Elsevier Science and the author.

As a means for evaluating the usefulness of inbred mouse strains, comparisons have been made of spiral ganglion cell loss in wild-type mice and other rodent outbred strains including guinea pigs and gerbils. Spiral ganglion cell loss in middle aged (18– to 19-month-old) wild type mice ranged from 16 to 49%, with the greatest loss in the basal turn (Dazert *et al.*, 1996). For old-age wild types (28–31 months) the drops relative to the young mice ranged from 50 to 76%, with the most loss in the apex and base, which were about the same. This time-dependent pattern of ganglion cell loss in the wild type

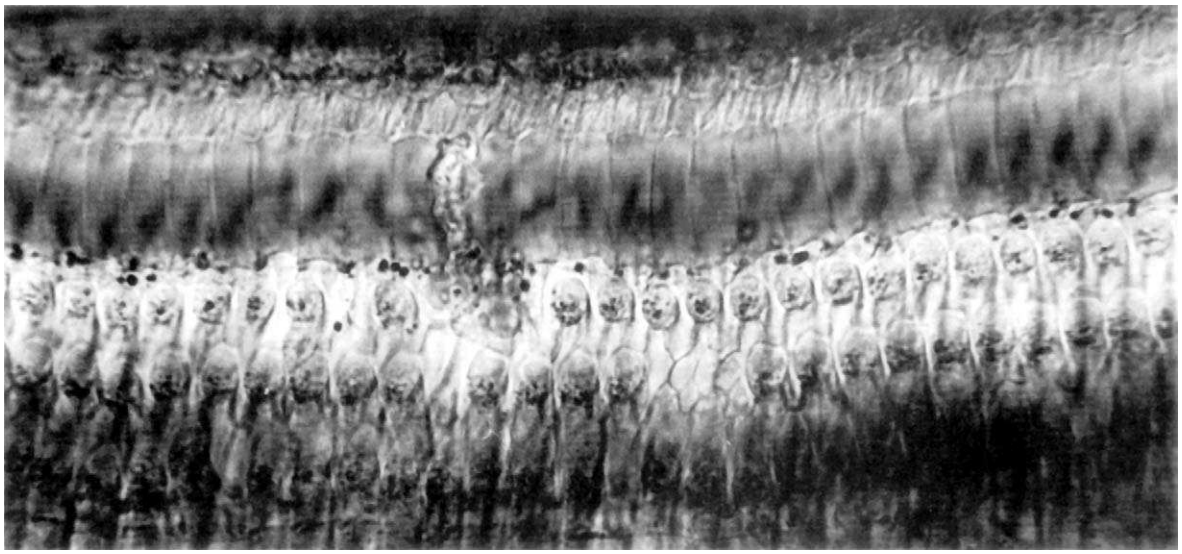

FIG. 37.3. Old chinchillas displayed increased occurrences of phalangeal scars and pigmented granules such as lipofuscin and melanin, throughout the extent of the organ of Corti. This photomicrograph displays a surface preparation from the basal turn of an 11-year-old chinchilla. From McFadden *et al.* (1997) with permission from Elsevier Science and the author.

mice, as shown in Fig. 37.4, was very similar to the pattern seen in gerbils, guinea pigs, and different strains of rats, where noticeable diminutions start to occur in middle age, and in old age the greatest losses are in the basal and apical turns of the cochlea (Keithley and Feldman, 1979; Keithley *et al*., 1989, 1992).

Last, Hoeffding and Feldman (1988) demonstrated in Sprague–Dawley rats that the median number of healthy, normal auditory nerve fibers (axons of spiral ganglion cells connecting the ear to the brain) was reduced by 21% at 27 months of age and by 24% at 36 months. The number of degenerating nerve fibers went up by 6 months of age, peaked at 27 months, and went back down at 36 months. Although the auditory nerve cross-sectional area rose over the life span due to increasing thickness of myelin sheaths, the packing density of healthy nerve fibers declined with age. Glial cells in the nerve were constant across the life span.

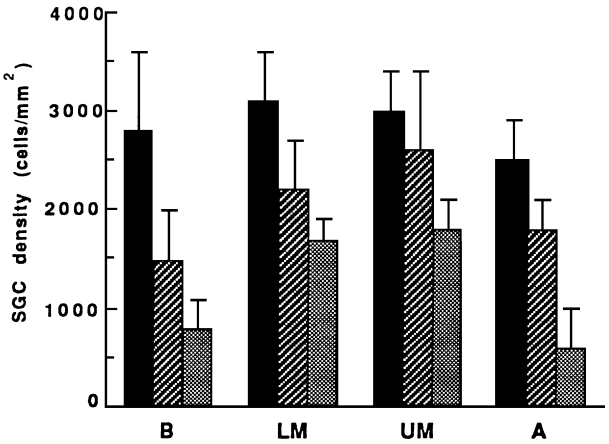

FIG. 37.4. Cochlear spiral ganglion cells decline with age in mice, with the greatest declines in the base and apex. This histogram displays the mean and standard deviation of the number of ganglion cells/mm^2 in the four half-turns of the mouse cochlea. Left bar (solid), young; middle bar (hatched), middle age; right bar (shaded), old; B, base; LM, lower middle turn; UM, upper middle turn; A, apex. Each turn was found to have a significant (ANOVA, $P < 0.001$) decline in spiral ganglion cells with age. From Dazert *et al.* (1996) with permission from Elsevier Science and the author.

c. Other Mammals and Primates. The usefulness of the rodent studies has precluded the necessity of performing extensive inner ear aging studies in other mammals. This is primarily due to the fact that what is known about presbycusis in other mammals is consistent with the findings of the rodents. For example, neuroanatomical studies of the aging cochlea conducted in monkeys, cats, and dogs (summarized by Willott, 1991) show slow progressive declines in the condition of the organ of Corti and in numbers of hair cells and spiral ganglion cells. In the absence of noise exposure and other environmental insults, higher mammals lose hair cells slowly, with the greatest losses in old age occurring in both the cochlear apex and base, and with outer hair cell diminutions exceeding and preceding inner hair cell degeneration. This was demonstrated quite exquisitely in the rhesus monkey by Hawkins and Johnsson (1985) whose cytocochleograms based on data from 15 monkeys aged 4–31 years showed the same U-shaped function as the chinchilla data displayed here in Fig. 37.2.

2. Human Studies

Elderly individuals, unlike laboratory animals housed in university vivariums, undergo environmental insults, which are overlaid or interact with general biological aging processes and any genetic susceptibilities to insults to the cochlea or brain coming from internal or external bioenvironments. Therefore, investigations of temporal bones from aged individuals have shown sensory and spiral ganglion cell losses throughout the cochlea, with usually the greatest severity in

the cochlear base and apex, and anomalies of the stria vascularis and inner ear microcirculation. For instance, Bredberg's (1968) study of old humans showed that inner hair cell loss was confined to the base and can be greater than a 50% reduction. However, organ of Corti abnormalities and outer hair cell loss generally took place throughout the auditory sensory epithelium with the greatest changes in both apex and base. Specifically, Bredberg (1968) reported a 75% reduction of outer hair cells in the base, and a 65% decrease in the apex. In some individuals, perhaps suffering from noise-induced hearing loss, patterns of degeneration did not follow the norm and were restricted to isolated points along the cochlear partition.

Bredberg (1968) along with Schuknecht (1974), also noted a finding that has been substantiated in animal models as mentioned above: destruction of outer hair cells generally starts with the outermost row, proceeds to the middle row, and finally reaches the inner row. Subsequently reported in animal preparations, Johnsson and Hawkins (1972) noted the presence of phalangeal scars in the organ of Corti of old humans. The scar apparently results from the growing together of apical processes of phalangeal (supporting) cells in the gap where the hair cell used to be. Johnsson (1971) in a study of the human Reissner's membrane (separating scala vestibuli [perilymph] from scala media [endolymph]), noted that in presbycusis the membrane degrades, producing vacuoles 5–10 μm in diameter which may be the product of pinocytosis. Breakdown of Reissner's membrane, which has been reported in some old animals, could compromise the separation of endolymph from perilymph, thus decreasing the endocochlear potential and interfering with normal hair cell functioning and the auditory mechanical-to-electrical transduction cascade. Engström *et al*. (1987), in a study of hair cell loss in the aged human cochlea, also reported abnormalities and fusing of stereocilia on the apical surfaces of hair cells that were still present.

In general, loss of spiral ganglion cells occurs sooner and to a greater degree with age in the base of the human cochlea relative to other turns of the cochlea, and is rarely seen in the absence of sensory-cell degeneration. An exception is the patchy loss of ganglion cells in certain restricted foci of the cochlea reported by Johnsson and Hawkins (1972), similar in configuration to the local hair cells losses mentioned in the previous paragraph. Schuknecht (1993) reports that in very old individuals (81–90 years old), ganglion cell loss is 54% in the apical turn and 48% in the basal turn, in contrast to ''younger'' old who have the greatest spiral ganglion cell losses in the basal turn of the cochlea. Felder and Schrott-Fischer (1995) also investigated relations between hair cell loss and myelinated nerve fiber degeneration in the human cochlea. They found that reductions of up 40% in spiral ganglion cells in aged (above 60 years of age) human temporal bones relative to cochleas from middle-aged persons with normal hearing. Interestingly, the spiral ganglion losses were throughout the cochlea, but the outer hair cell losses were most severe in the apex (up to 80% reductions), and declines in inner hair cells were minimal relative to the middle-aged controls. This study exemplifies another case where subjects have a classic, high-frequency presbycusic hearing loss that cannot be entirely understood in terms of hair cell or auditory nerve fiber degeneration, which themselves were not well correlated.

As Willott (1991) summarizes, in aging humans degeneration of spiral ganglion cell processes or cell bodies can occur sometimes where organ of Corti pathology or hair cell loss has not occurred. Therefore, it appears that loss of hair cells can induce ganglion cell loss with age, but the latter can also occur in some cases where a relatively healthy organ of Corti exists. It appears that organ of Corti pathology is a sufficient but not necessary condition for the atrophy of spiral ganglion cell distal (peripheral) dendrites or their perikarya. One possible mechanism for auditory nerve fiber death independent of organ of Corti pathology is destruction of nerve fibers due to disruption of their central projections to the cochlear nucleus resulting from central nervous system aging degeneration there (to be discussed later in this chapter). Indeed, consistent with reports of spiral ganglion cell loss, Rasmussen (1940) and Spoendlin and Schrott (1989, 1990) have counted nerve fibers in the auditory division of the eighth cranial nerve as a function of age in humans and found reductions of 8–25% and noted the presence of pathological axons in the old temporal bones.

An important ramification of ganglion cell loss, because their axons have the singular property of carrying all information about sound to the brain, is that decrements in speech recognition performance have been correlated with spiral ganglion cell degeneration in aged human subjects (Belal, 1975; Otte *et al*., 1978). These correlations are stronger than for pure tone sensitivity but can be quite variable overall. Interestingly, Pauler *et al*. (1986) found a surprisingly strong relationship between ganglion cell loss in the region of the human cochlea corresponding to sensitivity to 1–2 kHz (15–22 mm from the base) and speech recognition. This is intuitively appealing in that this frequency region is the most important band for human speech perception.

B. Metabolic and Blood-Flow Changes Affect Overall Sensitivity

In contrast to sensory and spiral ganglion cell losses that tend to occur in specific regions of the inner ear with age, metabolic and blood flow deficits with age can have a more generalized effect on the functioning of the cochlea. The stria vascularis is a highly vascularized region on the lateral wall of the cochlea whose primary function is to extract potassium chloride from cochlear capillaries to maintain the electrochemical gradient in scala media necessary for normal conversion of sound energy to nerve cell impulses.

1. Animal Models

The most systematic study of age-related metabolic and blood flow deficiencies of the cochlea have been accomplished by Schmiedt and Schulte's South Carolina investigative team using the gerbil as an animal model. For example, Schmiedt and Adams (1989), Schulte and Schmiedt (1993), and Gratton *et al*. (1997) demonstrated almost all of the reductions in the normal endocochlear potential with age can be accounted for by declines in ion transport capabilities of the stria vascularis marginal cells as visualized by Na,K-ATPase immunoreactivity in the gerbil inner ear. Figure 37.5 displays this functional relationship for 25 gerbil cochleas ranging in age from 3 to 32 months of age. The endocochlear potential is the voltage pre-

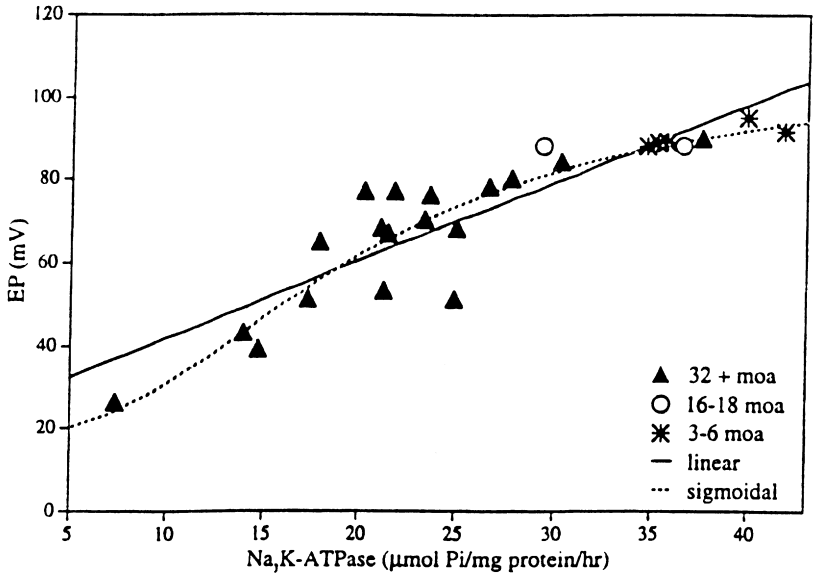

FIG. 37.5. A positive correlation was found for the magnitude of the endocochlear potential and the extent of Na^+,K^+-ATPase activity in gerbils of different ages. These data come from 25 ears, with the solid line representing the linear regression results while the dashed curve embodies a sigmoidal regression analysis. Data from 18 old gerbil (triangles), 2 middled age gerbils (circles), and 5 younger animal (asterisks) ears are displayed; moa, months of age. From Gratton *et al.* (1997) with permission from Elsevier Science and the author.

sent in scala media that provides the basis for acoustico-mechanico-electrical transduction (conversion of sound energy to the electrochemical code of the central nervous system). Another article reports, similar to what was explained above for hair cell loss, that aging gerbils first display stria vascularis lesions in the cochlear apex and base, rather than the middle turns, which then spread with age (Gratton and Schulte, 1995).

Gratton *et al.* (1996) looked for relations between changes in cochlear vasculature in quiet-aged gerbils at 36 months of age and their endocochlear potentials. The density and diameter of strial capillaries were assessed in wholemount preparations, and then histopathologic changes were studied with light and transmission electron microscopy. In the same animals, endocochlear potentials from all turns of the cochlea were recorded along with the endocochlear potential at the round window. No strong relations were found between the endocochlear potential in each turn and the amount of microvascular damage. However, there was a significant correlation between the overall, mean endocochlear and the amount of *normal* strial vasculature throughout the cochlea, both of which decline with age (see Fig. 37.6). In related investigations, Schmiedt (1993, 1996) found that despite age-related declines in the endochoclear potential (92 mV [$\pm$5.7 mV] in young adult gerbils to 65 mV [$\pm$15.8 mV] in 30-month-old animals to 61 mV at 36 months), scala media K^+ concentrations remained within normal limits until endocochlear potential voltages became very low (below 60 mV). Below, 60 mV, there was always a K^+ concentration below the normal range.

To better understand the etiology and temporal sequencing of age-related strial degeneration, the integrity of the basement membrane unique to strial capillaries was investigated in the gerbil inner ear. In general, the basement membrane is a thin layer associated with the plasmalemma of endothelial and epithelial cells as they contact other cell types, and provides mechanical support for proper cell attachment and differentiation, especially during development. It has many structural components, but a primary one is laminin, a multidomain glycoprotein composed of eight distinct chains. Sakaguchi *et al.* (1997) and Thomopoulos *et al.* (1997) found that starting in the cochlear apex of 6-month-old gerbils, the presence of laminin increased above normal levels in the basement membrane, causing it to become pathological. With advanced age (33 months and older), the cochlear base and apex showed abnormal basement membrane thickening in 65–85% of strial capillaries, which is about seven-fold the occurrence seen in young adult animals. This pathology was followed by capillary obstruction (in some ways similar to atherosclerotic closure of blood vessels with age), that resulted in degeneration of marginal cells and complete strial atrophy.

Fibrocytes of the spiral ligament, which contain K^+ transport enzymes, also play a critical role for endolymph homeostasis in scala media (Schulte and Adams, 1989; Spicer and Schulte, 1991). Spicer *et al.* (1997) demonstrated that changes in the concentration of K^+-ion transport enzymes are complex with age, but related to strial damage that starts first in the apical and basal regions, and then spreads to the middle turns in old age. For example, carbonic anhydrase isoenzyme II first upregulates itself in regions of strial damage, or regions adjacent to areas of strial damage, presumably to compensate for the failure of other ion transport systems and decreased blood flow. As presbycusis proceeds in gerbils, this upregulation ceases, and in very old gerbils with strial damage in most areas of the cochlea, the carbonic anhydrase immunoreactivity declines to levels below those of young adult animals.

Studies of stria vascularis integrity and patency of cochlear microcirculation in other animals, although not as comprehen-

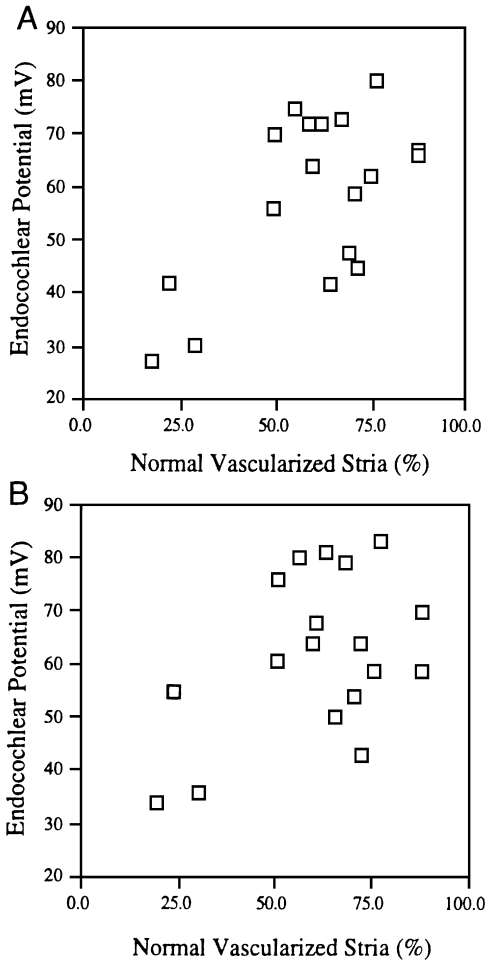

FIG. 37.6. Declines in the endocochlear potential follow reductions in the capillary integrity of the stria vascularis. Here endochoclear potential values are plotted versus the amount of normal capillary structure of the stria vascularis. (A) Data are from scala media of 18 gerbil inner ears ($P < 0.01$, $r = 0.61$, $n = 18$). (B) Similar data except data were collected at the round window, outside the cochlea ($P < 0.05$, $r = 0.44$, $n = 18$). From Gratton *et al.* (1997) with permission from Elsevier Science and the author.

sive and systematic, are important because they confirm that such changes discovered in the gerbil work are probably present to some degree in all mammals and therefore play a key role in understanding certain aspects of the biological bases of human presbycusis. For example, Bohne *et al.* (1990) performed a comprehensive investigation of cochlear changes involving over 80 chinchillas ranging in age from birth to 19.2 years. They found that substantial strial degeneration involving all three types of strial cells and capillaries occurred in 14% of chinchillas over 3 years of age. Willott (1991) reports increases in abnormal pigment inclusions in old C57 and CBA mice, with the occurrence much greater in the C57s, and summarizes the presence of age-induced strial degeneration in cats, dogs, and sometimes in old rhesus monkeys and chimpanzees. Ding *et al.* (1998) report aging effects in the cochleas of C57 and CBA mice regarding dehydrogenase activity and glycogen levels that in many aspects support the conclusions drawn from the gerbil studies.

2. Human Studies

Cochleas from persons suffering from presbycusis display pathologies of the stria vascularis as well as anomalies in cochlear microcirculation that parallel the age-related pathologies investigated in gerbils and other mammals. Based upon abnormalities of the stria vascularis and cochlear blood vessels in human temporal bones from elderly patients with hearing loss, Schuknecht (1964) and Schuknecht *et al.* (1974) put forth the concepts of "metabolic presbycusis" and "vascular presbycusis." Consistent with this are reports of significant loss of blood vessels in the spiral ligament, stria vascularis, and even in the organ of Corti in presbycusic ears (Johnsson and Hawkins, 1969). Like most mammals, these losses were greatest in the apical and basal turns of the cochlear spiral, and were accompanied by strial atrophy and declines in the diameters of remaining vessels. Interestingly, these vascular degenerations resemble those reported for the aging human retina. In addition, Fischel-Ghodsian *et al.* (1997) report that mitochondrial DNA from spiral ganglion cells and membranous labyrinths from old human cochleas had from 1 to 26 sequence changes in mitochondrial chromosomes versus a range of 1–3 sequence changes from control tissue (tissue from subjects with no hearing loss). These additional DNA sequence changes could disrupt normal oxidative phosphorylation in the ears of elderly patients, thus interfering with normal cochlear transduction functioning.

C. Summary of Cochlear Findings

The inner ear work shows some noteworthy similarities between the animal investigations and the human reports. In human pathological studies, hair cell loss occurs as a function of age, with declines first occurring in the cochlear base. The configuration of this degeneration is modeled well by hair cell loss in the C57 mouse strain. The rate of hair cell loss in humans, a slow progressive process with age, is better modeled by the slow decline of hair cells over the life span seen in the CBA mouse, the chinchilla, and other rodents. However, the animal losses for these species generally are more flat than that seen in classic cases of presbycusis. It appears that the high-frequency component of human presbycusis is due primarily to age-related loss of hair cells and spiral ganglion cells in the basal turn of the cochlea, starting with outer hair cells which are more susceptible to physiological insults such as noise, hyperthermia or other ototoxic agents. Superimposed on this may be a more general loss of sensory hair cells, and to a lesser extent spiral ganglion cells, from an aging process that affects the integrity of the stria vascularis or cochlear microcirculation, which may affect the entire cochlea. Strial atrophy and reductions in inner ear capillaries has been observed in many aged animals and demonstrated in detail in the gerbil. For reasons not completely understood, these vascular changes can induce hair cell loss in both the cochlear base and apex. Extensive apical damage is seen in most of the nongenetically inbred animal models and in the very old human temporal bones. Deficiencies in cochlear microcirculation have been reported in old human temporal bones, and in animal models such as the gerbil and the dog. Finally, an important lesson from these aging studies is that outer hair cell loss

in the cochlear base results in declines in hearing sensitivity, whereas reductions in outer hair cells in other turns of the cochlea are generally not well correlated with hearing loss.

III. Central Auditory System: Peripherally Induced Changes

Aging can affect the central auditory nervous system directly, by causing age-related problems in the structure and neurochemistry of brain cells. However, age can also cause damage to the inner ear, ultimately inducing degeneration of spiral ganglion cells and eighth nerve fibers that constitute the information channels connecting the cochlea to the cochlear nucleus, the first processing stage of the auditory central nervous system. At this point, peripherally induced central effects will be focused on first, and then this chapter will conclude with aging problems of the central auditory system that appear to be independent of damage to the inner ear. These concepts of differential effects of age on the auditory nervous system are explained graphically in another chapter (see Chapter 39, Fig. 1).

A. Animal Models Demonstrate Reorganization of the Brain Due to Reduced Peripheral Inputs

Contrary to classical notions of a hard-wired central nervous system in adult organisms, more recent neuroscientific research has unequivocally shown that the adult mammalian brain is capable of reorganization in response to normal or abnormal sensory input. In contrast to synaptic and pathway reorganizational capabilities of the brain, it is still the case that under normal conditions central nervous system nerve cells cannot regenerate themselves when lethally damaged or destroyed. In this section, we will focus on studies of the C57 mouse strain by Willott and colleagues and by our group. The rapid cochlear hearing loss of the C57 as a function of age provides a relatively unique model for investigating the effects of reduced peripheral input to the auditory central nervous system. A 6-month-old C57 mouse has an "old ear" with significant reductions in the information that gets relayed to the auditory central nervous system, but still has a young brain. In some sense then, the effects of an old ear can be studied for a brain that does not have its own age-related degradation.

1. Gross and Cellular Anatomical Measures of Central Nuclei

Anatomical measurements of the anteroventral cochlear nucleus were made by Willott *et al*. (1987) in C57 and CBA mice of different ages. No changes in anteroventral cochlear nucleus volume or dimensions were noted with age. For nerve cell number and packing density, there is a decline in the first 7 months of life for C57s and little change thereafter. However, these parameters do not decline in CBAs until the second year of life. C57 spherical and globular cells increase in size with age, whereas multipolars decline, and these changes take place earlier and largely in dorsal regions of anteroventral cochlear nucleus. These dorsal areas receive severely declining peripheral inputs as a function of age from the base (high-frequency turn) of the cochlea. All three cell types become smaller as a function of age for CBAs. These strain differences were interpreted to be the result of the different rates of hearing loss across the two strains. A follow-up study comparing anatomical changes in the AVCN for C57 and DBA mice revealed that for the DBA strain, which possesses a more rapid peripheral loss than the C57, neuronal losses were more rapid than in C57 mice, paralleling the accelerated time course of the peripheral hearing loss in DBAs (Willott and Bross, 1996). It was instructive to note that the final reductions in neuron size and packing density were of similar magnitude at the age of about 1 year for both strains. This suggested that although the rate of hearing loss is greater in the DBAs re the C57s, the anatomical changes asymptote at about the same level as both strains enter middle age, as shown here in Fig. 37.7.

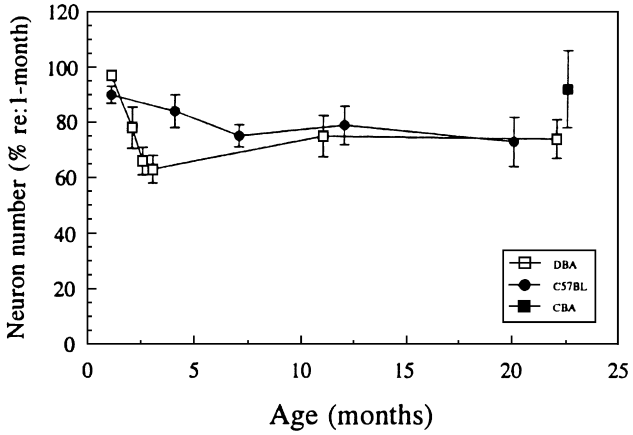

FIG. 37.7. Anteroventral cochlear nucleus neurons decrease in number with age at a faster rate in DBA mice compared to C57. The long-term reduction in DBA and C57 mice is the same (20+ months). For the DBA and C57 data, percentages are relative to 1-month-old values. Only 22-month-old CBA data are plotted, since CBA mice do not lose anteroventral cochlear nucleus neurons with age. From Willott and Bross (1996) with permission from Elsevier Science and the author.

Willott *et al*. (1992) measured properties of the dorsal cochlear nucleus layers I, II and III. They found that dorsal cochlear nucleus volume declined dramatically with age in C57s, but increased during the first year of life in CBAs and then declined slightly in very old age for CBAs; and declines in nerve cell numbers were greater for the C57 (primarily in layer III) than for the CBA. Comparison of the findings from the two strains suggests that loss of peripheral inputs that are exaggerated in the C57 cause the more noticeable reductions in this strain relative to the CBA. It makes sense that the changes were most evident in layer III of the dorsal choclear nucleus, since it is this lamina that receives inputs from the cochlea.

An important issue in presbycusis research, of which little is known, has to do with interactions between age and environmental insults such as noise exposure. Are old animals more susceptible to noise damage? Willott *et al*. (1994b) shed some light on this matter by exposing CBA mice of different ages to very loud, wideband noises of different magnitudes and then looked at the morphology of the cochlear nucleus at various time periods following the noise exposures. CBAs of the same age that were not exposed to noise served as controls.

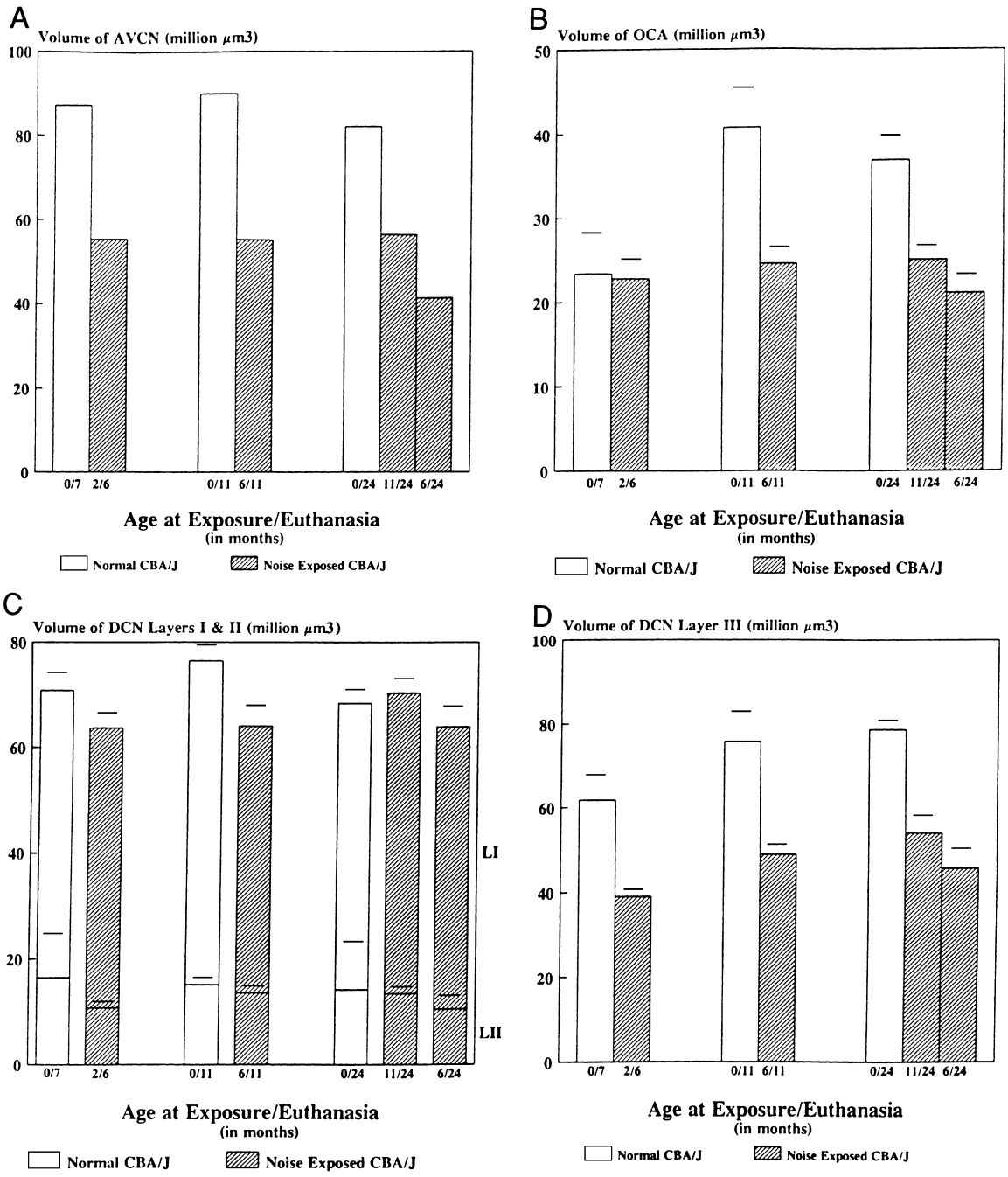

FIG. 37.8. Cochlear noise damage results in declines in volume of anteroventral cochlear nucleus (AVCN), octopus area (OCA), and dorsal cochlear nucleus (DCN) layer III in CBA mice. However, the age of the animals at the time of the noise exposure had no effect on the amount of damage (volume reductions). (A) Histograms showing relations between ages of noise exposure/euthanasia and the volume of the AVCN. (B) Same for the OCA. (C) Same for layers I and II of the DCN. Note that cochlear noise damage has little effect here because these portions of the DCN receive most inputs from noncochlear sources. (D) Similar to A and B for layer III of the DCN. Horizontal bars, standard deviations. From Willott *et al.* (1994b) with permission from Elsevier Science and the author.

They discovered that regions of the cochlear nucleus that are heavily innervated by peripheral inputs, such as the anteroventral division, the octopus cell area and layer III of the dorsal cochlear nucleus showed reductions in neuropil volume, declines in neuron size, and increases in neuron packing density, see Fig. 37.8. Only small declines in neuron number were present in all divisions, despite massive cochlear sensorineural damage. Relevant to the present chapter, there was no effect or interaction of age with the noise damage, i.e., a given noise-damage stimulus (peripheral pathology) produced the same amount of cochlear nucleus damage regardless of the animal's age.

Saada *et al.* (1996) examined differences in the light microscopic properties of the cochlear nucleus in congenitally deaf

white cats possessing early onset cochlear receptor loss with the cochlear nuclei of normally hearing pigmented cats, however, the ages of the subjects were not reported. They discovered that for the deaf cats (relative to the controls): the ventral and dorsal cochlear nucleus volumes were reduced by about 50%, spherical bushy cells of the anteroventral nucleus and pyramidal cells of the dorsal division were reduced in size by 31–40% (nonauditory neurons from adjacent brain areas were not changed), and astrocyte density was elevated in the anteroventral division by 40% and by 5% in the dorsal nucleus.

At the level of the inferior colliculus Kazee and colleagues (1995) demonstrated, using young adult, middle-aged, and old C57 mice, that there was a significant reduction in the number and size of synaptic contact areas on principal nerve cells of the central nucleus of the inferior colliculus and that the size of the principal cells declined with age. Interestingly, there was no difference in the magnitude of these age effects in different quadrants of the inferior colliculus. This means that the same synaptic reductions occurred in ventral (high-frequency) regions of the inferior colliculus that now receive more low-frequency information, as occurred in the dorsal (low frequency) areas. A follow-up investigation, using the same quantitative electron microscopy neuroanatomical procedures, found that no such age-related reductions occur in the inferior colliculus central nucleus of the CBA mouse (Kazee and West, 1999). Taken together, these two studies suggest that reductions in synaptic efficacy in the central nucleus of the auditory midbrain are a result of, or are in response to, synaptic reorganization or degradation caused by reductions in peripheral inputs, not to the aging brain itself. Put another way, it may be that to preserve the number and size of synaptic inputs to inferior colliculus nerve cells, relatively normal input from the auditory periphery is required.

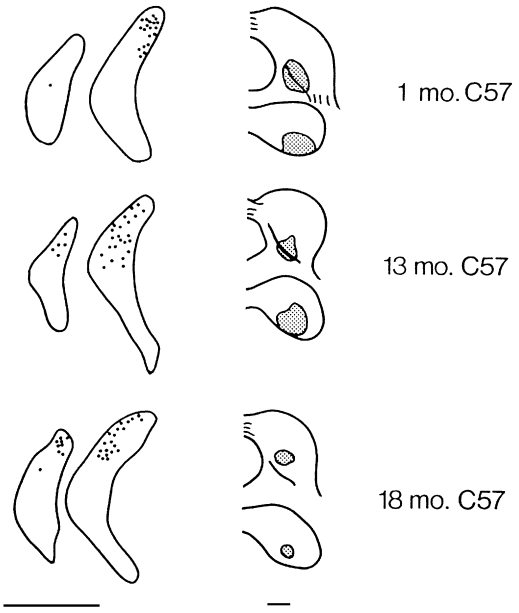

FIG. 37.9. Inputs to the ventral central nucleus of the inferior colliculus from the dorsal region of the anteroventral cochlear nucleus (AVCN) remain stable with age for C57 mice. (Right) Location of injection sites in the ventral region of the inferior colliculus in C57 mice of different ages. The upper inferior colliculus section is more rostral. (Left) Locations of retrogradely labeled cell bodies in dorsal regions of the contralateral AVCN. The left AVCN section is more rostral. Scale bars, 500 μm. From Willott *et al.* (1985) with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc., and the author.

2. Changes in Connectivity between Auditory Centers

Willott (1986) has shown that there is a functional reorganization of the tonotopic or cochlear map in the central nucleus of the inferior colliculus of the C57 mouse. The reorganization consists of nerve cells in the ventral portions of the inferior colliculus, most sensitive to high frequencies in normal hearing young animals, becoming preferentially sensitive to lower frequencies in old C57 mice, a phenomenon not seen in old CBA mice with a milder flat hearing loss. These physiological findings are explained in more detail in Walton and Burkard (Chapter 40). One important question concerning this functional reorganization is: at what level of the auditory central nervous does the transformation from high frequency sensitivity to low frequency sensitivity take place? To begin to answer this question, Willott *et al.* (1985) placed relatively focal horseradish peroxidase (a central nervous system anatomical tracer) injections into dorsal, intermediate, and ventral regions of the central nucleus of inferior colliculi of young adult, middle-aged, and old C57 mice. If the modification of inputs occurred above the level of the cochlear nucleus, then injections into the ventral inferior colliculus should label nerve cells of the ventral portions of the ventral cochlear nucleus, rather than the dorsal regions of the ventral cochlear nucleus as occurs in normal young adult mammals. As shown here in Fig. 37.9, Willott and colleagues found that regardless of the animal's age, horseradish peroxidase injections into ventral regions of inferior colliculi always retrogradely labeled nerve cell bodies in dorsal regions of the ventral cochlear nucleus. This suggests that the transformation of high-frequency pathways of the central auditory system to carry lower frequency information in old C57 mice takes place in the cochlear nucleus. It may be that auditory nerve axons or axonal collaterals reroute from the ventral portions of the ventral cochlear nucleus as a function of an age-related decline of auditory nerve fibers from the base of the C57 cochlea to innervate and synaptically terminate on nerve cells located more dorsally in the ventral cochlear nucleus. It could also be that the neuronal pathways are already present in young C57 mice, but they do not become preferentially activated until the high-frequencies inputs become attenuated and less dominant with age.

3. Neurochemical Responses in Auditory Neurons

Zettel *et al.* (1997) investigated the presence of the calcium-binding proteins calbindin and calretinin in the inferior colliculus of young and old C57 mice. The exact function of these proteins is not known; however, the available evidence suggests that they play critical roles in intracellular calcium regulation crucial for proper nerve cell functioning. Not unexpectedly, since declines in calbindin immunoreactivity have been reported in other systems of the aging brain of mammals, there was a statistically significant reduction in the number of nerve cells labeled with calbindin in the old C57s, with no change in calretinin. These quantitative data are shown in Fig. 16 of Fri-

sina *et al*. (Chapter 39). O'Neill *et al*. (1997) performed a similar investigation in the medial nucleus of the trapezoid body of C57 and CBA mice of different ages. O'Neill and coworkers found that the number of calbindin-immunoreactive neurons declined in the C57 strain but not in the CBA mouse. In addition, they measured the total number of nerve cells in the medial nucleus of the trapezoid body with age and found minimal declines that could not account for the more dramatic reductions in calbindin immunostaining. They concluded that for the medial nucleus of the trapezoidal body, the downregulation of calbindin was a result of the decline in peripheral inputs in the C57 with age.

B. Summary of Peripherally Induced Effects

Age-related reductions in peripheral inputs to the central auditory system oftentimes result in reductions in neuron numbers or size in regions of the cochlear nucleus that are heavily innervated by the synaptic endings of auditory nerve fibers. There may also be rewiring of axonal terminations and ramifications in response to reduced activity in high-frequency auditory nerve fibers with age. At the electron microscopy level, comparison of C57 and CBA strains indicate that synaptic reductions in the auditory midbrain may be primarily determined by declines in peripheral inputs, rather than a consequence of aging itself. The immunocytochemical studies examining the presence of calcium-binding proteins revealed that declines in calbindin sometimes occur with age in the central auditory system, but in other instances appear to be a result of reduced input from the cochlea.

IV. Central Auditory System: Aging Brain

A. Animal Models Exhibit Changes That Differ from Those Induced by the Aging Periphery

1. Alterations in Size and Pathway Connectivity

In an investigation of the octopus region of the posteroventral cochlear nucleus of CBA and C57 strains, Willott and Bross (1990) measured nerve cell sizes, packing density, and other light microscope features. Although there were differences in absolute numbers between the strains, both showed the same age-related trends, including declines in the overall volume of this area, loss of nerve cells, declines in nerve cell size, increases in glial cell packing density, and highly variable reductions in the size and presence of primary dendritic branches. In this case, taking results from the two strains into account, significant reduction in peripheral inputs did not exacerbate any central nervous system aging effects occurring in the brains of both strains.

Willott *et al*. (1994a) examined gross morphological features of the inferior colliculus as a function of age for CBAs and C57s. They measured the overall size of the inferior colliculus, the size of the inferior colliculus nerve cells, and the packing density of these cells and found no significant changes in either strain with age. They concluded that for these gross measures, declining peripheral input in the case of the C57s was not detrimental, and the aging of the brain itself did not affect these measurements either.

Frisina *et al*. (1997, 1998) explored the connections of the dorsomedial region of the CBA auditory midbrain, examples of which are displayed here in Fig. 12 of Frisina *et al*. (see Chapter 39). The anatomical region under study had previously been functionally characterized in a single-unit neurophysiological study by Walton *et al*. (1997) and explained in more detail in Chapters 40, and 39 (Fig. 8). Here, since the anatomy and physiology were conducted in the same animals, physiological maps could be literally overlaid with harsenadish peroxidase injection sites, as shown in Chapter 39 (Fig. 11). Walton *et al*. (1998) then went on to demonstrate that the sound gap encoding capabilities of single nerve cells in this region of the inferior colliculus became degraded with age. These neurophysiological temporal processing declines with age are more fully described in Chapters 39 (Figs. 9 and 10) and 40. We then examined how the inputs to this functionally characterized region changed with age in the CBAs. We found that some major inputs to dorsomedial inferior colliculi, such as those from the ipsilateral superior olivary complex and the ipsilateral nuclei of the lateral lemniscus remained stable with age, (see Figs. 14 and 15 in Chapter 39). Whereas the number of retrogradely labeled cell bodies from all three divisions of the contralateral cochlear nucleus and ipsilateral anterolateral periolivary nucleus declined dramatically as the CBAs aged, with statistically significant reductions starting in the middle-aged group of mice. These age-related drops are displayed in Figs. 13 and 14 in Chapter 39.

2. Changes in Intracellular Calcium Regulators

Zettel *et al*. (1997) examined the presence of the calcium-binding proteins calbindin and calretinin in the inferior colliculi of young and old CBA mice. Declines in calbindin immunoreactivity have been reported in other systems of the aging brain of mammals. Consistent with this, there was a statistically significant reduction in the number of nerve cells labeled with calbindin in the old CBAs. However, surprisingly, Zettel and coworkers observed a dramatic increase, or upregulation, in the number of principal cells labeled with a calretinin antibody. These data are shown in Fig. 16 in Chapter 39. Such upregulation as a function of age is much less common in the aging mammalian brain and had not been previously reported for any proteins that regulate intracellular calcium concentrations. Taken together with the Zettel *et al*. discovery that the number of calbindin-containing nerve cells in this region decline with age in C57 mice (described above), it appears that maintenance of peripheral input, sustenance of hearing in old age, is required or causes the upregulation of calretinin, but that the downregulation of calbindin may result from cellular aging processes of the central auditory system that occur on both mouse strains who have widely differing inputs in old age.

3. Declines in Inhibitory Neurotransmitters

Utilizing a variety of neuroanatomical and biochemical methodologies, Caspary, Helfert, and colleagues have uncovered evidence suggesting that there are significant declines in inhibitory synaptic circuitry in the rat auditory brain stem (Caspary *et al*., 1995). Specifically, in the cochlear nucleus,

glycine is the predominant inhibitory neurotransmitter and is likely essential for optimal auditory processing of biologically relevant acoustic signals in background noise. Milbrandt and Caspary (1995) showed that strychnine, a glycine antagonist, binding to glycine receptor sites significantly declined in the anteroventral and dorsal cochlear nucleus of old rats, with no significant change in affinity.

GABA (γ aminobutyric acid) is a primary inhibitory neurotransmitter in the central auditory system that is particularly prominent in nerve endings and nerve cells in the inferior colliculus, especially its central nucleus. It is very likely that this inhibitory transmitter plays a crucial role in several aspects of auditory functioning such as binaural coding, temporal processing, and discrimination of sounds from background noise. Caspary *et al*. (1990) immunolabeled inferior colliculus nerve cells against GABA in young (2- to 7-month) and old (18- to 29-month) Fischer-344 rats. Cell counts showed that in the ventrolateral central nucleus of the inferior colliculus (high-frequency region) the number of labeled neurons decreased by 36% with age. Neurochemical experiments measured the basal (resting) and K^+-evoked efflux of GABA from the central nucleus of the inferior colliculus. Tissue from central nucleus of the inferior colliculus old animals showed statistically significant reductions in both basal and K^+-evoked release of GABA relative to young rats. As a control, release of excitatory neurotransmitters, such as glutamate and aspartate, as well as another endogenous amino acid, tyrosine, and the inhibitory neurotransmitter acetylcholine, were measured and found to remain stable with age.

In a follow-up study, Caspary's group demonstrated an age-related decline in GABAB receptor binding in the rat inferior colliculus (Milbrandt *et al*., 1994). Using quantitative receptor autoradiography, reductions were demonstrated in the central nucleus, dorsal cortex, and external nucleus, (Fig. 37.10),

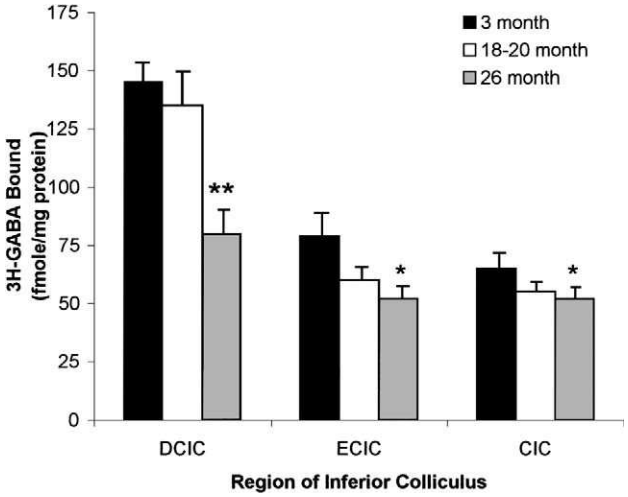

FIG. 37.10. GABAB receptor binding in the inferior colliculus declines with age in rats, with the biggest reduction occurring between middle age (18–20 months) and old age (26 months). Binding was significantly greater in the dorsal cortex nucleus of the inferior colliculus than in the other two divisions studied here. **, $P < 0.05$; *, $P < 0.05$; CIC, central nucleus of IC; DCIC, dorsal cortex of IC; ECIC, external nucleus of IC. From Milbrandt *et al.* (1994) with permission from Elsevier Science and the author.

whereas no age-related deficits were observed in neighboring cerebellar tissue. The inferior colliculus changes were seen despite no change in the cross-sectional area of the rat inferior colliculus with age. Milbrandt *et al*. (1994) went on to show that GABAB receptor binding decreased in old rats. In contrast, the number of GABAA receptors increased with age, perhaps as a partial compensation for the GABAB losses (Milbrandt *et al*., 1996).

Using immunogold electron microscopy methodologies, Helfert *et al*. (1999) quantitatively compared changes in the organization of GABA-containing and GABA-free terminals and synapses in the rat inferior colliculus. They discovered that the density of both types of terminals and synapses declined with age by 24–33% in old rats relative to young adults, with the range reduced by 9% if the densities are corrected for overall shrinkage of the inferior colliculus with age. The synaptic declines were correlated with reductions in dendritic size for inferior colliculus neurons, with the synaptic density remaining stable on those portions of GABA-negative dendrites that remained in old age. In GABA-containing dendrites, the pattern of synapses shifted to larger diameter dendrites, presumably closer to the cell body in the old animals. Since the age-related declines were similar for GABA-containing and GABA-free synaptic densities, the results of this study suggest that excitatory and inhibitory inputs decline proportionately with age, avoiding an age-related imbalance. In another investigation, Milbrandt *et al*. (1997) examined the GABAA receptor subunit composition. They found that this changes with age in the rat inferior colliculus such that enhanced responses to GABA can occur. Specifically, the gamma1 protein subunit *increased* with age, and the α_1 *decreased* with age (Caspary *et al*., 1999). They also found an age-related increase in GABA-mediated chloride influx, which is a functional consequence of these changes in the subunit composition. These varying effects are summarized in terms of functional implications (Fig. 37.11). If similar redistributions occur for excitatory synapses which enhance their responsiveness, then the relative balance and strength of excitation and inhibition could be somewhat maintained with age as an attempt to help preserve normal functioning of the inferior colliculus in the face of other age-related structural and neurochemical declines, such as those involving GABAB receptors.

An informative summary of Caspary and Helfert's investigations is given in an article by Caspary *et al*. (1995), (Figs. 37.12 and 37.13). The general finding is that there are declines in most aspects of GABA biochemistry in the inferior colliculus, including the presence of GABA, its receptors, and its activity levels. A limitation of this line of research is that these reductions in the presence of inhibitory neurotransmitters and their activity levels have rarely been reported in other species, so it is not clear how these results apply to auditory systems of other mammals and the human condition.

4. Other Neurochemical Investigations

Cransac *et al*. (1996) examined the presence of several important central nervous system neurotransmitters in the rat cochlear nucleus and medial vestibular nucleus as a function of age. They found that noradrenaline levels increased in the anteroventral cochlear nucleus and decreased in the vestibular nucleus. Serotonin levels were elevated with age in all divi-

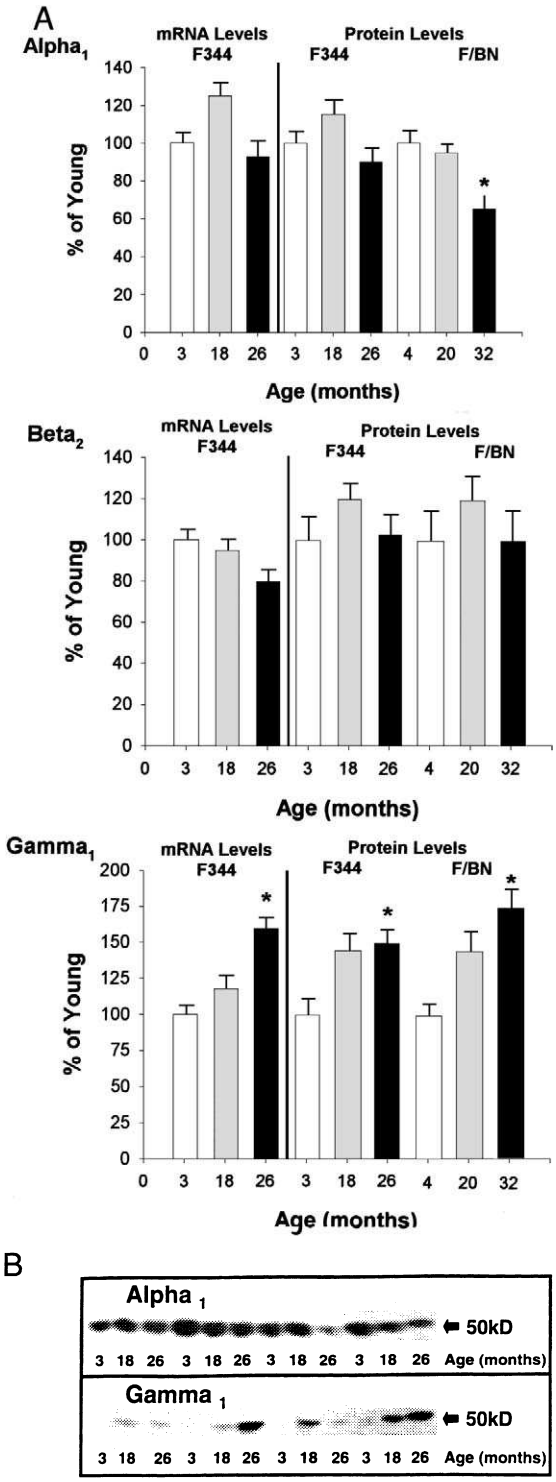

FIG. 37.11. GABAa receptor subunit composition changes with age in the rat central nucleus of the inferior colliculus, as demonstrated by Western blot and *in situ* hybridization studies. (A) For the α_1 subunit, mRNA and protein levels rise by middle age and then fall for old age. β_2 mRNA levels fall with age, and protein levels rise for middle age and then fall in the old animals. Gamma$_1$ mRNA and protein levels all rise with age. Only the γ_1 increases in old age, and the F/BN protein decline in old age were statistically significant (*). F/BN = Fischer 344/Brown-Norway F1 hybrid rats. (B) Western blot findings for FBN rats indicate age-related decreases for the α_1 subunit, and increases for the γ_1 subunit. From Caspary *et al*. (1999) with permission from Elsevier Science and the author.

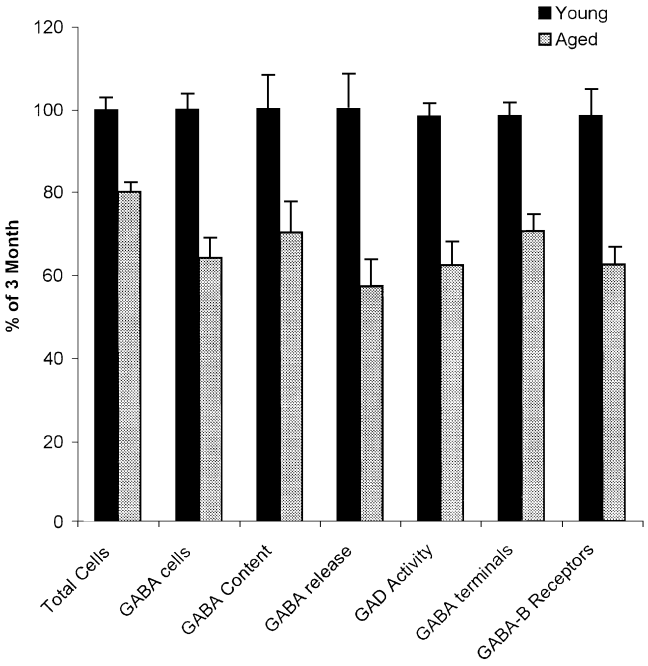

FIG. 37.12. Most aspects of GABA biochemistry in the rat inferior colliculus decline with age, as summarized on this histogram where aged (19–28 months) data are plotted as a percentage of levels from young animals (2.5–4 months). Differences between young and old data for each experiment are significant at the $P < 0.05$ level; the bars represent the standard errors of the mean. From Caspary *et al*. (1995) with permission from Elsevier Science and the author.

sions of the cochlear nucleus and the vestibular nucleus. These investigators concluded that elevation of serotonin may be widespread, whereas the noradrenaline changes are more varied in primary sensory centers of the brain stem with age. Increased levels of these modulatory neurotransmitters could be one way to compensate for decreased peripheral inputs that

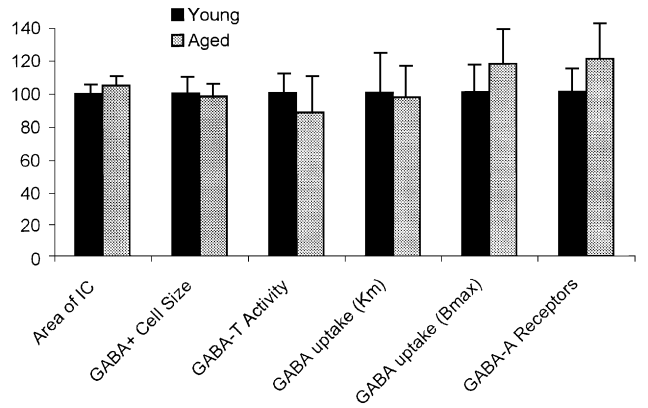

FIG. 37.13. Some aspects of the gross anatomy and GABA biochemistry in the rat inferior colliculus remained stable with age, as summarized on this histogram where aged (19–28 months) data are plotted as a percentage of levels from young animals (2.5–4 months). Differences between young and old data for each experiment were not statistically significant; the bars represent the standard errors of the mean. From Caspary *et al*. (1995) with permission from Elsevier Science and the author.

occur with age in the auditory and vestibular systems to improve the processing of acoustic signals in background noise.

Astrocytes and glial cells were traditionally thought to provide only a supportive, trophic function for nerve cells in the brain. However, more recent evidence suggests that they may play more active roles in real-time neural processing, as related to neurotransmitter uptake and regulation of synaptic density. Jalenques *et al*. (1995) analyzed the presence of glial fibrillary acidic protein (GFAP) immunoreactivity in the cochlear nuclei of young adult (3-month-old) and old (24-month-old) Sprague–Dawley rats, which have a mild peripheral hearing loss with age. GFAP is selectively located in astrocytes (Malhatra *et al*., 1990) and is an intermediate filament protein in astrocytes (Eng *et al*., 1971) that is differentially expressed in the face of brain aging or trauma (Eng, 1988). Jalenques and coworkers reported that there was a quantitative increase in GFAP immunoreactive astrocytes and processes with age and that the topographic distribution of label was altered. In the young adult animals, there is a concentration in the granular cell region, whereas this specificity disappears in the old rats and takes on a more homogenous distribution throughout the cochlear nucleus (Jalenques *et al*. 1997), (Fig. 37.14). The age-related increase in GFAP immunoreactive astrocytes actually peaks in middle age (12 months of age) and then declines a little with further aging. As a backdrop, they measured the total number of nerve cells in the cochlear nucleus and found no change with age, except for a decline in the anteroventral division with age.

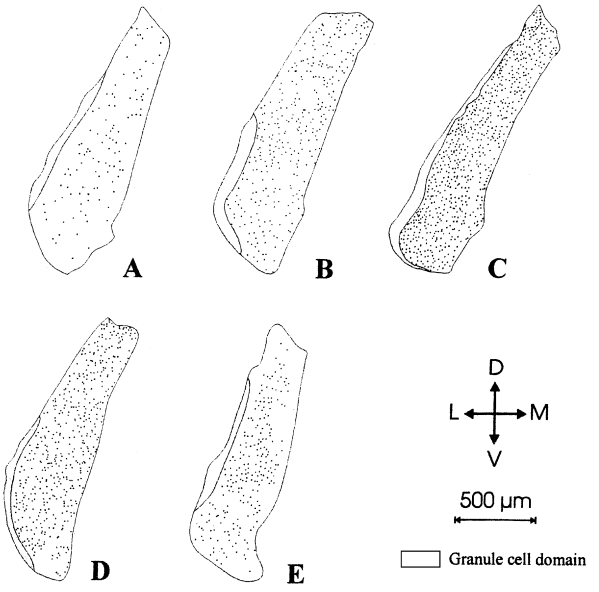

FIG. 37.14. In the anteroventral cochlear nucleus, GFAP-immunoreactive astrocytes increase in number and become topographically patterned from youth to middle age (3–12 months). As animals age beyond middle age to old age (18–24 months), the topographic distribution of GFAP astrocytes disappears and the number of astrocytes declines: (A) 3 months old, (B) 6 months old, (C) 12 months old, (D) 18 months old, (E) 24 months old. From Jalenques *et al*. (1997) with permission from Elsevier Science and the author.

B. Human Investigations

Relative to the major efforts put into anatomical and neurochemical studies of the aging brain related to age-related neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (e.g., Flood, 1993), very little is directly known about such matters for the human central auditory nervous system. However, what is known suggests that what has been discovered in the animal studies of the aging central auditory system probably occur to some extent in human presbycusis. For instance, in the human cochlear nucleus, age results in a reduction in ventral cochlear nucleus volume (Königsmark and Murphy, 1970, 1972) and neuron size (Seldon and Clark, 1991), without any significant reductions in neuron number, relative to that seen in young adults. However, increases in lipofuscin and decreases in the number of capillaries/unit area were reported with age.

In the lateral lemniscus, the major input tract to the inferior colliculus, Ferraro and Minckler (1977) report a significant decline in the number of nerve fibers with age, based upon analysis of 15 human brains ranging in age from birth to 97 years. At the midbrain level, McGeer and McGeer (1975) examined postmortem presence of GAD, a precursor to GABA important for its synthesis in neurons, and found that it significantly declines with age. At the level of the auditory cortex in the superior temporal gyrus, Brody (1955) reported a striking decline in the number of neurons with age (correlation coefficient $= -0.99$). This decline was much greater than reductions in neighboring cortical areas such as the inferior temporal cortex, striate (visual) cortex, and pre- and postcentral cortex.

Another hypothesis that has more recently been put forth states that mitochondrial DNA (mtDNA) deletions or point mutations, which result in declines of oxidative phosphorylation activity, play an important role in the eventual demise of postmitotic nerve cells (Corral-Debrinski *et al*., 1992), including those of the central auditory system (Keithley *et al*., 1999). The high metabolic requirements of nerve cells make mitochondrial dysfunction quite harmful for auditory system neurons. mtDNA is quite susceptible to mutation because it is exposed to high concentrations of free radicals that are byproducts of the respiratory chain reactions that take place in mitochondria.

C. Summary of Changes Due to the Aging Brain

As age occurs, one of the lessons learned from the animal model and human investigations is that loss of sensory cells and auditory nerve fibers is much greater in magnitude than the declines in numbers of nerve cells in the central auditory system, even at the level of the cochlear nucleus which directly receives the synaptic endings of the eighth nerve. Rather, age-related changes in the brain appear to be more subtle, such as shrinkage of nerve cells and overall volume, alterations in connectional pathways, or changes in the neurochemical makeup of nerve cells with age. For the neurochemical studies, like the anatomy, most changes are in the negative direction. For example, when we observed changes in the presence of calbindin with age, the alterations always involved a decline in the presence of calbindin. The same can be said for the inhibitory neurotransmitter studies at the level of the auditory midbrain.

Interestingly, whenever we have observed changes in calretinin in the aging auditory system, an *upregulation* has been discovered.

V. Overview and Future Directions

An understanding of the structural and chemical changes in the ear and the brain underlying presbycusis allows for development of prevention therapies and curative regimes to avoid or reverse age-related hearing loss. As with most biomedical aging problems, sorting out whether auditory pathologies are due to internal factors (i.e., cellular or systemic aging phenomena ultimately determined or programmed genetically) or external environments (such as noise, ototoxic drugs, fever, or diet) is important for moving toward effective ameliorations. Many of the structural and, to a lesser extent, chemical changes in the aging ear and brain are now known. However, the common and quintessential etiologies of these degradations are still poorly understood. Future successes of genetic therapeutic interventions—turning genes on and off to prevent or correct age-related pathologies—will depend on adequate knowledge of the causative chain of events that results in age-related deficits in sensory end organs like the ear and the sensory portions of the central nervous system.

Acknowledgments

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References

Belal, A. (1975). Presbycusis: Physiological or pathological. *J. Laryngol. Otol*. **89**, 1011–1025.

Bohne, B. A., Gruner, M. M., and Harding, G. W. (1990). Morphological correlates of aging in the chinchilla cochlea. *Hear. Res*. **48**, 79–92.

Bredberg, G. (1968). Cellular pattern and nerve supply of the human organ of Corti. *Acta Oto-Laryngol. (Suppl.)* **236**, 1–135.

Brody, H. (1955). Organization of the cerebral cortex: III. A study of aging in the human cerebral cortex. *J. Comp. Neurol.* **102**, 511–556.

Caspary, D. M., Raza, A., Armour, B. A. L., Pippin, J., and Arneric, S. P. (1990). Immunocytochemical and neurochemical evidence for age-related loss of GABA in the inferior colliculus: Implications for neural presbycusis. *J. Neurosci*. **10**, 2363–2372.

Caspary, D. M., Milbrandt, J. C., and Helfert, R. H. (1995). Central auditory aging: GABA changes in the inferior colliculus. *Exp. Gerontol*. **30**, 349–360.

Caspary, D. M., Holder, T. M., Hughes, L. F., Milbrandt, J. C., McKernan, R. M., and Naritoku, D. K. (1999). Age-related changes in GABAa receptor subunit composition and function in rat auditory system. *Neuroscience* **93**, 307–312.

Cohen, G. M., Park, J. C., and Grasso, J. S. (1990). Comparison of demyelination and neural degeneration in spiral and Scarpa's ganglia of C57BL/6 mice. *J. Electron Microsc. Tech*. **15**, 165–172.

Corral-Debrinski, M., Horton, T., Lott, M. T., Shoffner, J. M., Beal, M. F., and Wallace, D. C. (1992). Mitochondrial DNA deletions in human brain: Regional variability and increase with advanced age. *Nat. Genet*. **2**, 324–329.

Covell, W. P., and Rogers, J. B. (1957). Pathologic changes in the inner ears of senile guinea pigs. *Laryngoscope* **67**, 118–129.

Cransac, H., Peyrin, L., Cottet-Emard, J. M., Farhat, F., Pequignot, J. M., and Reber, A. (1996). Aging effects on monoamines in rat medial vestibular and cochlear nuclei. *Hear. Res*. **100**, 150–156.

Dazert, S., Feldman, M. L., and Keithley, E. M. (1996). Cochlear spiral ganglion cell degeneration in wild-caught mice as a function of age. *Hear. Res*. **100**, 101–106.

Ding, D. L., McFadden, S. L., Wang, J., Hu, B. H., and Salvi, R. J. (1998). Age-and strain-related differences in dehydrogenase activity and glycogen levels in CBA and C57 mouse cochleas. *Audiol. Neuro-Otol*. **2**, 1–14.

Eng, L. F. (1988). Astrocytic response to injury. *In* "Current Issues in Neuronal Regeneration Research" (P. Reier, R. Bunge, and F. Seil, eds.), pp. 247–255. Liss, New York.

Eng, L. F., Vanderhaeghen, J. J., Bignami, A., and Gersti, B. (1971). An acid protein isolated from fibrous astrocytes. *Brain Res*. **28**, 351–354.

Engström, B., Hillerdal, M., and Laurell, G. (1987). Selected pathological findings in the human cochlea. *Acta Oto-Laryngol., Suppl*. **436**, 110–116.

Erway, L. C., Willott, J. F., Archer, J. R., and Harrison, D. E. (1993). Genetics of age-related hearing loss in mice: I. Inbred and F1 hybrid strains. *Hear. Res*. **65**, 125–132.

Felder, E., and Schrott-Fischer, A. (1995). Quantitative evaluation of myelinated nerve fibres and hair cells in cochleae of humans with age-related high-tone hearing loss. *Hear. Res*. **91**, 19–32.

Ferraro, J. A., and Minckler, J. (1977). The human lateral lemniscus and its nuclei. *Brain Lang*. **4**, 277–294.

Fischel-Ghodsian, N., Bykhovskaya, Y., Taylor, K., Kahen, T., Cantor, R., Ehrenman, K., Smith, R., and Keithley, E. M. (1997). Temporal bone analysis of patients with presbycusis reveals high frequency of mitochondrial mutations. *Hear. Res*. **110**, 147–154.

Flood, D. G. (1993). Critical issues in the analysis of dendritic extent in aging humans, primates, and rodents. *Neurobiol. Aging* **14**, 649–654.

Frisina, R. D., Walton, J., Lynch-Armour, M. A., and Klotz, D. A. (1997). Efferent projections of a physiologically characterized region of the inferior colliculus of the young adult CBA mouse. *J. Acoust. Soc. Am*. **101**, 2741–2753.

Frisina, R. D., Walton, J., Lynch-Armour, M. A., and Byrd, J. D. (1998). Inputs to a physiologically characterized region of the inferior colliculus of the young adult CBA mouse. *Hear. Res*. **115**, 61–81.

Gratton, M. A., and Schulte, B. A. (1995). Alterations in microvasculature are associated with atrophy of the stria vascularis in quiet-aged gerbils. *Hear. Res*. **82**, 44–52.

Gratton, M. A., Schmiedt, R. A., and Schulte, B. A. (1996). Age-related decreases in endocochlear potential are associated with vascular abnormalities in the stria vascularis. *Hear. Res*. **102**, 181–190.

Gratton, M. A., Smyth, B. J., Lam, C. F., Boettcher, F. A., and Schmiedt, R. A. (1997). Decline in the endocochlear potential corresponds to decreased Na,K-ATPase activity in the lateral wall of quiet-aged gerbils. *Hear. Res*. **108**, 9–16.

Hawkins, J. E., and Johnsson, L. G. (1985). Otopathological changes associated with presbycusis. *Semin. Hear.* **6**, 115–133.

Helfert, R. H., Sommer, T. J., Meeks, J., Hofstetter, P., and Hughes, L. F. (1999). Age-related synaptic changes in the central nucleus of the inferior colliculus of Fischer-344 rats. *J. Comp. Neurol*. **406**, 285–298.

Henry, K. R., and Chole, R. (1980). Genotypic differences in behavioral, physiological and anatomical expressions of age-related hearing loss on the laboratory mouse. *Audiology* **19**, 369–383.

Hoeffding, V., and Feldman, M. L. (1988). Changes with age in the morphology of the cochlear nerve in rats: Light microscopy. *J. Comp. Neurol.* **276**, 537–546.

Ingham, N. J., Comis, S. D., and Withington, D. J. (1999). Hair cell loss in the aged guinea pig cochlea. *Acta Oto-Laryngol.* **119**, 42–47.

Jalenques, I., Albuisson, E., Despres, G., and Romand, R. (1995). Distribution of glial fibrillary acidic protein (GFAP) in the cochlear nucleus of adult and aged rats. *Brain Res*. **686**, 223–232.

Jalenques, I., Burette, A., Albuisson, E., and Romand, R. (1997). Age-related changes in GFAP-immunoreactive astrocytes in the rat ventral cochlear nucleus. *Hear. Res*. **107**, 113–124.

Johnsson, L. G. (1971). Reissner's membrane in the human cochlea. *Ann. Otol. Rhinol. Laryngol*. **80**, 425–439.

Johnsson, L. G., and Hawkins, J. E. (1969). Nerve degeneration and vascular changes in Corti's organ based on surface preparations of the human cochlea. *Oto- Rhino- Laryngol. (Amsterdam)* **206**, 655–656.

Johnsson, L. G., and Hawkins, J. E. (1972). Sensory and neural degeneration with aging, as seen in microdissections of the human inner ear. *Ann. Otol. Rhinol. Laryngol*. **81**, 179–194.

Kazee, A. M., and West, N. R. (1999). Preservation of synapses on principal cells of the central nucleus of the inferior colliculus with aging in the CBA mouse. *Hear. Res*. **133**, 98–106.

Kazee, A. M., Han, L. Y., Spongr, V. P., Walton, J. P., Salvi, R. J., and Flood, D. G. (1995). Synaptic loss in the central nucleus of the inferior colliculus correlates with sensorineural hearing loss in the C57BL/6 mouse model of presbycusis. *Hear. Res*. **89**, 109–120.

Keithley, E. M., and Feldman, M. L. (1979). Spiral ganglion cell counts in an age-graded series of rat cochleas. *J. Comp. Neurol*. **188**, 429–444.

Keithley, E. M., Ryan, A. F., and Woolf, N. K. (1988). Spiral ganglion cell density in young and old gerbils. *Hear. Res*. **38**, 125–134.

Keithley, E. M., Ryan, A. F., and Woolf, N. K. (1989). Spiral ganglion cell density in young and old gerbils. *Hear. Res*. **38**, 125–134.

Keithley, E. M., Ryan, A. F., and Feldman, M. L. (1992). Cochlear degeneration in aged rats of four strains. *Hear. Res*. **59**, 171–178.

Keithley, E. M., Linthicum, F., and Fischel-Ghodsian, N. (1999). Mitochondrial DNA, cytochrome oxidase and presbycusis. *Registry* **7**, 1–6.

Königsmark, B. W., and Murphy, E. A. (1970). Neuronal populations in the human brain. *Nature (London)* **228**, 1335–1336.

Königsmark, B. W., and Murphy, E. A. (1972). Volume of the ventral cochlear nucleus in man: Its relationship to neuronal populations and age. *J. Neuropathol. Exp. Neurol*. **31**, 304–316.

Malhatra, S. K., Shnitka, T. K., and Elbrluk, J. (1990). Reactive astrocytes: A review. *Cytobios* **61**, 133–160.

McFadden, S. L., Campo, P., Quaranta, N., and Henderson, D. (1997). Age-related decline of auditory function in the chinchilla (*Chinchilla laniger*). *Hear. Res*. **111**, 114–126.

McGeer, E. G., and McGeer, P. L. (1975). Age changes in the human for some enzymes associated with metabolism of catecholamines, GABA and acetylcholine. *In* ''Neurobiology of Aging'' (J. M. Ordy and K. R. Brizzee, eds.), pp. 287–305, Plenum, New York.

Milbrandt, J. C., and Caspary, D. M. (1995). Age-related reduction of [^{3}H]strychnine binding sites in the cochlear nucleus of the Fischer 344 rat. *Neuroscience* **67**, 713–719.

Milbrandt, J. C., Albin, R. L., and Caspary, D. M. (1994). Age-related decrease in GABAb receptor binding in the Fischer 344 rat inferior colliculus. *Neurobiol. Aging* **15**, 699–703.

Milbrandt, J. C., Albin, R. L., Turgeon, S. M., and Caspary, D. M. (1996). GABAa receptor binding in the aging rat inferior colliculus. *Neuroscience* **73**, 449–458.

Milbrandt, J. C., Hunter, C., and Caspary, D. M. (1997). Alterations of GABAa receptor subunit mRNA levels in the aging Fischer rat inferior colliculus. *J. Comp. Neurol*. **379**, 455–465.

O'Neill, W. E., Zettel, M. L., Whittemore, K. R., and Frisina, R. D. (1997). Calbindin D-28k immunoreactivity in the medial nucleus of the trapezoid body declines with age in C57BL/6, but not CBA/CaJ, mice. *Hear. Res*. **112**, 158–166.

Otte, J., Schuknecht, H. F., and Kerr, A. G. (1978). Ganglion cell populations in normal and pathological human cochleae. Implications for cochlear implantation. *Laryngoscope* **38**, 1231–1246.

Pauler, M., Schuknecht, H. F., and Thornton, A. R. (1986). Correlative studies of cochlear neuronal loss with speech discrimination and pure-tone thresholds. *Arch. Oto-Rhino-Laryngol*. **243**, 200–206.

Rasmussen, A. T. (1940). Studies of the VIIIth cranial nerve of man. *Laryngoscope* **50**, 67–83.

Saada, A. A., Niparko, J. K., and Ryugo, D. K. (1996). Morphological changes in the cochlear nucleus of congenitally deaf white cats. *Brain Res*. **736**, 315–328.

Sakaguchi, N., Spicer, S. S., Thomopoulos, G. N., and Schulte, B. A. (1997). Increased laminin deposition in capillaries of the stria vascularis of quiet-aged gerbils. *Hear. Res*. **105**, 44–56.

Schmiedt, R. A. (1993). Cochlear potentials in gerbils: Does the aging cochlea need a jump start? *In* ''Sensory Research: Multimodal Perspectives'' (R. T. Verrillo, ed.), pp. 91–103. Erlbaum, Hillsdale, NJ.

Schmiedt, R. A. (1996). Effects of aging on potassium homeostasis and the endocochlear potential in the gerbil cochlea. *Hear. Res*. **102**, 125–132.

Schmiedt, B. A., and Adams, J. A. (1989). Distribution of immunoreactive Na^+K^+-ATPase in gerbil cochlea. *J. Histochem. Cytochem*. **37**, 127–134.

Schmiedt, R. A., Mills, J. H., and Adams, J. A. (1990). Tuning and suppression in auditory nerve fibers of aged gerbils raised in quiet or noise. *Hear. Res*. **45**, 221–236.

Schuknecht, H. F. (1964). Further observations on the pathology of presbycusis. *Arch. Otolarygol*. **80**, 369–382.

Schuknecht, H. F. (1974). ''Pathology of the Ear.'' Harvard University Press, Cambridge, MA.

Schuknecht, H. F. (1993). ''Pathology of the Ear.'' Lea & Febiger, Philadelphia.

Schuknecht, H. F., Watanuki, K., Takahashi, T., Belal, A., Kimura, R. S., and Jones, D. D. (1974). Atrophy of the stria vascularis, a common cause for hearing loss. *Laryngoscope* **84**, 1777–1821.

Schulte, B. A., and Adams, J. A. (1989). Distribution of immunoreactive Na^+,K^+-ATPase in gerbil cochlea. *J. Histochem. Cytochem*. **37**, 127–134.

Schulte, B. A., and Schmiedt, R. A. (1992). Lateral wall Na^+, K^+-ATPase and endocochlear potentials decline with age in quiet-reared gerbils. *Hear. Res*. **61**, 35–46.

Seldon, H. L., and Clark, G. M. (1991). Human cochlear nucleus: Comparison of Nissl-stained neurons from deaf and hearing patients. *Brain Res*. **551**, 185–194.

Spicer, S. S., and Schulte, B. A. (1991). Differentiation of inner ear fibrocytes according to their ion transport related activity. *Hear. Res*. **56**, 53–64.

Spicer, S. S., Gratton, M. A., and Schulte, B. A. (1997). Expression patterns of ion transport enzymes in spiral ligament fibrocytes change in relation to strial atrophy in the aged gerbil cochlea. *Hear. Res*. **111**, 93–102.

Spoendlin, H., and Schrott, A. (1989). Analysis of the human auditory nerve. *Hear. Res*. **43**, 25–38.

Spoendlin, H., and Schrott, A. (1990). Quantitative evaluation of the human cochlear nerve. *Acta Oto-Laryngol., Suppl*. **470**, 61–70.

Spongr, V. P., Flood, D. G., Frisina, R. D., and Salvi, R. J. (1997a). Quantitative measures of hair cell loss in CBA and C57BL/6 mice throughout their life spans. *J. Acoust. Soc. Am*. **101**, 3546–3553.

Spongr, V. P., Walton, J. P., Frisina, R. D., Kazee, A. M., Flood, D. G., and Salvi, R. J. (1997b). Hair cell loss and synaptic loss in inferior colliculus of C57BL/6 mice. *In* ''Acoustical Signal Processing in the Central Auditory System'' (J. Syka, ed.), pp. 535–542. Plenum, New York.

Tarnowski, B. I., Schmiedt, R. A., Hellström, L. I., Lee, F. S., and Adams, J. C. (1991). Age-related changes in cochleas of Mongolian gerbils. *Hear. Res*. **54**, 123–134.

Thomopoulos, G. N., Spicer, S. S., Gratton, M. A., and Schulte, B. A. (1997). Age-related thickening of basement membrane in stria vascularis capillaries. *Hear. Res*. **111**, 31–41.

Walton, J. P., Frisina, R. D., Ison, J. R., and O'Neill, W. E. (1997). Neural correlates of behavioral gap detection in the inferior colliculus of the young CBA mouse. *J. Comp. Physiol. A* **181**, 161–176.

Walton, J. P., Frisina, R. D., and O'Neill, W. E. (1998). Age-related alteration in processing of temporal sound features in the auditory midbrain of the CBA mouse. *J. Neurosci*. **18**, 2764–2776.

Willott, J. F. (1986). Effects of aging, hearing loss, and anatomical location on thresholds of inferior colliculus neurons in C57BL/6 and CBA mice. *J. Neurophysiol*. **56**, 391–408.

Willott, J. F. (1991). "Aging and the Auditory System: Anatomy, Physiology, and Psychophysics." Singular Publishing Group, San Diego, CA.

Willott, J. F., and Bross, L. S. (1990). Morphology of the octopus cell area of the cochlear nucleus in young and aging C57BL/6J and CBA/J mice. *J. Comp. Neurol*. **300**, 61–81.

Willott, J. F., and Bross, L. S. (1996). Morphological changes in the anteroventral cochlear nucleus that accompany sensorineural hearing loss in DBA/2J and C57Bl/6J mice. *Dev. Brain Res*. **91**, 218–226.

Willott, J. F., and Erway, L. C. (1998). Genetics of age-related hearing loss in mice. IV. Cochlear pathology and hearing loss in 25 BXD recombinant inbred mouse strains. *Hear. Res*. **119**, 27–36.

Willott, J. F., Demuth, R. M., and Lu, S. M. (1984). Excitability of auditory neurons in the dorsal and ventral cochlear nuclei of DBA/2 and C57BL/6 mice. *Exp. Neurol*. **83**, 495–506.

Willott, J. F., Pankow, D., Hunter, K. P., and Kordyban, M. (1985). Projections from the anterior ventral cochlear nucleus to the central nucleus of the inferior colliculus in young and aging C57Bl/6 mice. *J. Comp. Neurol*. **237**, 545–551.

Willott, J. F., Jackson, L. M., and Hunter, K. P. (1987). Morphometric study of the anteroventral cochlear nucleus of two mouse models of presbycusis. *J. Comp. Neurol*. **260**, 472–480.

Willott, J. F., Bross, L. S., and McFadden, S. L. (1992). Morphology of the dorsal cochlear nucleus in C57BL/6J and CBA/J mice across the life span. *J. Comp. Neurol*. **321**, 666–678.

Willott, J. F., Bross, L. S., and McFadden, S. L. (1994a). Morphology of the inferior colliculus in C57BL/6J and CBA/J mice across the life span. *Neurobiol. Aging* **25**, 175–183.

Willott, J. F., Bross, L. S., and McFadden, S. L. (1994b). Morphology of the cochlear nucleus in CBA/J mice with chronic, severe sensorineural cochlear pathology induced during adulthood. *Hear. Res*. **74**, 1–21.

Willott, J. F., Erway, L. C., Archer, J. R., and Harrison, D. (1995). Genetics of age-related hearing loss in mice: II. Strain differences and effects of caloric restriction on cochlear pathology and evoked response thresholds. *Hear. Res*. **88**, 143–155.

Zettel, M. L., Frisina, R. D., Haider, S. E. A., and O'Neill, W. E. (1997). Age-related changes in calbindin D-28K and calretinin immunoreactivity in the inferior colliculus of CBA/CaJ and C57B1/6 mice. *J. Comp. Neurol*. **386**, 92–110.

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38

Age, Noise, and Ototoxic Agents

As the population ages and life expectancy increases, more and more individuals will be at risk for developing age-related hearing loss, or presbycusis. Presbycusis can greatly impair interpersonal communication and lead to social isolation in the elderly. Age-related damage to various sensory and structural elements in the inner ear gives rise to different types of hearing loss. The type of damage that develops in the ear and the rate at which presbycusis progresses undoubtedly have a genetic basis. Rapid advances in molecular biology and in sequencing the human and mouse genomes will enhance our understanding of the genes that contribute to age-related hearing loss. Hearing loss in the elderly can have other causes as well. In particular, hearing loss can be caused by exposure to loud sounds and various therapeutic drugs, including several antibiotics and anti-tumor agents. Importantly, it is likely that these three agents—biological aging, noise exposure, and exposure to ototoxic drugs—interact in some manner (e.g., additive, subadditive, multiplicative). Regardless of the exact nature of the interaction, the hearing loss is likely to occur earlier and be more severe than that produced by any one agent alone. Strategies for reducing the risk of age-related hearing loss, ototoxicity and noise-induced hearing loss require an understanding of the underlying mechanisms of cell death in the inner ear. Recent studies with neurotrophic compounds and antioxidants may lead to new intervention strategies.

I. Introduction

The auditory system, like other sensory and motor systems, shows a gradual deterioration in function over time. Presbycusis, or the age-related decline in hearing function, is presumably the result of many factors, some endogenous and others exogenous. For example, the deterioration of other systems in the body, such as the cardiovascular system or the kidneys, could conceivably exacerbate age-related deterioration of cellular components in the inner ear. Several exogenous factors are likely to contribute to the loss of hearing with advancing age. For example, the accumulative effect of many years of noise exposure or short-term treatments with ototoxic drugs could simply add to the effect of age-related hearing loss or it could exacerbate age-related hearing loss. Because humans are constantly bombarded by different environmental stressors, it is difficult to distinguish what proportion of hearing loss is purely the result of aging or environmental factors (Corso, 1976; Gates *et al*., 1989; Cruickshanks *et al*., 1998). In highly industrialized societies, one important environmental factor that causes hearing loss is incessant exposure to a barrage of environmental sounds. Hearing loss can also be caused by certain drugs used to treat life-threatening illnesses. For example, several anti-cancer and anti-microbial drugs are ototoxic, causing permanent damage to the delicate sensory hair cells in the inner ear. These three agents—aging, noise, and ototoxic drugs—can each cause hearing loss independently. However, hearing loss in the elderly is probably always due to a combination of factors, or to interactions among them. Like aging, noise exposure and ototoxic drugs exert their primary effects on the inner ear. Therefore, it is instructive to begin by reviewing cochlear anatomy and a model of presbycusis that is based on the types of cochlear pathologies observed in aged subjects.

II. The Cochlea and Cochlear Presbycusis

The cochlea, or inner ear, consists of a bony, spiral-shaped capsule containing the sensory epithelium. Although there are many different cell types within the cochlea, the three most essential groups are the sensory cells, cells of the stria vascularis, and the spiral ganglion neurons. The sensory cells reside in the organ of Corti, which lies on the basilar membrane (Fig. 38.1). The organ of Corti spirals around the bony modiolus from the base to the apex of the cochlea. The organ of Corti contains two types of sensory cells, inner and outer hair cells. A single row of inner hair cells and three rows of outer hair cells are separated by inner and outer pillar cells. The stereocilia bundles (actually microvilli) on the apical surface of the hair cells contain the mechanically gated ion channels respon-

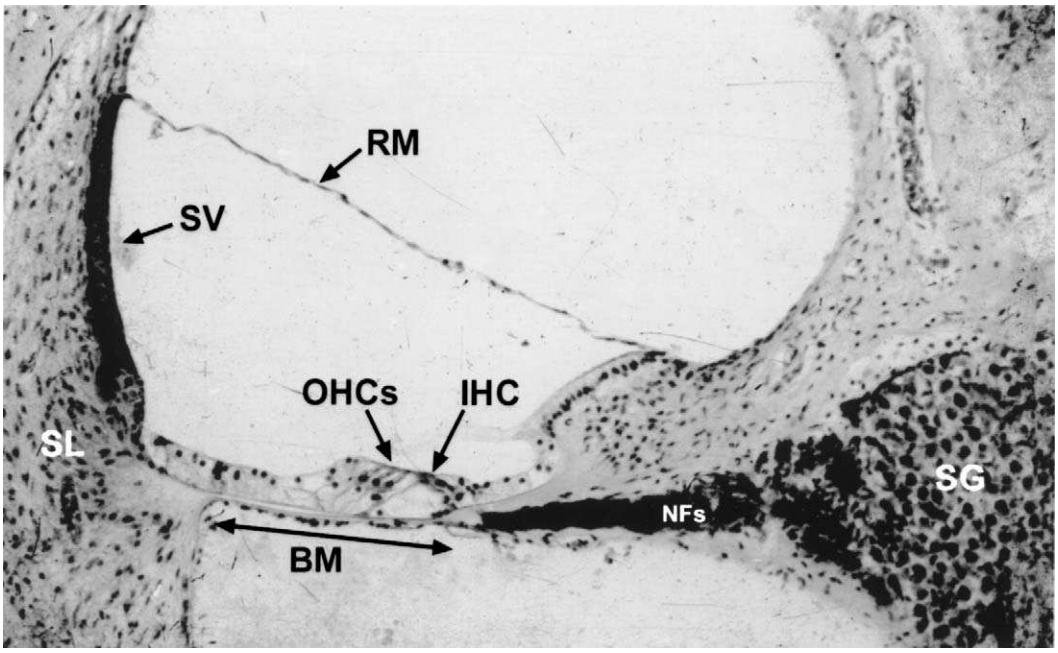

FIG. 38.1. Radial section of the cochlea showing the organ of Corti resting on the basilar membrane (BM). Inner hair cell (IHC), three rows of outer hair cells (OHCs), nerve fibers (NFs), spiral ganglion (SG), stria vascularis (SV), spiral ligament (SL), and Reissner's membrane (RM).

sible for converting the mechanical vibration of sound into a neural response. The stereocilia and apical surfaces of the hair cells are bathed by endolymph. The endolymph is a specialized extracellular fluid whose composition is regulated by marginal cells in the stria vascularis, a vascular layer of tissue that lies along the lateral wall of the cochlea. The endolymphatic space, bounded by Reissner's membrane and the reticular lamina at the top of the hair cells, contains a high concentration of potassium ions and has a potential of approximately +80 mV. This provides the hair cells with a powerful electrochemical gradient that plays a major role in generating the receptor potential when stereocilia bundles are deflected by sound-induced mechanical movement in the cochlea.

The output of the hair cells is transmitted to the central auditory system by approximately 50,000 auditory nerve fibers whose cell bodies form the spiral ganglion within the bony core of the modiolus. Most (~90%) auditory nerve fibers (type I) contact inner hair cells in a one-to-one fashion (Spoendlin, 1969) and are largely, if not exclusively, involved in transmitting acoustic information to the central auditory nervous system (Liberman, 1982). Only about 5 to 10% of auditory nerve fibers (type II) contact outer hair cells, but they do not appear to transmit information about sound to the central auditory pathway (Robertson, 1984).

Sounds that are transmitted to the cochlea cause the basilar membrane to vibrate, producing a traveling wave vibration pattern. The traveling wave vibration pattern that develops in the inner ear arises from the mass and stiffness gradient along the length of the cochlea (Bekesy, 1960). The stiffness of the basilar membrane decreases from base to apex, whereas mass increases from base to apex. As shown in Fig. 38.2, the traveling wave vibration pattern for a high-frequency sound produces its maximum vibration near the basal end of the cochlea. The maximum vibration amplitude for low-frequency sounds occurs near the apex of the cochlea whereas mid-frequency sounds, such as 2 kHz, produce maximum vibration near the middle of the cochlea. Thus, frequency is distributed spatially along the length of the cochlea.

The degenerative changes observed in the inner ear of older subjects are numerous and varied. Schuknecht (1976) origin-

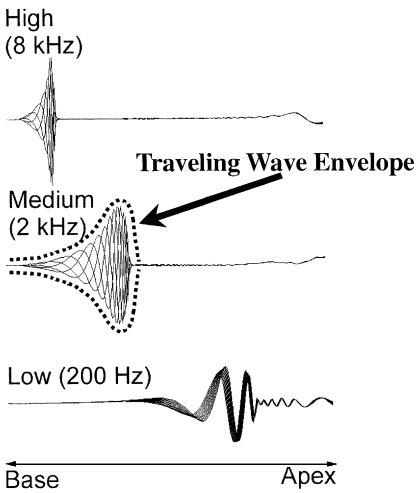

FIG. 38.2. Instantaneous traveling wave vibration pattern for a high-frequency (8 kHz), mid-frequency (2 kHz), and low-frequency (200 Hz) sound. The dotted line shows the envelope of the traveling wave vibration pattern for a 2 kHz tone. Note that high-frequency sounds produce maximum vibration near base of cochlea, whereas low-frequency sounds produce maximum vibration near the apex

ally classified the degenerative changes into four categories according to the primary target of damage—sensory cells, nerve fibers or ganglion cells, strial tissue, or structural elements of the organ of Corti and basilar membrane. Each type of inner ear histopathology would be expected to have a unique clinical outcome, as described briefly below. Schuknecht's classification of human presbycusis into four major types has been extremely helpful in conceptualizing the histopathological changes that produce hearing loss. However, it is important to keep in mind that inner ear degeneration during aging generally involves more than one type of tissue. "Pure" forms of sensory, neural, strial, and cochlear conductive presbycusis rarely, if ever, occur.

A. Sensory Presbycusis

Correlation of audiometric data with postmortem analysis of the cochleas from aged patients and animals has provided compelling evidence that degeneration of inner and outer hair cells plays an extremely important role in many cases of presbycusis (Johnson *et al*., 1997). Sensory presbycusis is generally associated with a steep high-frequency hearing loss (Fig. 38.3A). In most cases, the outer hair cells degenerate first, followed by the inner hair cells (Fig. 38.4) (Schuknecht, 1976; Spongr *et al*., 1997). In humans, sensory cell degeneration typically begins around middle age and spreads from the basal high-frequency region of the cochlea toward the apical low-frequency region with advancing age. Sensory cell loss is often accompanied by degeneration of supporting cells such as Deiters', pillar, and Hensen cells. The loss of sensory and supporting cells results in flattening of the organ of Corti.

Animal studies can provide valuable insights into the anatomical basis of presbycusis independent of extraneous variables such as noise, diet, and ototoxic drugs. From a practical viewpoint, animals with short life spans, such as mice, gerbils, and rats, allow one to study the effects of aging over a period of a few years. Mice have certain advantages because of the genetic homogeneity in inbred strains (thereby controlling for the effects of genetic background on inner ear degeneration and hearing loss), the large number of mutant and transgenic strains, and the rapid progress being made in sequencing the mouse genome. Studies with various strains of mice are likely to provide important clues regarding the genetic basis of presbycusis. CBA mice, for example, show little hair cell loss until the very late stages of life (Henry and Chole, 1980; Li and Borg, 1991; Willott and Mortenson, 1991; Spongr *et al*.,

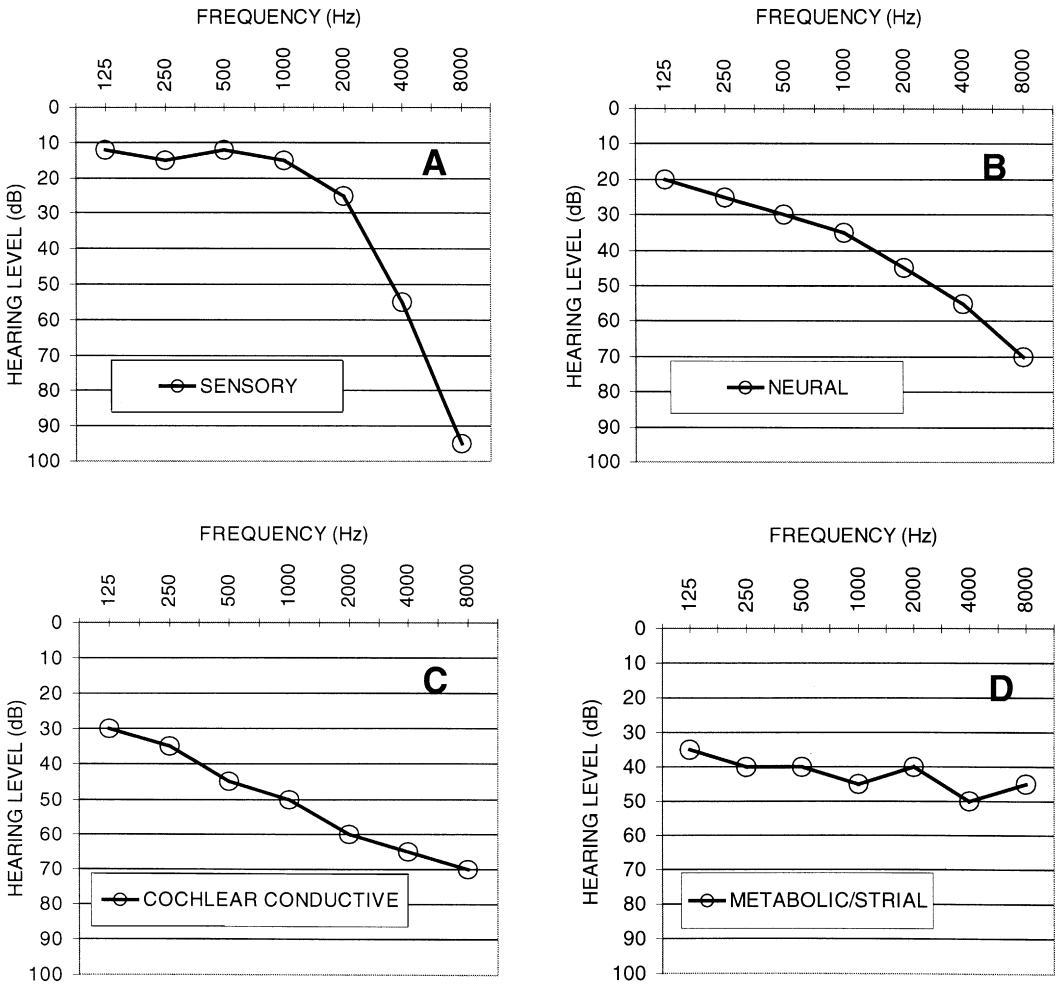

FIG. 38.3. Typical pattern of age-related hearing loss according to Schuknecht (1976). (A) Sensory presbycusis, (B) neural presbycusis, (C) cochlear conductive presbycusis, and (D) metabolic/strial presbycusis (see text for details).

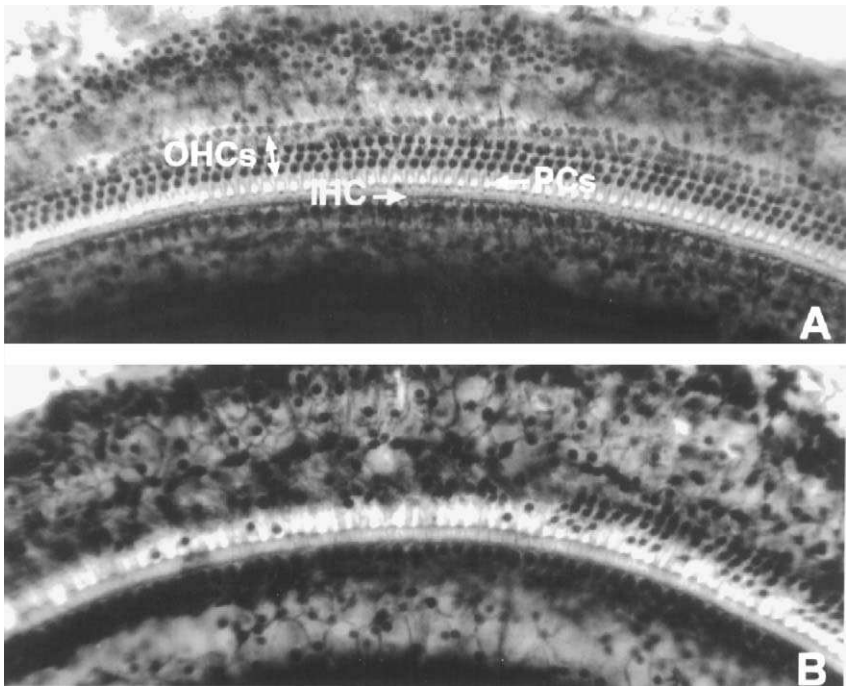

FIG. 38.4. (A) Surface preparation view of the organ of Corti from a young wild type SOD1 mouse. Note three rows of outer hair cells (OHC) and one row of inner hair cells (IHC) separated by pillar cells (PCs). (B) Surface preparation of the organ of Corti from an old wild type SOD1 mouse. Compare to (A). Note absence of outer hair cells.

1997). C57BL/6J mice, by contrast, start to exhibit hair cell loss in the base of the cochlea in early adult life. The lesion begins in the basal cochlea and rapidly progresses toward the apex of the cochlea with advancing age (Mikaelian, 1979; Henry and Chole, 1980; Li and Borg, 1991; Spongr *et al*., 1997). The sensory cells in the Bronx Waltzer mutant mouse also begin to degenerate in early adulthood. However, the lesion pattern is unique in that the inner hair cell loss precedes outer hair cell damage (Schrott *et al*., 1989, 1991).

Rats are also commonly used in studies of aging, and the effect of aging on hair cell integrity has been examined in a few strains. Some strains, such as Sprague–Dawley, show minimal hair cell loss, confined mainly in the apex of the cochlea, around 30 months of age (Keithley and Feldman, 1982). Other strains, such as the Wag–Rij rat, exhibit significant outer and inner hair cell loss around 30 months of age. The damage in this strain decreased from base to apex, a pattern typical of humans and many strains of mice.

Although hair cell loss is likely to play an important role in presbycusis, it is not clear how prevalent this type of hearing loss is in the human population. In addition, it is not clear what proportion of presbycusis is due to sensory cell damage versus some other functional or structural deficits in the inner ear. Finally, while animal models can provide some insights into the mechanisms underlying presbycusis, it is not clear which animal model is most relevant to the human condition.

B. Neural Presbycusis

Presbycusis of a neural basis was proposed on the basis of human studies showing speech discrimination scores far worse than expected from the hearing loss (Schuknecht, 1976). Hearing losses often have a steeply sloping or gradually sloping configuration (Fig. 38.3B). Analysis of temporal bones in these patients revealed a disproportionate loss of spiral ganglion neurons (Fig. 38.5) (Bredberg, 1968; Nadol, 1979). A disproportionately large loss of spiral ganglion cells has also been seen in some cases with steep high-frequency hearing loss (Suga and Lindsay, 1976). While hair cells are often missing, the ganglion cell loss is far greater than would be expected from the hair cell lesions. Neuronal losses were generally more severe in the base of the cochlea than in the apex; however, losses in the apex are clearly more important for processing speech sounds that are located in the mid-to-low frequency region (Otte *et al*., 1978). In aged chinchillas, the dendrites of spiral ganglion neurons were degenerated in regions of the cochlea with intact hair cells; however, only a small proportion (5%) of animals exhibited such changes (Bohne *et al*., 1990). Thus, some cases of neural presbycusis can be found in chinchillas.

The survival of the auditory nerve probably depends on many different factors, but recent studies indicate that several neurotrophic factors promote the survival of spiral ganglion neurons. Neurotrophin 3 (NT-3), which is synthesized and released by inner hair cells, binds to trk-C receptors on spiral ganglion neurons and promotes their survival (Ernfors *et al*., 1995). Brain-derived neurotrophic factor (BDNF), which binds to the trk-B receptor on spiral ganglion neurons, also enhances the survival of spiral ganglion neurons (Zheng *et al*., 1995). Thus, the long-term survival of spiral ganglion neuron may depend in part on an intact population of inner hair cells that release neurotrophins. Deficiencies in these neurotrophins or their receptors could lead to abnormally rapid degeneration of spiral ganglion neurons.

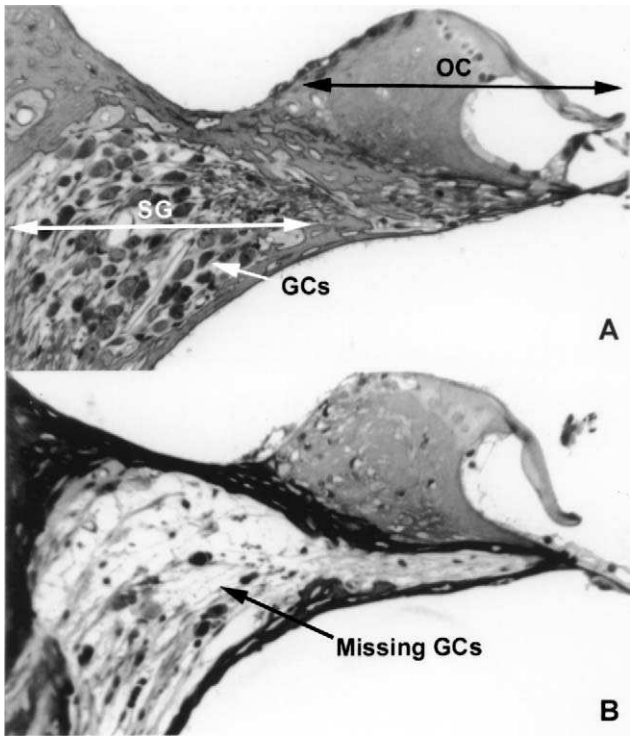

FIG. 38.5. Radial section of the organ of Corti (OC) and spiral ganglion (SG) of the modiolus. (A) Ganglion cells (GCs) present in the spiral ganglion of young SOD1 mouse. (B) Note loss of ganglion cells in old SOD1 mouse.

Certain types of health problems could exacerbate neural presbycusis. For example, ganglion cell loss seems to be extremely severe in individuals with hearing loss resulting from measles, bacterial labyrinthitis and congenital syphilis (Otte *et al*., 1978). Studies with mice lacking copper-zinc superoxide dismutase (Cu/Zn SOD), an important antioxidant enzyme found in the cytosol of virtually all cells, suggests that antioxidant levels can influence ganglion cell and nerve fiber survival during aging. Aged (13-month-old) mice lacking Cu/Zn SOD had significantly fewer ganglion cells and nerve fibers than like-aged wild type (normal) mice (McFadden *et al*., 1999a).

C. Metabolic or Strial Presbycusis

Schuknecht's model of metabolic presbycusis is based on clinical observations from patients with a relatively flat hearing loss (Fig. 38.3C) and good speech discrimination (Schuknecht and Ishii, 1966). Postmortem analysis of temporal bones revealed significant atrophy of the cells and blood vessels in the stria vascularis, which is responsible for generating the endolymphatic potential. The pattern of strial atrophy is varied. In some cases, the degeneration is patchy with the greatest atrophy in the apex and base of the cochlea (Takahashi, 1971). Strial atrophy can involve all cell types, but the marginal cells often show the greatest damage. In other cases, the atrophy is diffuse; although the stria maintains its normal thickness, there are many large intercellular spaces. Studies with aged gerbils lend support for a strial model of presbycusis (Schulte and Schmiedt, 1992; Gratton *et al*., 1996). The vascular bed of the stria showed a patchy degeneration that was most severe in the base and apex of the cochlea. Significantly, there was a strong correlation between the total stria vascular bed and the endolymphatic potential. Na,K-ATPase immunolabeling, an indirect measure of cochlear ion transport, also declined with age most notably in the apical turn followed by the middle and basal turns of the cochlea. The decline in the endolymphatic potential and Na,K-ATPase immunolabeling were positively correlated.

Another common finding in aged ears is the presence of numerous lipofusion or pigment granules (Schuknecht, 1976). Numerous pigment granules are often found in the stria vascularis of old chinchillas (Fig. 38.6). In addition, many pigment granules are found near the cuticular plate of outer hair cells and pillar cells and near the apical surface of supporting cells that line the endolymphatic space (Bohne *et al*., 1990).

Strial atrophy is sometimes considered the only form of metabolic presbycusis; however, other, more subtle, metabolic changes may also play a role. Dehydrogenase histochemistry can be used to assess the status of important enzymes involved in aerobic metabolism in normal and pathologic inner ear

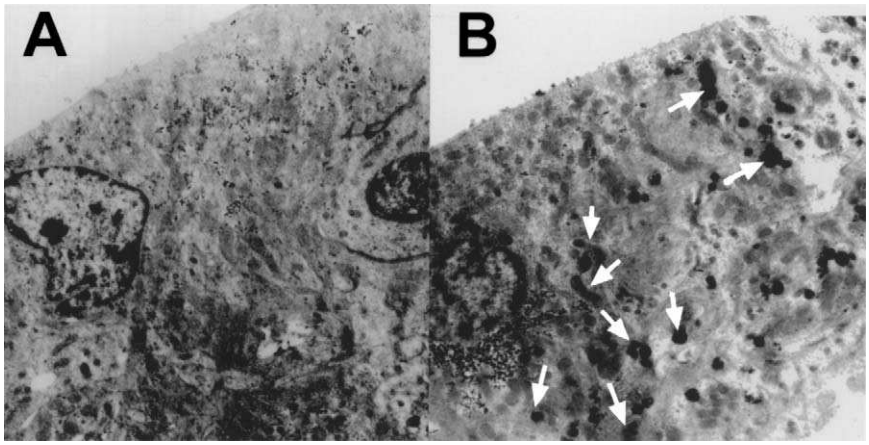

FIG. 38.6. Section of stria vascularis in young (A) and 15-year-old (B) chinchilla. Note increase in the number and size of pigment granules in old chinchilla (arrows).

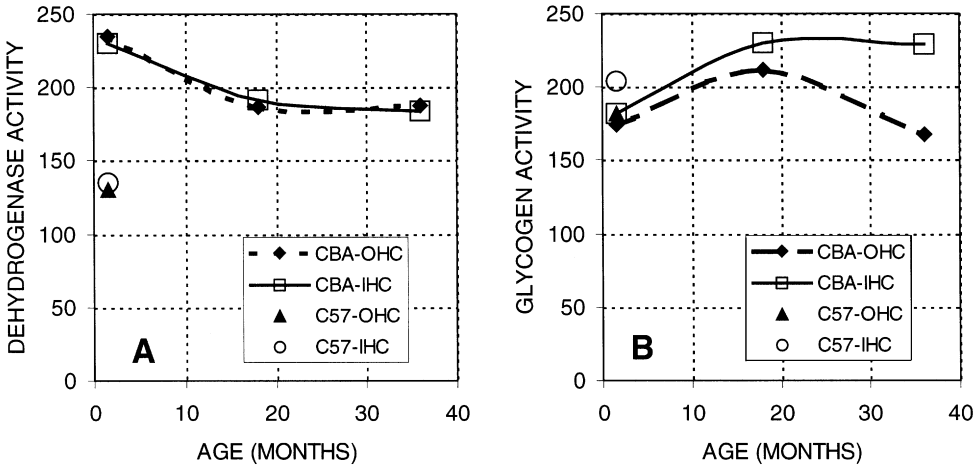

FIG. 38.7. (A) Mean dehydrogenase staining levels for outer hair cells and inner hair cells in CBA mice as a function of age. Data for young C57 mice also shown. (B) Mean glycogen-staining levels for hair cells and stria vascularis of CBA mice as a function of age. Data for young C57 mice are also shown.

tissue. Significant age-related changes in energy metabolism have been observed in the mitochondria of hair cells and stria vascularis with dehydrogenase and glycogen histochemistry (Ding *et al*., 1999). CBA mice, a strain that maintains normal hearing until very old age, show a significant decrease in hair cell dehydrogenase activity between young adulthood and middle age (Fig. 38.7A), coupled with increased glycogen levels in hair cell and stria vascularis over the same time period (Fig. 38.7B). Interestingly, young C57BL/6J mice, a strain that starts to show hearing loss in early adulthood, have significantly less dehydrogenase activity in hair cells than CBA mice of any age (Fig. 38.7A). Young C57 mice also showed less glycogen activity in hair cells and stria vascularis (Fig. 38.7B) than middle aged CBA mice. Changes in the metabolic activity of mitochondria in hair cells and the stria vacularis could be either a cause or a consequence of other inner ear histopathology. One possible interpretation is that impaired metabolism in C57 mice leads to increased cochlear histopathology and susceptibility to noise as compared to CBA mice.

The decline in dehydrogenase activity noted above may be related to age-related changes in the mitochondrial genes involved with energy metabolism. Damage to mitochondrial DNA (mtDNA) has been associated with a number of pathologies, including a form of nonsyndromic hearing loss in which deafness occurs only after the use of aminoglycosides. Recent studies by Seidman and colleagues (1996, 1997) have shown a correlation between a common mtDNA deletion (involving the base pair at position 4834) and age-related cochlear pathology. A corresponding mtDNA deletion in humans (involving the 4977 base pair, which is homologous to the 4834 base pair in the rat) has also been observed in temporal bones obtained from aged humans. However, the 4977-base-pair deletion is observed in humans without hearing loss as well as those with presbycusis. Thus, the common mtDNA deletion may be a better marker for aging than for presbycusis per se. Much more work is required for an understanding of the relationship between mitochondrial dysfunction and presbycusis.

D. Cochlear Conductive Presbycusis

The category of cochlear conductive presbycusis is primarily one of exclusion, reserved for cases in which there is no gross cochlear histopathology present to account for the observed hearing loss (Schuknecht, 1976; Ramadan and Schuknecht, 1989; Schuknecht and Gacek, 1993). The hearing loss associated with cochlear conductive presbycusis is usually bilateral, and gradually increases from low to high frequencies (Fig. 3D). Steeper losses in such patients are associated with poorer speech discrimination. Hearing loss of this type is presumably due to a change in the physical properties of the cochlea that modifies its mechanical response. It has been suggested that remote masking might serve as a test of cochlear conductive presbycusis (Quaranta *et al*., 1978). However, as Schuknecht notes, this category does not have a proven pathology and thus remains largely a speculative category. The only structural pathology consistently seen in ears with gradually sloping hearing loss is atrophy of the spiral ligament (Wright and Schuknecht, 1972). Recent studies suggest that subtle changes in the cochlea can contribute to cochlear conductive presbycusis. Saha and Slepecky (2000) have found age-related changes in microtubules in the guinea pig organ of Corti. Specifically, there is a shift in tubulin isoforms with increasing age that could affect the micromechanical properties of the sensory epithelium. Other recent evidence points to the involvement of fibrocytes in the spiral ligament. Fibrocytes in the spiral ligament play a fundamental role in the recycling of potassium from the organ of Corti to the stria vascularis and then to the endolymph (Minowa *et al*., 1999; Steel, 1999). Mice lacking the Brn-4 gene, which encodes a POW transcription factor, exhibit profound deafness, but do not exhibit any gross pathologies of the middle ear, hair cells, stria vascularis, or spiral ganglion. The lack of gross anatomical pathology in these ears is consistent with Schuknecht's description of patients with conductive presbycusis. However, careful ultrastructural examination revealed changes in the spiral ligament fibrocytes that were associated with a large reduction in the endolympha-

tic potential and broad hearing loss. Thus, subtle anatomical changes in the spiral ligament could conceivably be a factor in the so-called conductive presbycusis.

Viable Dominant Spotting (Wv/Wv) mutant mice show significant hearing loss which is associated with the absence of melanocytes within the stria vascularis (SV) and an endocochlear potential near zero (Cable *et al*., 1994; Kitamura *et al*., 1994). Furthermore, degree of pigmentation of the stria in other W-allele mouse strains was positively correlated with the magnitude of the endolymphatic potential (Cable *et al*., 1994). These results indicate that melanocytes are required to maintain normal stria function. Mice carrying the Light mutation (Blt), a dominant allele, show a gradual age-related whitening of hair coat color due to degeneration of melanocytes in the hair follicles (Cable *et al*., 1993). The age-related reduction in coat color was correlated with loss of melanocytes in the stria and a decline in the endolymphatic potential; no other ultrastructural pathology of the stria was noted. Thus, age-related loss of strial melanocytes associated with the Light mutation may be another factor contributing to strial presbycusis.

III. Age-Related Hearing Loss

Despite its limitations, the pure-tone audiogram remains the most commonly used metric for assessing presbycusis and other forms of hearing loss. The onset and time course of hearing loss varies considerably from one individual to the next presumably because of differences in genetic and environmental factors such as an individual's history of exposure to noise and ototoxic agents. The United States Public Health Service conducted a survey of hearing thresholds as a function of age using subjects representative of the general population (i.e., individuals not screened for hearing loss) (Glorig and Roberts, 1965). High-frequency hearing loss increases with age and is considerably greater for males than females (Fig. 38.8). The gender difference in age-related hearing loss has typically been attributed to greater exposure to noise in males. However, recent studies with chinchillas (McFadden *et al*., 1999c) suggest that factors other than noise exposure may play important roles. Careful epidemiological studies of so-called "pure" age-related hearing loss, that is, studies which exclude individuals with a history of noise exposure or hearing loss from other sources, show less age-related hearing loss (Robinson and Sutton, 1979) (Fig. 38.9). Nevertheless, it is clear that hearing loss progresses and accelerates with advancing age. Males also continue to exhibit more high-frequency age-related hearing loss than females, while females tend to show more low-frequency hearing loss than males. This has been referred to as the gender reversal phenomenon (Jerger *et al*., 1993). When considering data on the prevalence and rate of age-related hearing loss among men and women, one should keep in mind that although the subjects were screened, other subtle forms of acquired hearing loss cannot be completely ruled out.

IV. Acoustic Trauma and Age-Related Hearing Loss

Exposure to loud sounds is the most common cause of hearing loss in the industrialized societies, particularly among young people. Approximately million Americans are routinely exposed to hazardous noise in the workplace (von Gierke and Johnson, 1976; National Institute of Health (NIH), 1990, 1991). In addition, noise-induced hearing loss is rated by the National Occupational Safety and Health as one of the 10 most serious occupational hazards. An understanding of the similarities, differences, and interactions between noise-induced hearing loss and age-related hearing loss is critical for a series of important

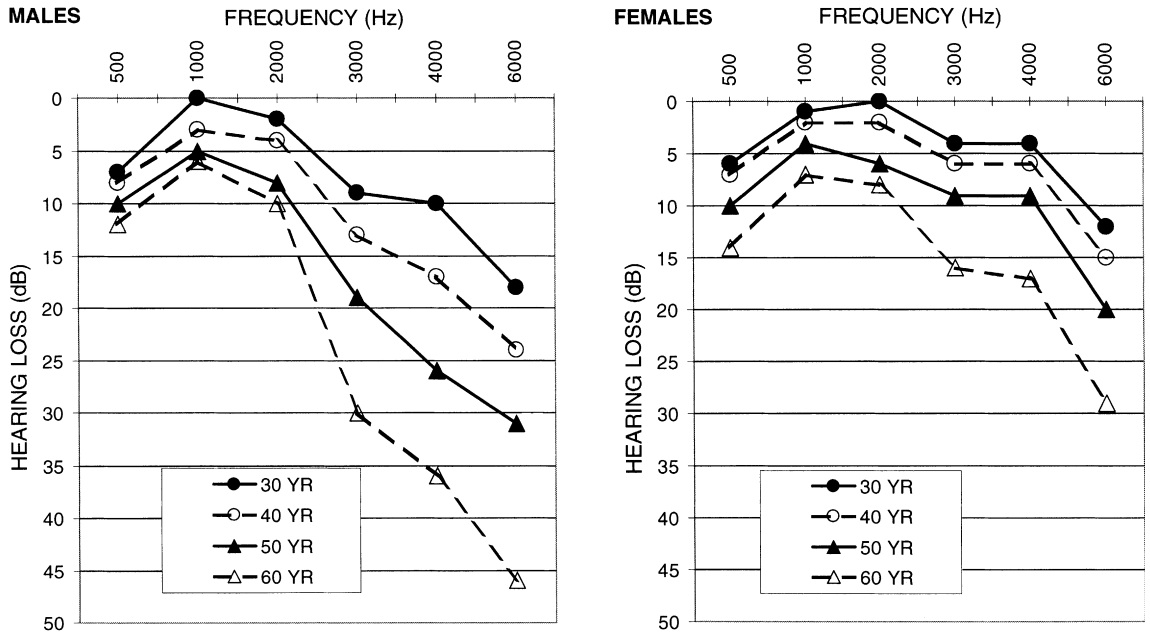

FIG. 38.8. Mean hearing loss as a function of age in male (left) and female (right) subjects representative of the general population (Glorig and Roberts, 1965). (Inset) Loss for each decade.

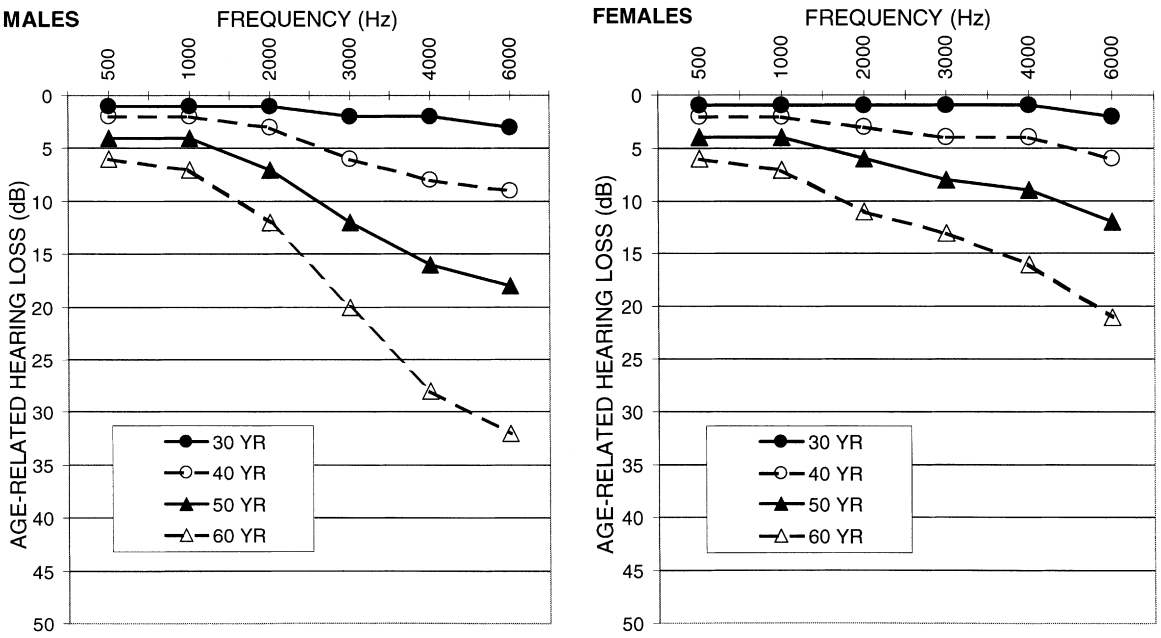

FIG. 38.9. Mean age-related hearing loss in a population of male (left) and female (right) subjects which excluded individuals with a history of noise exposures or other hearing problems (Robinson and Sutton, 1979). (Inset) Loss for each decade.

clinical legal questions. Is the aged ear more vulnerable to the effects of noise? Is an individual's susceptibility to noise-induced hearing loss and age-related hearing loss positively correlated? How does one separate the age and noise components of an individual's hearing loss? These are fundamental questions when managing risks from hearing loss in occupational settings or when one is trying to discern the degree of noise-induced hearing loss for purposes of compensation or other legal matters.

Answers to these questions are difficult to discern from human studies because the process takes place over decades. Epidemiological studies show that occupational noise exposures initially produce hearing losses around 3 to 6 kHz. The hearing loss grows to a plateau over a 10- to 15-year period. Lower and higher frequencies accumulate losses at a slower rate, but the losses continue to accumulate over a 30- to 40-year period (Burns and Robinson, 1970). In males, age-related hearing loss begins to accumulate between the ages of 20 to 30 years (Pearson *et al*., 1995). At higher frequencies (8 and 16 kHz), hearing loss accumulates at a rate of 0.8 to 2 dB per year. At low frequencies (<4 kHz), it increases at a rate of 0.2 to 0.8 dB per year. If one is interested in understanding the relation between noise-induced hearing loss and age-related hearing loss, controlled animal experiments provide a more direct and manageable perspective.

A. Animal Models of Noise-Induced Hearing Loss and Age-Related Hearing Loss

Animal studies of noise-induced hearing loss provide insights into the relation between the acoustic parameters of a noise exposure and amount of hearing loss. In addition, they provide the most direct information on the biological and pathological processes associated with high-level noise exposure. Animal studies of noise-induced hearing loss have generally been conducted with mice, rats, guinea pigs, rabbits, cats, and chinchillas. However, almost all these animals are more susceptible to the effects of noise than humans; consequently the results from animal models are difficult to extrapolate to humans.

An appropriate animal model of age-related hearing loss is difficult to choose. The most popular animal models are the Mongolian gerbil (Mills *et al*., 1990; Schmiedt *et al*., 1990; Adams and Schulte, 1997) and the C57 mouse (Henry, 1983; Willott *et al*., 1994). The gerbil's audiogram is similar to humans (Ryan, 1976; Mills *et al*., 1990). The gerbil, like humans, is outbred and, like humans, shows a tremendous variability in the amount of age-related hearing loss (Mills *et al*., 1990). In terms of shape of the audiogram and cochlear pathology, the gerbil, by 36 months, typically shows a flat hearing loss that is accompanied by a few missing outer hair cells. However, there is significant degeneration of the stria vascularis (Gratton *et al*., 1996) and a significant reduction of Na,K-ATPase and the endocochlear potential (Schulte and Schmiedt, 1992). Thus, the gerbil appears to be a good model of metabolic or strial presbycusis.

The C57 mouse begins to develop a hearing loss at 1 to 2 months of age and is essentially deaf long before the end of its life span (Henry, 1983; Kazee *et al*., 1995). The pattern of hearing loss differs from the gerbil and is more like human sensory presbycusis. C57 mice begin to lose hearing at the highest frequencies and the pattern of cochlear degeneration starts with outer hair cells in the base of the cochlea (Hunter and Willott, 1987; Spongr *et al*., 1997). The hearing loss in the C57 mouse is different from humans in that it develops faster over a very short time period.

The chinchilla is an alternative model of age-related hearing loss (McFadden *et al*., 1997). Its audiogram is similar to that of

humans (Miller, 1970) and its life span is approximately 20 years—10 times longer than the mouse and roughly 25% of the human life span. The chinchilla begins to show a hearing loss, mainly due to sensory cell loss, at about 10 years of age and it accumulates hearing loss at about the same rate as humans (Bohne *et al*., 1990; McFadden *et al*., 1997). Thus, the pattern and rate of hearing loss in the chinchilla resembles that in humans.

B. Mechanism of Hearing Loss

The mechanisms underlying noise-induced hearing loss and age-related hearing loss are not fully understood; however, recent research suggests that free radicals may be a factor in both types of hearing loss. For example, when the ear is exposed to traumatic levels of noise, the metabolic rate increases along with increased production of reactive oxygen species and ischemia (Yamane *et al*., 1995). Exposure to high levels of music reduced the number of red blood cells in the capillaries of the stria vascularis and increased free radical levels along the marginal cells of the stria in guinea pigs. Two hours after the exposure, strial capillaries and free radical levels returned to normal. Nicotera and colleagues (1999) reported that chinchillas exposed to impulse noise show evidence of outer hair cell swelling, distortion of the cell body, and an accumulation of reactive oxygen species at the perimeter of the cells. Glutathione monoethyl ester, a powerful antioxidant, reduced reactive oxygen species levels and reduced the amount of hair cell damage from impulse noise (Hight *et al*., 1999).

Free radical formation occurs during many normal physiological processes. The toxic effects of free radicals are neutralized with cellular antioxidants. Thus, a normal balance between the formation of reactive oxygen species and their inactivation by antioxidants is necessary for normal cellular function. Increasing reactive oxygen species or decreasing cellular antioxidants leads to greater cell damage (Halliwell, 1992). The effects of various antioxidant molecules on age-related hearing loss can be evaluated in transgenic mice. For example, mutant mice with a targeted deletion of the gene that codes for Cu/Zn SOD, an important endogenous antioxidant enzyme, developed greater loss of hair cells and spiral ganglion neurons than wild type mice as they aged (McFadden *et al*., 1999a,b). Homozygous mice (−/−) with 100% loss of SOD develop more hearing loss than heterozygous mice (−/+) with 50% Cu/Zn SOD and heterozygous mice develop more loss than wild type mice (+/+) with normal levels of Cu/Zn SOD (Fig. 38.10). These results suggest that reactive oxygen species can influence the magnitude of age-related hearing loss in susceptible individuals, because the absence of Cu/Zn SOD, the primary antioxidant for the superoxide radical, exacerbated cochlear degeneration.

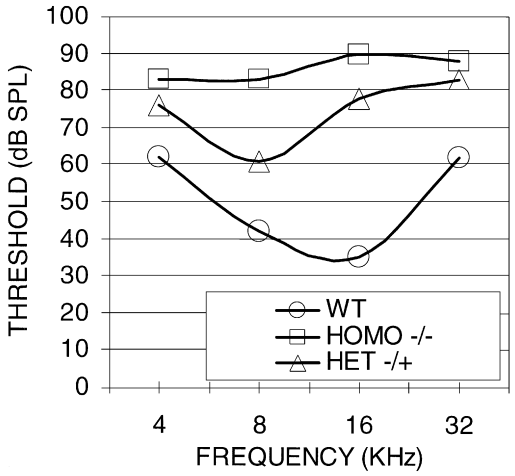

FIG. 38.10. Hearing loss in WT mice and heterozygous (HET −/+) and homozygous (HOMO −/−) Cu/Zn deficient-SOD mice (see text for details) (McFadden *et al*., 1999a).

If reactive oxygen species generation is a common factor in both noise-induced hearing loss and age-related hearing loss, then one might expect that susceptibility to noise-induced hearing loss and age-related hearing loss would be correlated. To test this hypothesis, 18-month-old gerbils were exposed to a monaural traumatizing sound. The animals were allowed to age to 36 months and then hearing was evaluated in the unexposed, aged ear. There was essentially no relationship between the amount of noise-induced hearing loss and the age-related hearing loss in the opposite, unexposed ear (F. Boettcher, personal communication). These results are consistent with data from mice lacking Cu/Zn SOD that did not show a strong relationship between susceptibility to noise and presbycusis. Clearly, this area requires further investigation before strong conclusions can be drawn.

Dietary restriction has been shown to prolong life span and reduce the incidence of cancer (Pugh *et al*., 1999). Thus, it is conceivable that dietary restriction might reduce susceptibility to age-related hearing loss. To test this hypothesis, mice were maintained on a high-energy or a low-energy diet. The low-energy diet increased longevity in several strains of mice without ameliorating age-related changes in cochlear pathology. Interestingly, caloric restriction decreased age-related cochlear degeneration in C57 mice, but had no effect on longevity in this strain (Willott *et al*., 1995). This study demonstrates the complex relationship between an individual's genetic background and the response of the auditory system to environmental factors.

C. Aging and Susceptibility to Noise-Induced Hearing Loss

How do you separate noise-induced hearing loss from age-related hearing loss? The hearing loss of a 65-year-old is most likely the combination of hearing loss and aging. ISO standard 1999 provides normative data for estimating the amount of hearing loss for aging in men and women (Kryter, 1991; Dobie, 1992). The ISO 1999 strategy is based on the assumption that noise-induced hearing loss and age-related hearing loss are additive (Mills *et al*., 1998). Consequently, the worker's noise-induced hearing is calculated to be the total hearing loss minus the hearing loss due to presbycusis. This approach is based more on logic than data; in fact, the data are equivocal in several respects. First, the average age correction is open to error because of the large variability in presbycusis. In a well-controlled laboratory study of gerbils raised in quiet, the hearing levels in 36-month-old animals ranged from normal to a

flat 65 dB loss (Mills *et al*., 1990). Thus, the range of hearing loss was extremely large despite tight control of other extraneous variable (noise exposure, diet, light–dark cycle). Second, experiments to measure the sum of age-related and noise-induced hearing loss have yielded results that agree and disagree with the additivity principle. Mills and colleagues (1997) exposed (3.5 kHz, 113 dB SPL) gerbils monaurally for 1 hr at 18 months and measured hearing levels in both ears at 36 months. If the additivity rule was appropriate, the hearing loss in the exposed ear should be the sum of the permanent hearing loss from the noise exposure and the age-related hearing loss in the unexposed ear at 36 months of age. The additivity rule significantly overestimated the total loss from noise plus aging. However, when long-duration noise exposures were used, the additivity rule was a reasonable predictor of the total hearing loss (Mills *et al*., 1996). Finally, if age-related hearing loss and noise-induced hearing loss covaried, the additivity rule would have to be modified to accommodate the degree of hearing loss. For example, for workers with a hearing loss 1 standard deviation above the mean, the correction for age-related hearing loss would need to be 1 standard deviation above the mean. Obviously, in practice, this would be a highly charged issue that would be unwise to promote given the limited and often contradictory data.

Is the aged ear more susceptible to noise? The data on this question are mixed. CBA/J mice develop normal hearing within the first 3 weeks of birth and then maintain relatively normal hearing until advanced age. Susceptibility to noise-induced hearing loss was tested from birth out to advanced age by exposing animals to a single 5 min exposure at 124 dB SPL. Susceptibility to noise-induced hearing loss increased out to 20 days of age. Susceptibility to noise-induced hearing loss remained high from 20 to 90 days, but the maximum loss shifted from 16 to 32 kHz. Between 120 days and until the end of the life span, susceptibility remained high, but the maximum hearing loss shifted to 64 kHz. These results were independent of exposure level. CBA/Ca mice also showed the same susceptibility to noise-induced hearing loss over their life span (Shone *et al*., 1991). C57BL/6, which possess a recessive gene for adult-onset age-related hearing loss (*ahl* on chromosome 10) (Johnson *et al*., 1997), were also exposed to noise and the hearing loss was compared to CBA/Ca mice were more susceptible to noise-induced hearing loss than CBA/Ca mice. This finding has been replicated in studies by Erway and colleagues (Erway and Willott, 1996; Davis *et al*., 1999), and interpreted as evidence for a common genetic predisposition to presbycusis and noise-induced hearing loss in the C57 mouse strain.

McFadden *et al*. (1998) and McFadden and Campo (1998) studied susceptibility to noise-induced hearing loss in young and old chinchillas and used two noise intensities (95 and 106 dB SPL) to determine if sound level had an effect on the age–noise interaction. The 95 dB exposure produced approximately the same amount of hearing loss in aged and young chinchillas. However, the old animals developed more hearing loss than the young animals when the high level exposure was used. In summary, the results suggest that there may be a complex interaction between age, noise, genetic background, and sound exposure level. Further work is needed to clarify the complex nature of these interaction effects.

V. Ototoxicity and Aging

The effects of drugs, both positive and negative, depend on factors such as their rate of accumulation, the concentration of the drug at its sites of action, and the duration of contact at those sites. These factors are influenced not only by the amount of drug administered, but also by pharmacokinetics—i.e., the dynamic processes involved in drug absorption, distribution, binding or localization in tissues, metabolic transformations, and excretion from the body. Many factors related to aging can have a profound impact on drug pharmacokinetics, leading to increased toxicity and the potential for negative side-effects in elderly patients. For instance, changes in fat or blood protein content can alter the transport and delivery of drugs to their sites of action; changes in kidney or liver function can alter drug clearance rates. The relationship between age and ototoxicity of commonly prescribed drugs has not been adequately explored. However, it is reasonable to expect that ototoxicity may be enhanced in elderly individuals.

A. Aminoglycoside Ototoxicity

Aminoglycoside antibiotics, such as streptomycin, gentamicin, tobramycin, and kanamycin, are potent antibiotics used in the treatment of serious infections caused by Gram-negative bacteria. The major drawbacks to their clinical use are their nephrotoxic and ototoxic side-effects. Consequently, aminoglycoside antibiotics are used sparingly in most developed countries except in cases of life-threatening illness. However, in many underdeveloped countries, aminoglycoside antibiotics are used extensively because of their ready availability and low cost. Aminoglycoside antibiotics could conceivably exacerbate sensorineural hearing loss in the aged population, particularly in individuals with impaired renal function.

Aminoglycoside antibiotics preferentially damage the sensory hair cells in the inner ear (Dallos *et al*., 1972; Worthingon *et al*., 1973; Ryan and Dallos, 1975). In general, hair cell damage progresses from the base (high frequencies) toward the apex (low frequencies) of the cochlea. In addition, aminoglycoside antibiotics tend to destroy outer hair cells before destroying inner hair cells. In cases where the dose of aminoglycoside antibiotic selectively destroys all of the outer hair cells, a hearing loss on the order of 50–60 dB will develop (Ryan and Dallos, 1975). In addition, frequency selectivity, or the ability to discriminate a signal in a background of noise will be greatly impaired (Ryan *et al*., 1979; Schmiedt *et al*., 1980). When the dose of aminoglycoside is high enough to destroy all of the inner and outer hair cells, a profound hearing loss will develop.

1. Mechanisms

The mechanisms by which aminoglycoside antibiotics exert their ototoxic effects are not fully understood; however, the progressive onset of hearing loss and hair cell degeneration provides some clues regarding the mechanism of cell death. Aminoglycoside antibiotics are rapidly taken up by the fluid compartments of the inner ear (Tran Ba Huy *et al*., 1986). However, the drugs are slowly incorporated into hair cells by

receptor-mediated endocytosis (Hashino *et al*., 1997; Richardson *et al*., 1997) and are sequestered in lysosomal-like vesicles. The vesicles increase in size and eventually rupture, releasing their toxic content into the cytoplasm, and leading to the demise of the hair cell. Sulfhydryl compounds, such as glutathione, and antioxidants can reduce aminoglycoside ototoxicity (Garetz *et al*., 1994). Moreover, transgenic mice overexpressing the antioxidant enzyme, superoxide dismutase, showed increased resistance to aminoglycoside ototoxicity (Sha *et al*., 1997). These results suggest that the antioxidant defense system may play a role in ototoxicity; however, the effects of age on the antioxidant defense system are unclear (Cristiano *et al*., 1995; Bhagwat, 1997; Campisi *et al*., 1999).

2. Effect of Age

Several studies have examined the effects of advancing age on aminoglycoside ototoxicity. One clinical study suggests that the concentration of tobramycin in serum varies with age (Cipolle *et al*., 1980). In general, the dose needed to produce a given serum concentration was higher for young patients than for old patients, because drug clearance was faster in young versus old subjects. These results suggest that aminoglycoside ototoxicity might be greater in older subjects due to slower drug clearance. However, the variability between subjects was quite high. In fact, the three factors, age, weight, and creatinine clearance, accounted for only 42% of the variance in amount of hearing loss. These results suggest that age may play only a minor role in regulating clearance rates of aminoglycosides.

Young animals show a critical period for aminoglycoside ototoxicity. Developing rats treated with amikacin between 10 and 25 days of age, during the onset and development of hearing function, showed substantially more hearing loss than younger or older animals (Carlier and Pujol, 1980). Results similar to these have also been reported for the cat, another altricial animal that develops normal hearing 2–3 weeks following birth.

Aminoglycoside ototoxicity has been examined over the life span of the CBA/J mouse (Henry *et al*., 1981). Since this strain of mouse maintains relatively normal hearing and cochlear anatomy into advanced age (Spongr *et al*., 1997), the effects of aminoglycoside antibiotics will not be confounded by any age-related hearing loss or hair cell loss. Preweanling mice treated with kanamycin showed significant hair cell loss throughout the cochlea and significant threshold shifts over a broad range of frequencies. In contrast, postweanling mice showed minimal hair cell loss and cochlear damage. Finally, post-middle-aged mice treated with kanamycin developed inner and outer hair cell lesions in the base of the cochlea and showed elevated high-frequency thresholds. These results suggest that there is a sensitive period for aminoglycoside ototoxicity around the time of onset and development of hearing. However, older subjects are more susceptible to high-frequency hearing loss from aminoglycoside ototoxicity.

The anatomical effects of the aminoglycoside antibiotic, tobramycin, were examined over a broader age range, 3 and 30 months, in the Wag–Rij rat (Dormans *et al*., 1996). Unlike the CBA mouse, the Wag–Rij rat showed increasing hair cell damage with advancing age that spread from the base toward the apex of the cochlea. Consequently, the amount of hair cell damage induced by the tobramycin must be compared to age-matched controls. As expected, hair cell loss and stereocilia damage increased with tobramycin dose. However, the amount of hair cell damage observed in old rats treated with tobramycin was not significantly different from that seen in age-matched controls. One interpretation of these results is that aging does not increase susceptibility to aminoglycoside ototoxicity if there is preexisting hearing loss or hair cell loss. However, in the case of the CBA mouse, which does not exhibit significant age-related hearing loss or cochlear pathology, aging does appear to increase susceptibility to aminoglycoside antibiotics. Clearly, the case is more complicated with elderly humans, since a host of nonauditory factors must also be taken into account.

B. Cisplatin Ototoxicity

Cisplatin (*cis*-diaminedichloro-platinum) and other platinum-based antineoplastic agents are frequently used to treat squamous cell carcinomas of the head and neck and genitourinary system. Cisplatin and its derivatives, which are inorganic, heavy metal compounds, inhibit cell division by forming intrastrand cross-links between guanine bases, thereby inhibiting DNA synthesis (Dedon and Borch, 1987). Cisplatin is taken up readily by cells in the liver, kidney, and inner ear. In humans and some animals, the hearing loss begins in the high-frequency regions and gradually spreads to the low frequencies with increasing dose (van der Hulst *et al*., 1988).

1. Mechanisms

The mechanisms underlying carboplatin ototoxicity are not fully understood; however, results from other systems have provided some preliminary insights. Histochemical studies indicate that cisplatin and its related compounds result in uncoupling of oxidative phosphorylation, inhibition of ATP synthesis, loss of calcium from mitochondria, and loss of calcium-associated transport enzymes and membrane transport enzymes. Increased calcium intake protected these enzymes and preserved kidney function (Aggarwal, 1993).

Carboplatin-induced damage to mitochondria, which may in some cases lead to an increase in the production of reactive oxygen species, could overwhelm the cell's free radical defense system and increase the risk of cell death. Cisplatin treatment of guinea pigs caused a significant hearing loss and a significant reduction of glutathione and glutathione *S*-transferase, compounds that protect against oxidative damage. Interestingly, glutathione levels in the rat auditory nerve declined by 86%, whereas cochlear levels remained unchanged (Lautermann *et al*., 1997).

Compounds that up- or downregulate the free radical defense system could modulate cisplatin ototoxicity. Buthionine sulfoximine (BSO), which inhibits glutathione synthesis, exacerbates hearing loss and inner and outer hair cell damage from carboplatin (Hu *et al*., 1999). Conversely, D-methionine, which upregulates the antioxidant defense system, provides significant protection against cisplatin and carboplatin ototoxicity *in vivo* and *in vitro* (Campbell *et al*., 1996; Gabaizadeh *et al*., 1997; Lockwood *et al*., 2000). Diethyldithiocarbamate,

which upregulates cochlear glutathione levels, also protects against cisplatin ototoxicity (Rybak *et al*., 1995). In addition to damaging sensory cells, cisplatin and related compounds can destroy spiral ganglion neurons (Zheng *et al*., 1995; Ding *et al*., 1997). BDNF, NT-3, and neurotrophin-4/5 (NT-4/5) promote the survival of spiral ganglion neurons (Zheng *et al*., 1995; Gabaizadeh *et al*., 1997). These results suggest that an age-related reduction in the level of cochlear antioxidants and neurotrophin could increase the ear's susceptibility to ototoxic drugs. For example, an age-related loss of inner hair cells would decrease NT-3 levels in the cochlea and presumably decrease the survival of spiral ganglion neurons.

2. Effects of Age

A heightened period of sensitivity to cisplatin has been observed in developing gerbils (Sie *et al*., 1999), an altricial species whose onset of hearing occurs around 12 days of age (McFadden *et al*., 1996). Decreases in high-frequency distortion product otoacoustic emissions, a measure of outer hair cell function, were greatest around 14 days after birth. These results suggest that there is a sensitive period for cisplatin ototoxicity in the developing gerbil ear. A comparable period in humans would occur *in utero* around the second trimester.

Hearing loss and cisplatin pharmacokinetics have been compared in children (1–7 years) and adolescents (12–15 years) with solid tumors (Murakami *et al*., 1990). The dose of cisplatin that caused hearing loss in the adolescent group was roughly three times higher than in children. The increased susceptibility in children was attributed to slower elimination of the drug compared to that seen in adolescents.

Many organs in the human body, such as the ear, liver, and kidney, gradually deteriorate with age. Therefore, the ototoxic effects of cisplatin and related compounds could conceivably be more severe in elderly patients. Several studies have found that the risk of hearing loss does indeed increase with age (Helson *et al*., 1978; Laurell and Borg, 1988; Hallmark *et al*., 1992) and with the drug dose (Helson *et al*., 1978). The increased susceptibility with age was not correlated with the drug dose or the use of furosemide or aminoglycosides (Hallmark *et al*., 1992).

3. Effect of Prior Hearing Loss

Is the age-related increase in cisplatin ototoxicity related to hearing loss that accompanies aging or some other factor? In gynecologic cancer patients, cisplatin ototoxicity was significantly correlated with a history of otologic problems (van der Hulst *et al*., 1988). However, others have reported that hearing loss caused by cisplatin was uncorrelated with prior hearing loss (Melamed *et al*., 1985).

VI. Summary

As the human life span continues to increase, age-related hearing loss will become an increasingly serious problem for health care professionals. Older individuals who develop severe to profound age-related hearing loss experience immense social isolation that can lead to secondary symptoms such as depression. While hearing aids can improve the threshold for hearing and sound quality in a quiet room for some individuals, they often provide little or no benefit in noisy social situations such as a restaurant. The inability to discriminate complex speech sounds in a noise background is largely due to two factors. One of the most important factors, which impacts the vast majority of patients, is damage to the outer hair cells that results in a loss of frequency selectivity and an elevation of threshold (Evans, 1976; Liberman and Mulroy, 1982). Hearing aids can overcome the threshold deficit, but cannot restore the ear's ability to distinguish one sound frequency from another particularly in moderate to high levels of background noise. The second factor that seems to have a profound impact on speech discrimination is the loss of spiral ganglion neurons (Schuknecht and Woellner, 1953; Schuknecht, 1976). Loss of inner hair cells and spiral ganglion neurons has little impact on threshold and frequency selectivity (Wang *et al*., 1997). However, the number of neuronal channels funneling information to the brain is greatly reduced, depriving the brain of the full array of information it needs to discriminate complex sounds such as speech (Salvi *et al*., 1999).

The range of hearing loss among individuals of the same age (Taylor *et al*., 1965) or individuals exposed to the same noise is enormous (Taylor *et al*., 1965). One of the factors that is likely to contribute to this enormous variability is genetic susceptibility to presbycusis, acoustic trauma, ototoxicity or some combination of these (Fischel-Ghodsian *et al*., 1993; Erway *et al*., 1996; Johnson *et al*., 1997; McFadden *et al*., 1999a,b). Rapid advances being made on the human and mouse genomes will undoubtedly accelerate our understanding of the genetic factors that predispose an individual to developing a hearing loss. Individuals armed with such information can take the necessary precautionary measures to reduce the risk of age-related hearing loss.

Over the past two decades, important advances have been made in understanding the mechanisms that can lead to cell death in the inner ear. Using this information, scientists have identified some promising intervention strategies aimed at protecting the inner ear from damage due to aging, ototoxicity and noise exposure (Boettcher *et al*., 1992; Seidman *et al*., 1993; Zheng *et al*., 1995; Campbell *et al*., 1996; Hu *et al*., 1997; McFadden *et al*., 1997; Willott and Turner, 1999). Many obstacles, such as efficacy of drugs in humans, drug delivery routes, inherent toxicity, and convenience, must be overcome before these otoprotective agents can be used to slow or prevent age-related hearing loss.

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References

Adams, J. C., and Schulte, B. A. (1997). Histopathologic observations of the aging gerbil cochlea. *Hear. Res.* **104**, 101–111.

Aggarwal, S. K. (1993). A histochemical approach to the mechanism of action of cisplatin and its analogues. *J. Histochem. Cytochem.* **41**, 1053–1073.

Bekesy, G. (1960). "Experiments in Hearing." Wiley, New York.
Bhagwat, V. R. (1997). Relationship of erythrocyte superoxide dismutase, serum lipid peroxides and age. *Indian J. Med. Sci.* **51**, 45–51.
Boettcher, F. A., Spongr, V. P., and Salvi, R. J. (1992). Physiological and histological changes associated with the reduction in threshold shift during interrupted noise exposure. *Hear. Res.* **62**, 217–236.
Bohne, B. A., Gruner, M. M., and Harding, G. W. (1990). Morphological correlates of aging in the chinchilla cochlea. *Hear. Res.* **48**, 79–91.
Bredberg, G. (1968). Cellular pattern and nerve supply of the human organ of Corti. *Acta Oto-Laryngol., Suppl.*, pp. 1–124.
Burns, W., and Robinson, D. W. (1970). An investigation of the effects of occupational noise on hearing. *In* "Sensorineural Hearing Loss," pp. 177–192. Churchill, London.
Cable, J., Jackson, I. J., and Steel, K. P. (1993). Light (Blt), a mutation that causes melanocyte death, affects stria vascularis function in the mouse inner ear. *Pigm. Cell Res.* **6**, 215–225.
Cable, J., Huszar, D., Jaenisch, R., and Steel, K. P. (1994). Effects of mutations at the W locus (c-kit) on inner ear pigmentation and function in the mouse. *Pigm. Cell Res.* **7**, 17–32.
Campbell, K. C., Rybak, L. P., Meech, R. P., and Hughes, L. (1996). D-methionine provides excellent protection from cisplatin ototoxicity in the rat. *Hear. Res.* **102**, 90–98.
Campisi, A., Di Giacomo, C., Russo, A., Sorrenti, V., Vanella, G., Acquaviva, R., Li Volti, G., and Vanella, A. (1999). Antioxidant systems in rat lens as a function of age: Effect of chronic administration of vitamin E and ascorbate. *Aging (Milano)* **11**, 39–43.
Carlier, E., and Pujol, R. (1980). Supra-normal sensitivity to ototoxic antibiotic of the developing rat cochlea. *Arch. Otolaryngol.* **226**, 129–133.
Cipolle, R. J., Seifert, R. D., Zaske, D. E., and Strate, R. G. (1980). Systematically individualizing tobramycin dosage regimens. *J. Clin. Pharmacol.* **20**, 570–580.
Corso, J. F. (1976). Presbycusis as a complicating factor in evaluating noise-induced hearing loss. *In* "Effects of Noise on Hearing" (D. Henderson, R. P. Hamernik, D. Dosanjh, and J. Mills, eds.), pp. 497–524, Raven Press, New York.
Cristiano, F., de Haan, J. B., Iannello, R. C., and Kola, I. (1995). Changes in the levels of enzymes which modulate the antioxidant balance occur during aging and correlate with cellular damage. *Mech. Ageing Dev.* **80**, 93–105.
Cruickshanks, K. J., Klein, R., Klein, B. E., Wiley, T. L., Nondahl, D. M., and Tweed, T. S. (1998). Cigarette smoking and hearing loss: The epidemiology of hearing loss study. *JAMA, J. Am. Med. Assoc.* **279**, 1715–1719.
Dallos, P., Billone, M. C., Durrant, J. D., Wang, C., and Raynor, S. (1972). Cochlear inner and outer hair cells: Functional differences. *Science* **177**, 356–358.
Davis, R. R., Cheever, M. L., Krieg, E. F., and Erway, L. C. (1999). Quantitative measure of genetic differences in susceptibility to noise-induced hearing loss in two strains of mice. *Hear. Res.* **134**, 9–15.
Dedon, P. C., and Borch, R. F. (1987). Characterization of the reactions of platinum antitumor agents with biologic and nonbiologic sulfur-containing nucleophiles. *Biochem. Pharmacol.* **36**, 1955–1964.
Ding, D., Wang, J., and Salvi, R. J. (1997). Early damage in the chinchilla vestibular sensory epithelium from carboplatin. *Audiol. Neuro-Otol.* **2**, 155–167.
Ding, D. L., McFadden, S. L., Wang, J., Hu, B. H., and Salvi, R. J. (1999). Age- and strain-related differences in dehydrogenase activity and glycogen levels in CBA and C57 mouse cochleas. *Audiol. Neuro-Otol.* **4**, 55–63.
Dobie, R. A. (1992). The relative contributions of occupational noise and aging in individual cases of hearing loss. *Ear Hear.* **13**, 19–27.
Dormans, J. A., Peters-Volleberg, G. W., Dortant, P. M., and Speijers, G. J. (1996). Ototoxicity of tobramycin in young adult and old rats. *Toxicol. Appl. Pharmacol.* **136**, 179–185.
Ernfors, P., Van De Water, T., Loring, J., and Jaenisch, R. (1995). Complementary roles of BDNF and NT-3 in vestibular and auditory development. *Neuron* **14**, 1153–1164.
Erway, L. C., and Willott, J. F. (1996). Genetic susceptibility to noise-induced hearing loss in mice. *In* "Scientific Basis of Noise-induced Hearing Loss" (A. Axelsson, H. Borchgrevink, R. P. Hamernik, P. A. Hellström, D. Henderson, and R. J. Salvi, eds.), pp. 56–64. Thieme, New York.
Erway, L. C., Shiau, Y. W., Davis, R. R., and Krieg, E. F. (1996). Genetics of age-related hearing loss in mice. III. Susceptibility of inbred and F1 hybrid strains to noise-induced hearing loss. *Hear. Res.* **93**, 181–187.
Evans, E. F. (1976). Temporary sensorineural hearing losses and 8th nerve changes. *In* "Effects of Noise on Hearing" (D. Henderson, R. P. Hamernik, S. Dosanjh, and J. H. Mills, eds.), pp. 199–224. Raven Press, New York.
Fischel-Ghodsian, N., Prezant, T. R., Bu, X., and Oztas, S. (1993). Mitochondrial ribosomal RNA gene mutation in a patient with sporadic aminoglycoside ototoxicity. *Am. J. Otolarygol.* **14**, 399–403.
Gabaizadeh, R., Staecker, H., Liu, W., Kopke, R., Malgrange, B., Lefebvre, P. P., and Van de Water, T. R. (1997). Protection of both auditory hair cells and auditory neurons from cisplatin induced damage. *Acta Oto-Laryngol.* **117**, 232–238.
Garetz, S. L., Rhee, D. J., and Schacht, J. (1994). Sulfhydryl compounds and antioxidants inhibit cytotoxicity to outer hair cells of gentamicin metabolite in vitro. *Hear. Res.* **77**, 75–80.
Gates, G. A., Caspary, D. M., Clark, W., Pillsbury, H. C. D., Brown, S. C., and Dobie, R. A. (1989). Presbycusis. *Otolaryngol. Head Neck Surg.* **100**, 266–271.
Glorig, A., and Roberts, J. (1965). Hearing levels of adults by age and sex. United States. 1960–1962. *Vital Health Stat.* **11**, 1–34.
Gratton, M. A., Schmiedt, R. A., and Schulte, B. A. (1996). Age-related decreases in endocochlear potential are associated with vascular abnormalities in the stria vascularis. *Hear. Res.* **102**, 181–190.
Halliwell, B. (1992). Oxygen radicals as key mediators in neurological disease: Fact or fiction? *Ann. Neurol.* **32**, S10–S15.
Hallmark, R. J., Snyder, J. M., Jusenius, K., and Tamimi, H. K. (1992). Factors influencing ototoxicity in ovarian cancer patients treated with Cis-platinum based chemotherapy. *Eur. J. Gynaecol. Oncol.* **13**, 35–44.
Hashino, E., Shero, M., and Salvi, R. J. (1997). Lysosomal targeting and accumulation of aminoglycoside antibiotics in sensory hair cells. *Brain Res.* **777**, 75–85.
Helson, L., Okonkwo, E., Anton, L., and Cvitkovic, E. (1978). cis-Platinum ototoxicity. *Clin. Toxicol.* **13**, 469–478.
Henry, K. R. (1983). Lifelong susceptibility to acoustic trauma: Changing patterns of cochlear damage over the life span of the mouse. *Audiology* **22**, 372–383.
Henry, K. R., and Chole, R. A. (1980). Genotypic differences in behavioral, physiological and anatomical expressions of age-related hearing loss in the laboratory mouse. *Audiology* **19**, 369–383.
Henry, K. R., Chole, R. A., McGinn, M. D., and Frush, D. P. (1981). Increased ototoxicity in both young and old mice. *Arch. Otolaryngol.* **107**, 92–95.
Hight, N. G., Henderson, D., McFadden, S. L., Zheng, X.-Y., and Ding, D.-L. (1999). The effect of glutathione monoethyl ester (GSHee) on hearing loss and hair cell loss in chinchillas exposed to impulse noise. *Abstr. Assoc. Res. Otolaryngol.* **22**, 602.
Hu, B. H., Zheng, X. Y., McFadden, S. L., Kopke, R. D., and Henderson, D. (1997). R-phenylisopropyladenosine attenuates noise-induced hearing loss in the chinchilla. *Hear. Res.* **113**, 198–206.
Hu, B. H., McFadden, S. L., Salvi, R. J., and Henderson, D. (1999). Intracochlear infusion of buthionine sulfoximine potentiates carboplatin ototoxicity in the chinchilla. *Hear. Res.* **128**, 125–134.
Hunter, K. P., and Willott, J. F. (1987). Aging and the auditory brainstem response in mice with severe or minimal presbycusis. *Hear. Res.* **30**, 207–218.

Jerger, J., Chmiel, R., Stach, B., and Spretnjak, M. (1993). Gender affects audiometric shape in presbyacusis. *J. Am. Acad. Audiol.* **4**, 42–49.

Johnson, K. R., Erway, L. C., Cook, S. A., Willott, J. F., and Zheng, Q. Y. (1997). A major gene affecting age-related hearing loss in C57BL/6J mice. *Hear. Res.* **114**, 83–92.

Kazee, A. M., Han, L. Y., Spongr, V. P., Walton, J. P., Salvi, R. J., and Flood, D. G. (1995). Synaptic loss in the central nucleus of the inferior colliculus correlates with sensorineural hearing loss in the C57BL/6 mouse model of presbycusis. *Hear. Res.* **89**, 109–120.

Keithley, E. M., and Feldman, M. L. (1982). Hair cell counts in an age-graded series of rat cochleas. *Hear. Res.* **8**, 249–262.

Kitamura, K., Sakagami, M., Umemoto, M., Takeda, N., Doi, K., Kasugai, T., and Kitamura, Y. (1994). Strial dysfunction in a melanocyte deficient mutant rat (Ws/Ws rat). *Acta Oto-Laryngol.* **114**, 177–181.

Kryter, K. D. (1991). Hearing loss from gun and railroad noise—relations with ISO standard 1999. *J. Acoust. Soc. Am.* **90**, 3180–3195.

Laurell, G., and Borg, E. (1988). Ototoxicity of cisplatin in gynaecological cancer patients. *Scand. Audiol.* **17**, 241–247.

Lautermann, J., Crann, S. A., McLaren, J., and Schacht, J. (1997). Glutathione-dependent antioxidant systems in the mammalian inner ear: Effects of aging, ototoxic drugs and noise. *Hear. Res.* **114**, 75–82.

Li, H. S., and Borg, E. (1991). Age-related loss of auditory sensitivity in two mouse genotypes. *Acta Oto-Rhino-Larygol. Belg.* **111**, 827–834.

Liberman, M. C. (1982). Single-neuron labeling in the cat auditory nerve. *Science* **216**, 1239–1241.

Liberman, M. C., and Mulroy, M. J. (1982). Acute and chronic effects of acoustic trauma: Cochlear pathology and auditory nerve pathophysiology. *In* "New Perspectives on Noise-Induced Hearing Loss" (R. P. Hamernik, D. Henderson, and R. J. Salvi, eds.), pp. 105–135. Raven Press, New York.

Lockwood, D., Ding, D.-L., Wang, J., and Salvi, R. J. (2000). D-methionine attenuates inner hair cell loss in carboplatin treated chinchillas. *Audiol. Neuro-Otol.* (in press).

McDowell, B. (1982). Patterns of cochlear degeneration following gentamicin administration in both old and young guinea pigs. *Br. J. Audiol.* **16**, 123–129.

McFadden, S. L., and Campo, P. (1998). Cubic distortion product otoacoustic emissions in young and aged chinchillas exposed to low-frequency noise. *J. Acoust. Soc. Am.* **104**, 2290–2297.

McFadden, S. L., Walsh, E. J., and McGee, J. (1996). Onset and development of auditory brainstem responses in the Mongolian gerbil *(Meriones unguiculatus). Hear. Res.* **100**, 68–79.

McFadden, S. L., Campo, P., Quaranta, N., and Henderson, D. (1997). Age-related decline of auditory function in the chinchilla *(Chinchilla laniger). Hear. Res.*

McFadden, S. L., Campo, P., Ding, D., and Quaranta, N. (1998). Effects of noise on inferior colliculus evoked potentials and cochlear anatomy in young and aged chinchillas. *Hear. Res.* **117**, 81–96.

McFadden, S. L., Ding, D., Burkard, R. F., Jiang, H., Réaume, A. G., Flood, D. G., and Salvi, R. J. (1999a). Cu/Zn SOD deficiency potentiates hearing loss and cochlear pathology in aged 129,CD-1 mice. *J. Comp. Neurol.* **413**, 101–112.

McFadden, S. L., Ding, D., Réaume, A. G., Flood, D. G., and Salvi, R. J. (1999b). Age-related cochlear hair cell loss is enhanced in mice lacking copper/zinc superoxide dismutase. *Neurobiol. Aging* **20**, 1–8.

McFadden, S. L., Henselman, L. W., and Zheng, X. Y. (1999c). Sex differences in auditory sensitivity of chinchillas before and after exposure to impulse noise. *Ear Hear.* **20**, 164–174.

Melamed, L. B., Selim, M. A., and Schuchman, D. (1985). Cisplatin ototoxicity in gynecologic cancer patients. A preliminary report. *Cancer (Philadelphia)* **55**, 41–43.

Mikaelian, D. O. (1979). Development and degeneration of hearing in the C57/b16 mouse: Relation of electrophysiologic responses from the round window and cochlear nucleus to cochlear anatomy and behavioral responses. *Laryngoscope* **89**, 1–15.

Miller, J. D. (1970). Audibility curve of the chinchilla. *J. Acoust. Soc. Am.* **48**, 513–523.

Mills, J. H., Schmiedt, R. A., and Kulish, L. F. (1990). Age-related changes in auditory potentials of Mongolian gerbil. *Hear. Res.* **46**, 201–210.

Mills, J. H., Lee, F.-S., Dubno, J. R., and Boettcher, F. A. (1996). Interactions between age-related and noise-induced hearing loss. *In* "Scientific Basis of Noise-induced Hearing Loss" (A. Axelsson, H. Borchgrevink, R. P. Hamernik, P.-A. Hellström, D. Henderson, and R. J. Salvi, eds.), pp. 193–212, Thieme, New York.

Mills, J. H., Boettcher, F. A., and Dubno, J. R. (1997). Interaction of noise-induced permanent threshold shift and age-related threshold shift. *J. Acoust. Soc. Am.* **101**, 1681–1686.

Mills, J. H., Dubno, J. R., and Boettcher, F. A. (1998). Interaction of noise-induced hearing loss and presbyacusis. *Scand. Audiol. Suppl.* **48**, 117–122.

Minowa, O., Ikeda, K., Sugitani, Y., Oshima, T., Nakai, S., Katori, Y., Suzuki, M., Furukawa, M., Kawase, T., Zheng, Y., Ogura, M., Asada, Y., Watanabe, K., Yamanaka, H., Gotoh, S., Nishi-Takeshima, M., Sugimoto, T., Kikuchi, T., Takasaka, T., and Noda, T. (1999). Altered cochlear fibrocytes in a mouse model of DFN3 nonsyndromic deafness. *Science* **285**, 1408–1411.

Murakami, T., Inoue, S., Sasaki, K., and Fujimoto, T. (1990). Studies on age-dependent plasma platinum pharmacokinetics and ototoxicity of cisplatin. *Sel. Cancer Ther.* **6**, 145–151.

Nadol, J. B., Jr. (1979). Electron microscopic findings in presbycusic degeneration of the basal turn of the human cochlea. *Otolaryngol. Head Neck Surg.* **87**, 818–836.

National Institute of Health (NIH). (1990). Noise and hearing loss. NIH Consensus Statement 1990, Jan 22–24. **8**, 1–24.

National Institute of Health (NIH) (1991). NIH consensus conference. Noise and hearing loss. *N. J. Nurse* **21**, 10.

Nicotera, T., Henderson, D., Zheng, X.-Y., Ding, D., and McFadden, S. L. (1999). Reactive oxygen species, apoptosis and necrosis in noise-exposed cochleas of chinchillas. *Abstr. Assoc. Res. Otolaryngol.* **22**, 626.

Otte, J., Schunknecht, H. F., and Kerr, A. G. (1978). Ganglion cell populations in normal and pathological human cochleae. Implications for cochlear implantation. *Laryngoscope* **88**, 1231–1246.

Pearson, J. D., Morrell, C. H., Gordon-Salant, S., Brant, L. J., Metter, E. J., Klein, L. L., and Fozard, J. L. (1995). Gender differences in a longitudinal study of age-associated hearing loss. *J. Acoust. Soc. Am.* **97**, 1196–1205.

Pugh, T. D., Oberley, T. D., and Weindruch, R. (1999). Dietary intervention at middle age: Caloric restriction but not dehydroepiandrosterone sulfate increases lifespan and lifetime cancer incidence in mice. *Cancer Res.* **59**, 1642–1648.

Quaranta, A., Amoroso, C., and Cervellera, G. (1978). Remote masking in presbycusis. *J. Aud. Res.* **18**, 125–129.

Ramadan, H. H., and Schuknecht, H. F. (1989). Is there a conductive type of presbycusis? *Otolaryngol. Head Neck Surg.* **100**, 30–34.

Richardson, G. P., Forge, A., Kros, C. J., Fleming, J., Brown, S. D., and Steel, K. P. (1997). Myosin VIIA is required for aminoglycoside accumulation in cochlear hair cells. *J. Neurosci.* **17**, 9506–9519.

Robertson, D. (1984). Horseradish peroxidase injection of physiologically characterized afferent and efferent neurons in the guinea pig spiral ganglion. *Hear. Res.* **15**, 113–121.

Robinson, D. W., and Sutton, G. J. (1979). Age effect in hearing—a comparative analysis of published threshold data. *Audiology.* **18**, 320–334.

Ryan, A. (1976). Hearing sensitivity of the mongolian gerbil, *Meriones unguiculatis. J. Acoust. Soc. Am.* **59**, 1222–1226.

Ryan, A., and Dallos, P. (1975). Effect of absence of cochlear outer hair cells on behavioural auditory threshold. *Nature (London)* **253**, 44–46.

Ryan, A., Dallos, P., and McGee, T. (1979). Psychophysical tuning curves and auditory thresholds after hair cell damage in the chinchilla. *J. Acoust. Soc. Am.* **66**, 370–378.

Rybak, L. P., Ravi, R., and Somani, S. M. (1995). Mechanism of protection by diethyldithiocarbamate against cisplatin ototoxicity: Antioxidant system. *Fundam. Appl. Toxicol.* **26**, 293–300.

Saha, S., and Slepecky, N. B. (2000). Age-related changes in microtubules in the guinea pig organ of Corti. Tubulin isoform shifts with increasing age suggest changes in micromechanical properties of the sensory epithelium. *Cell Tissue Res.* **300**, 29–46.

Salvi, R. J., Wang, J., Ding, D.-L., Stecker, N., and Arnold, S. (1999). Auditory deprivation of the central auditory system resulting from selective inner hair cell loss: Animal model of auditory neuropathy. *Scand. Audiol.*, Suppl. **51**, 1–12.

Schmiedt, R. A., Zwislocki, J. J., and Hamernik, R. P. (1980). Effects of hair cell lesions on responses of cochlear nerve fibers. I. Lesions, tuning curves, two-tone inhibition, and responses to trapezoidal-wave patterns. *J. Neurophysiol.* **43**, 1367–1389.

Schmiedt, R. A., Mills, J. H., and Adams, J. C. (1990). Tuning and suppression in auditory nerve fibers of aged gerbils raised in quiet or noise. *Hear. Res.* **45**, 221–236.

Schrott, A., Stephan, K., and Spoendlin, H. (1989). Hearing with selective inner hair cell loss. *Hear. Res.* **40**, 213–220.

Schrott, A., Puel, J. L., and Rebillard, G. (1991). Cochlear origin of 2f1-f2 distortion products assessed by using 2 types of mutant mice. *Hear. Res.* **52**, 245–253.

Schuknecht, H. F. (1976). "Pathology of the Ear." Harvard University Press, Cambridge, MA.

Schuknecht, H. F., and Gacek, M. R. (1993). Cochlear pathology in presbycusis. *Ann. Otol. Rhinol. Laryngol.* **102**, 1–16.

Schuknecht, H. F., and Ishii, T. (1966). Hearing loss caused by atrophy of the stria vascularis. *Nippon Jibi Inkoka Gakkai Kaiho* **69**, 1825–1833.

Schuknecht, H. F., and Woellner, R. C. (1953). Hearing losses following partial section of the cochlear nerve. *Laryngoscope* **63**, 441–465.

Schulte, B. A., and Schmiedt, R. A. (1992). Lateral wall Na,K-ATPase and endocochlear potentials decline with age in quiet-reared gerbils. *Hear. Res.* **61**, 35–46.

Seidman, M. D., Shivapuja, B. G., and Quirk, W. S. (1993). The protective effects of allopurinol and superoxide dismutase on noise- induced cochlear damage. *Otolaryngol. Head Neck Surg.* **109**, 1052–1056.

Seidman, M. D., Bai, U., Khan, M. J., Murphy, M. J., Quirk, W. S., Castora, F. L., and Hinojosa, R. (1996). Association of mitochondrial DNA deletions and cochlear pathology: A molecular biologic tool. *Laryngoscope* **106**, 777–783.

Seidman, M. D., Bai, U., Khan, M. J., and Quirk, W. S. (1997). Mitochondrial DNA deletions associated with aging and presbyacusis. *Arch. Otolaryngol. Head Neck Surg.* **123**, 1039–1045.

Sha, S.-H., Zajic, G., and Schacht, J. (1997). Transgenic mice over-expressing copper-zinc superoxide dismutase are protected from kanamycin-induced ototoxicity. *Abstr. Assoc. Res. Otolaryngol.* **20**, 122.

Shone, G., Altschuler, R. A., Miller, J. M., and Nuttall, A. L. (1991). The effect of noise exposure on the aging ear. *Hear. Res.* **56**, 173–178.

Sie, K. C., deSerres, L. M., and Norton, S. J. (1999). Age-related sensitivity to cisplatin ototoxicity in gerbils. *Hear. Res.* **134**, 39–47.

Spoendlin, H. (1969). Innervation patterns in the organ of Corti of the cat. *Acta Oto-Laryngol.* **67**, 239–254.

Spongr, V. P., Flood, D. G., Frisina, R. D., and Salvi, R. J. (1997). Quantitative measures of hair cell loss in CBA and C57BL/6 mice throughout their life spans. *J. Acoust. Soc. Am.* **101**, 3546–3553.

Steel, K. P. (1999). Perspectives: Biomedicine. The benefits of recycling. *Science* **285**, 1363–1364.

Suga, F., and Lindsay, J. R. (1976). Histopathological observations of presbycusis. *Ann. Otol. Rhinol. Laryngol.* **85**, 169–184.

Takahashi, T. (1971). Electron microscopic figures of the abnormal stria vascularis and spiral prominence in man. *Nippon Jibi Inkoka Gakkai Kaiho* **74**, 512–513.

Taylor, W. J., Pearson, A., Mair, W., and Burns, W. (1965). Study of noise and hearing in jute weaving. *J. Acoust. Soc. Am.* **38**, 113–120.

Tran Ba Huy, P., Bernard, P., and Schacht, J. (1986). Kinetics of gentamicin uptake and release in the rat. Comparison of inner ear tissues and fluids with other organs. *J. Clin. Invest.* **77**, 1492–1500.

van der Hulst, R. J., Dreschler, W. A., and Urbanus, N. A. (1988). High frequency audiometry in prospective clinical research of ototoxicity due to platinum derivatives. *Ann. Otol. Rhinol. Laryngol.* **97**, 133–137.

von Gierke, H. E., and Johnson, D. L. (1976). Summary of present damage risk criteria. *In* "Effects of Noise on Hearing" (D. Henderson, R. P. Hamernik, D. Dosanjh, and J. Mills, eds.), pp. 547–560. Raven Press, New York.

Wang, J., Powers, N. L., Hofstetter, P., Trautwein, P., Ding, D., and Salvi, R. (1997). Effects of selective inner hair cell loss on auditory nerve fiber threshold, tuning and spontaneous and driven discharge rate. *Hear. Res.* **107**, 67–82.

Willott, J. F., and Mortenson, V. (1991). Age-related cochlear histopathology in C57BL/6J and CBA/J mice. *Abstr. Assoc. Res. Otolaryngol.* **14**, 16.

Willott, J. F., and Turner, J. G. (1999). Prolonged exposure to an augmented acoustic environment ameliorates age-related auditory changes in C57BL/6J and DBA/2J mice. *Hear. Res.* **135**, 78–88.

Willott, J. F., Carlson, S., and Chen, H. (1994). Prepulse inhibition of the startle response in mice: Relationship to hearing loss and auditory system plasticity. *Behav. Neurosci.* **108**, 703–713.

Willott, J. F., Erway, L. C., Archer, J. R., and Harrison, D. E. (1995). Genetics of age-related hearing loss in mice. II. Strain differences and effects of caloric restriction on cochlear pathology and evoked response thresholds. *Hear. Res.* **88**, 143–155.

Worthingon, E. L., Lunin, L. F., Heath, M., and Catalin, F. I. (1973). "Index-Handbook of Ototoxic Agents 1966–1971." Johns Hopkins University Press, Baltimore, MD.

Wright, J. L., and Schuknecht, H. F. (1972). Atrophy of the spiral ligament. *Arch. Otolaryngol.* **96**, 16–21.

Yamane, H., Nakai, Y., Takayama, M., Konishi, K., Iguchi, H., Nakagawa, T., Shibata, S., Kato, A., Sunami, K., and Kawakatsu, C. (1995). The emergence of free radicals after acoustic trauma and strial blood flow. *Acta Oto-Laryngol., Suppl.* **519**, 87–92.

Zheng, J. L., Stewart, R. R., and Gao, W. Q. (1995). Neurotrophin-4/5 enhances survival of cultured spiral ganglion neurons and protects them from cisplatin neurotoxicity. *J. Neurosci.* **15**, 5079–5087.

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39

Auditory Temporal Processing during Aging

This chapter presents results of a thematic interdisciplinary approach to characterizing and determining the neural bases of presbycusis. Research audiology, psychoacoustics, behavior and experimental psychology, neuroimaging and neurology, single-cell neurophysiology, neuroanatomy and neurochemistry, and evoked potential neurophysiology contributed to cross-project experiments in humans and animals. By using psychoacoustic and evoked potential neurophysiological paradigms such as forward and backward masking (gaps), interstimulus intervals and rates, and by manipulating naturally occurring pauses and voice-onset times in speech, we were able to gain insights into age-related slowing of central nervous system timing mechanisms. The peripheral auditory system with its extensive bank of filters is responsible for the spectral analysis of simple and complex environmental sounds. Thus, inner ear dysfunction is characterized principally and initially by deficiencies in frequency analysis and sensitivity. In contrast, most sounds, and especially suprathreshold complex sounds such as speech, vary over time. Therefore, measures of temporal resolution that can reflect the integrity of the central auditory system have become especially useful in our research seeking to determine the effects of age, per se, on hearing. Here we present results of our temporal resolution research utilizing human and animal subjects aimed at determining neural sites of hearing loss due to aging. This chapter also reinforces how findings from animal models can assist in understanding the human condition and thus lead to future interventions.

I. Themes and Specific Aims of Presbycusis Research Program

Cross-project experiments in humans and animals have brought together the disciplines of research audiology, psychoacoustics, behavior and experimental psychology, neuroimaging and neurology, single-cell neurophysiology, neuroanatomy and neurochemistry, and evoked potential neurophysiology. Strengths of this multidisciplinary approach include the ability to (a) conduct parallel animal and human experiments, (b) obtain repeated measures on the same subjects (human/animal) or subject groups, (c) obtain behavioral and physiological measures on the same subjects, and (d) study central as well as peripheral components/interactions in the auditory system (see Fig. 39.1).

We examined auditory temporal resolution, which is a fundamental acoustic feature for speech perception. This auditory dimension was chosen because aged listeners have difficulty understanding speech in noisy or reverberant environments, and because temporal processing is largely the domain of the central auditory nervous system. By using psychoacoustic and evoked potential neurophysiological paradigms such as forward and backward masking (gaps) and interstimulus intervals and rates and by manipulating naturally occurring pauses and voice-onset times in speech, we were able to gain insights into age-related slowing of central nervous system timing mechanisms.

The peripheral auditory system with its extensive bank of filters is responsible for the spectral analysis of simple and complex environmental sounds. Thus, inner ear dysfunction is characterized principally and initially by deficiencies in frequency analysis and sensitivity. In contrast, most sounds, and especially complex sounds such as speech, vary over time. Therefore, measures of temporal resolution that can reflect the integrity of the central auditory system have become especially useful in our research seeking to determine the effects of age, per se, on hearing. This chapter presents results of our temporal resolution research utilizing human and animal subjects aimed at determining neural sites of hearing loss due to aging. It also represents how findings from animal models can assist in understanding the human condition and thus lead to future interventions.

II. Neurobiology of Temporal Processing: Human Subjects

A. Speech Recognition in the Elderly

We were guided in the design of our speech recognition experiments by the extensive report prepared by the Commit-

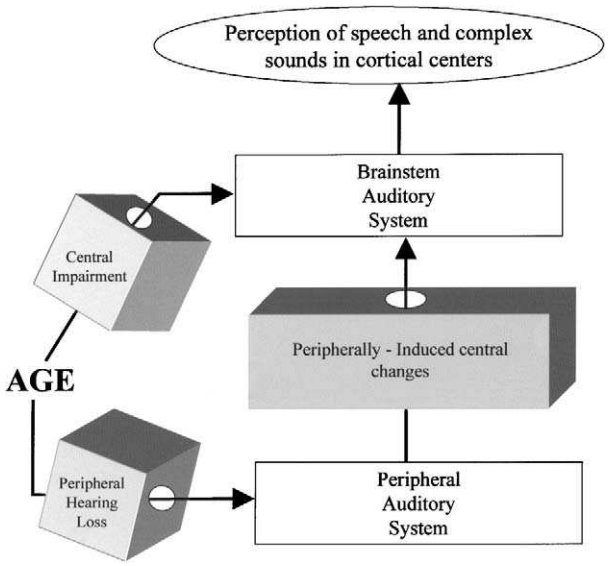

FIG. 39.1. Schematic representation of auditory system illustrating possible neural sites underlying age-related hearing loss. Age-related central changes can occur independently of peripheral hearing loss or in conjunction with peripherally induced central changes.

tee on Hearing, Bioacoustics and Biomechanics (CHABA) of the Acoustical Society of America in 1988. That report expressed the lack of certainty regarding auditory neural site(s) of biological aging because much of the prevailing research focused on the peripheral auditory system. It also encouraged more careful subject selection criteria and accurate control of materials and experimental procedures when comparing adult subject groups of different ages. In general, the committee's review of studies dealing with speech perception performance in quiet and simple tones tended to confirm the vital role played by the peripheral auditory nervous system irrespective of subject age. However, the results of studies measuring speech recognition performance in noise and complex signals were equivocal with respect to differences due to age versus those due to inner ear damage.

It is characteristic of most people 60 years of age and older to show some hearing loss for pure tones and speech stimuli (Gates *et al*., 1990). Their expressed concern, however, is difficulty in understanding the speech of others especially when in noisy or reverberant acoustic environments. In order to test for an age effect in the central auditory system, it was necessary to find those relatively few elderly subjects 60 years of age and older who demonstrated normal speech-frequency pure-tone sensitivities and speech perception thresholds in quiet. For every 100 elderly women and men screened, we found 5 or 6 women and 3 or 4 men with normal auditory sensitivity thresholds. These ''golden ears'' provided the basis for comparing speech recognition in noise performance of young and old adult normal hearers and thus helped determine the existence of a central auditory nervous system age effect. It was clear from prior research that temporal resolution dysfunction would most likely be revealed under conditions of adaptively varying background noise rather than presenting speech signals at fixed signal-to-noise levels (Gordon-Salant, 1987).

Here we evaluated the effects of age and/or hearing loss by comparing monaural speech recognition performance in five groups consisting of young adult normal listeners (ages 18–30), old adult normal listeners (ages 60–81), and three groups of old adults (ages 60–81) with sensorineural hearing loss (Frisina and Frisina, 1997a). The young and old normal listeners were matched on absolute thresholds for pure tones and speech. The old sensorineural hearing loss groups represented three levels of hearing loss. Three types of speech stimuli were employed, spondees (words of two equally stressed syllables, e.g., eardrum), sentences in which context contributed to target word identification, and sentences where context did not aid in the identification of target words. Speech perception performance results in the quiet conditions indicated that the young and old normal hearers did not differ, thereby confirming that the auditory sensitivity of these two groups was similar. By implication, peripheral auditory system function did not differ. In addition, comparisons of performance of the two groups on ability to use context yielded no difference, thereby eliminating cognitive function as causation for speech recognition (in noise) performance differences. However, when the noise background, 12-speaker multitalker noise (Kalikow *et al*., 1977), was used, the performance of the young normal hearing group was superior to that of the old normal hearers. Thus implicating age as a factor contributing to this difference. The effect of hearing loss was revealed by comparing speech-recognition performance of the old normal hearers to that of the three old hearing loss groups. The combined effects of age and hearing loss were revealed in the relative performances of the young normal hearers and the three old groups with hearing loss. These findings pointed to a neural dysfunction originating in the central auditory system. Gap detection thresholds were later obtained in these subject groups as a measure of auditory temporal resolution the results of which were consistent with these findings.

We also conducted a study of free field perception of speech in noise to test for a central effect of age on binaural hearing in a simulated everyday environment (Frisina and Frisina, 1997b). Speech recognition performance of young normal hearers and old normal hearers with equivalent hearing sensitivity similar to those in the above study were compared. Sentences from the Hearing-in-Noise Test (HINT), (Nilsson *et al*., 1994) were presented adaptively. Speech signals and speech-spectrum noise were presented simultaneously at ear level 1 m from the subject seated in a double-walled sound booth. Audiometric thresholds of the young (18–27 years of age) and old normal hearers (60–81 years of age) did not differ. Speech was presented at 0° azimuth while noise was presented simultaneously at each of three positions, 0°, 90°, or 270°. An age effect was found in each of the speech-in-noise performance comparisons between the young and the old normal hearers. Moreover, spatial separation of speech and noise resulted in greater release from masking for the younger group in all conditions. We suspect that an age effect exists because the older human listener is unable to use timing cues as effectively as the younger human listener. In addition, in both groups noise interference was less when speech was delivered at 0° and noise at 270° than when speech was located at 0° and noise at 90°. This laterality effect in which a masker is presented to the right ear (and thus favoring the left and dominant

speech cortex) was the more effective masker for both young and old subjects. Of added importance was the finding (in a companion study, Ison *et al*., 1998) that it was a significantly more effective masker for the oldest of the old subjects. This result occurred in a context in which there was no significant relationship within this particular older group of subjects between age and audiometric loss. This suggests that the processing of speech signals in noise lateralized to the right ear is particularly affected by old age, strongly implicating a central effect in the genesis of this aspect of presbycusis.

In summary, most adults beyond the age of 60 years experience difficulty in understanding speech when conversing in the presence of background noise. One obvious reason for this difficulty is a change in the spectral resolving capacity of the inner ear. However, our findings utilizing ''golden ear'' subjects suggest that changes in the central auditory nervous system, characterized as temporal resolution dysfunction, is due to biological aging and exists independently or in conjunction with peripheral changes in the inner ear. To test this hypothesis further, our colleagues in psychoacoustics, evoked potential neurophysiology, behavior and experimental psychology, and neurology and neuroimaging conducted parallel studies in humans. In this way, we hoped to identify and elucidate the neural substrates underlying speech recognition problems reported by and corroborated in older adults.

B. Psychoacoustic Declines in Temporal Gap Detection

We explored declines in processing rate by examining temporal acuity on an elemental level in a simple paradigm, an auditory gap-detection task. We did so because age-related changes in the processing of speech, especially in acoustically complex conditions (van Rooij and Plomp, 1992), had been linked to declines in rate processing.

Because gap resolution had been demonstrated to be strongly dependent on the audibility of high-frequency energy in a test signal (Buus and Florentine, 1985) and generally gap thresholds in people with cochlear hearing loss were reported to be higher (Moore and Glasberg, 1988), the need to measure temporal acuity in older adults without hearing loss was clear. Other investigators who explored temporal resolution in older subjects using a gap-detection paradigm (e.g., Moore *et al*., 1992; Schneider *et al*., 1994) noted the importance of not confounding age and hearing loss. Nevertheless, the absolute thresholds of older subjects in previous studies were usually poorer than those of young subjects. Thus, it remained unclear whether the decreased temporal acuity reported for the older subjects reflected age-related changes alone or interactions between age and hearing loss.

Also, previous studies of gap detection and aging used sinusoidal signals (Moore *et al*., 1992, with 400 msec sinusoids; Schneider *et al*., 1994, with tone pips). While frequency specificity is often desirable, age-related changes in temporal resolution measured in tasks other than gap detection have been reported to increase with the complexity of the stimulus condition (Fitzgibbons and Gordon-Salant, 1995). This finding is consistent with those of other studies which indicated that the magnitude of the age-related slowing was proportional to task complexity (Sliwinski *et al*., 1994). Therefore, the use of more complex sets of conditions in studying gap detection in aging adults increases the likelihood of detecting age-related differences. Of practical consequence, results with acoustically complex signals in complex background may be more directly relevant to understanding the speech perception problems of older adults (CHABA, 1988).

Our study sought to clarify and extend understanding of age-related changes in temporal resolution by matching pairs of young and old subjects for absolute sensitivity across a wider range of frequencies than in previous studies and by measuring temporal resolution in conditions varying in stimulus and background complexity. If previously reported age-related differences reflected primarily differences in absolute sensitivity or its interactions with age, groups matched audiometrically should have comparable gap thresholds in all conditions. On the other hand, comparable gap detection in quiet and group performance differences in complex backgrounds would be consistent with reports of relatively greater difficulty understanding speech in noisy or complex listening environments that cannot be explained by audiometric thresholds alone. Finally, differences between groups in all conditions would support the hypothesis that a more generalized age-related change occurs in the speed of auditory processing.

One of our recent studies (Snell, 1997) demonstrated that gap detection is strongly influenced by age; i.e., mean gap thresholds of the younger subjects were smaller than those of older subjects. Mean gap thresholds appeared to be especially larger in conditions in which the carrier of the gap signal was more complex, e.g., amplitude-modulated, or in which the acoustic background was complex, for example, when high-frequency noise was present. Mean gap thresholds were significantly longer for the older subjects in 24 experimental conditions. The robustness of the effect given the variety of signals and listening conditions was striking. The mean gap thresholds of the older subjects were about 27 and 37% larger with 1 and 6 kHz noise-burst signals, respectively, than those of the younger subjects. These results are important for several reasons. First, the differences cannot be easily attributed to differences in absolute sensitivity, as can the differences reported in previous studies. Half of the conditions used a noise-burst signal with an upper cutoff frequency of 1 kHz and encompassed frequencies for which absolute sensitivity was precisely the same in each group. Second, the magnitude of the difference in gap thresholds was similar to age-related differences that have been recently reported in our neurophysiology team's recordings from young and old mice (Walton *et al*., 1998). Third, regardless of the marked differences in method and subject selection, results from different labs point to the same mechanism (Moore *et al*., 1992; Schneider *et al*., 1994).

Background condition had little effect on mean gap thresholds for the low-passed noise-bursts with a cutoff frequency of 6 kHz. For example, the mean gap thresholds in quiet, with a noise floor, and with a noise floor and high-frequency masker were 2.8, 2.7, and 2.9 msec, respectively. Pairwise comparisons for the 6 kHz signal indicated that mean gap thresholds of neither group increased with the addition of a noise floor. Gap thresholds increased only when both a noise floor and a gated-high-frequency masker were present, and then only slightly (although significantly at $P < 0.05$). Mean gap thresholds of the young subjects in the three background conditions

were 2.3 (quiet), 2.3 (noise floor), and 2.5 msec (noise floor and gated-high-frequency masker). The mean gap thresholds of the older subjects in the corresponding background conditions were 3.2, 3.2, 3.3 msec, respectively.

The high-frequency masker conditions were used to provide a straightforward interpretation of between-group differences in the noise-floor conditions even if high-frequency absolute thresholds were not exactly matched. That is, the gap thresholds of the two groups were expected to be comparable in the quiet condition and increase in the noise-floor conditions for the older group only. If the relatively better performance in the noise-floor condition reflected the more sensitive high-frequency hearing of the young group, then addition of a high-frequency masker should have eliminated their relative advantage. As a result, group performance should have been equivalent in the noise-floor plus high-frequency masker conditions. In fact, the addition of the high-pass masker resulted in increased mean gap thresholds for the 6 kHz signal in both groups of subjects. To estimate the contribution of high-frequency hearing, gap thresholds were correlated with absolute thresholds at 8 kHz. Correlations were insignificant, ranging from 0.010 to 0.143. Correlations with 6 kHz absolute thresholds were also calculated and were similarly small. While this probably reflects, in part, the narrow range of absolute thresholds at 6 and 8 kHz, the lack of significant correlations suggests that it is unlikely that the age effect is primarily related to the very small differences in high-frequency sensitivity.

Individual data of the 40 subjects were examined in 24 scatterplots representing each of the experimental conditions. Two representative conditions are shown in Fig. 39.2. Subject age in years is plotted on the abscissa; gap thresholds in milliseconds on the ordinate. Each data point represents the mean gap threshold of one subject. There are several aspects worth noting, which have been discussed in previous studies. First, there is considerable overlap between age groups in the distributions of gap thresholds. Second, the range of gap thresholds of the older group is broader than for the younger group, although the variability is similar when examined on a log scale. Finally, several older subjects appeared to have exceptionally poor temporal resolution in each condition.

One aspect of the data that has not been previously noted is shown in both panels of Fig. 39.2. Note that gap detection by several younger subjects appears exceptionally acute even when compared to the smallest gap thresholds of the older subjects. Interestingly, small (1 to 2 msec) neural correlates of gap thresholds measured in onset and primary-like neurons in the inferior colliculus of young mice have also been reported (see below, and Walton *et al*., 1997). These scatterplots illustrate that the age-related increase in mean gap thresholds reflects not just larger gap thresholds in a few older subjects but an overall shift in the distribution of gap thresholds with age. While there is considerable overlap between the two distributions, roughly a third of the gap thresholds of the older subjects fall well outside the range of the younger subjects and the gap thresholds of a few younger subjects are considerably smaller than the smallest old gap thresholds.

While pairs of subjects had been matched for absolute sensitivity at audiometric frequencies, it is possible that age-related differences in absolute sensitivity to the wideband signals could have contributed to between-group differences

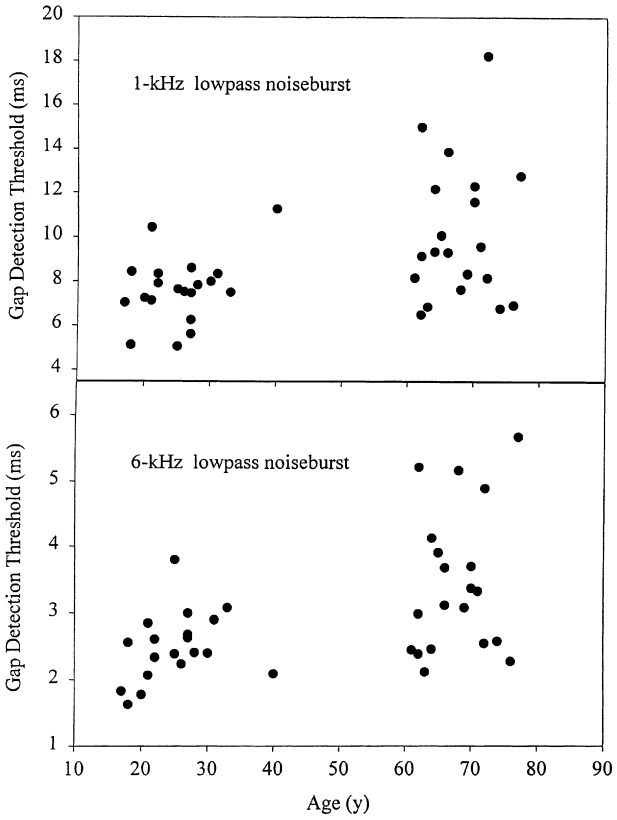

FIG. 39.2. Scatterplots of gap thresholds as a function of subject age. The top plot contains the gap thresholds obtained with a modulated noiseburst lowpassed at 1 kHz presented in a complex background. The bottom plot shows the gap thresholds obtained with a modulated noiseburst lowpassed at 6 kHz presented in a quiet background. (From Snell, 1997, with permission from the American Institute of Physics.)

in gap thresholds. To examine this possibility, individual absolute and masked thresholds obtained for sample signals in three background conditions were examined using ANOVA and pairwise comparisons. The results of these analyses indicated that the mean masked thresholds did not significantly differ between groups and therefore could not have contributed to the between-group differences in the noise-floor conditions. Also, the masked thresholds (dB SPL) for each subject were higher than the corresponding absolute thresholds, ensuring that the gap thresholds obtained in the noise-floor condition were actually masked gap thresholds. Sensation levels for the unmasked signals were slightly higher for the younger subjects but exceeded 30 dB SL for all subjects.

Our results indicate that age-related changes in temporal acuity occur across a range of stimulus characteristics and background conditions. Mean gap thresholds of older subjects were larger when compared to mean gap thresholds of younger subjects with matched audiograms for signals varying in spectra, intensity, and depth of modulation. Although the gap thresholds of younger and older subjects showed similar patterns in response to various signal manipulations, older subjects were more susceptible to the effects of background noise in most conditions. For instance, when a noise floor

was added to a low-passed noise-burst signal with a cutoff frequency of 1 kHz, the gap thresholds of the older subjects increased, whereas the gap thresholds of the younger subjects remained stable. Mean thresholds of both groups increased slightly when a high-frequency masker was gated with the signal and allowed to partially fill the gap.

C. Age-Related Changes in Temporal Processing: Auditory Brain-Stem Response

We evaluated temporal processing in young and aged adults using the auditory brain-stem response (ABR) (Walton *et al*., 1999). The ABR is a surface-recorded evoked potential that reflects activity arising from the auditory nerve and auditory brain stem (Møller, 1994). This response is ideal for primary sensory measurements as the response is only minimally affected by attention and arousal (Amadeo and Shagass, 1973; Picton and Hillyard, 1974), but is strongly dependent on numerous variables, including stimulus level, rate, and level of a continuous masking noise (Burkard and Hecox, 1983, 1987). In addition, the ABR is affected by some subject variables, including development (Hecox and Burkard, 1982). Walton *et al*. (1999) used an ABR forward masking paradigm to investigate temporal processing. In this paradigm, a primary signal, called the masker, is presented, followed by a second signal, called the probe, which elicits the ABR. Walton *et al*. (1999) used constant-level sinusoid maskers and probes, and varied the time interval between the end of the masker and the onset of the probe. This latter interval is referred to as the forward-masker interval. As with many recent auditory studies from the Rochester group, they used young and old subjects that were audiometrically normal. Specifically, they had thresholds of 20 dB HL or less for the octave frequencies from 250 to 8000 Hz, inclusive. Masker/probe frequencies included 1, 4, and 8 kHz. Probes were presented at 40 dB above perceptual threshold (40 dB sensation level), while maskers were presented at a level that just masked the ABR response when the probe was presented at masker offset. Probes were presented alone, and with forward-masker intervals of 0, 2, 4, 8, 16, 32, and 64 msec. Figure 39.3 shows responses to 4 kHz probes and maskers, for one young and one old subject, for all masker conditions. Note that wave V, indicated by the arrow heads, is the only ABR peak consistently seen in these recordings. Wave V is currently thought to arise from rostral brain-stem structures such as the lateral lemniscus (Møller, 1994), and hence any age-related differences up to and including the pons/midbrain level for auditory temporal processing should be manifested in wave V behavior.

Figure 39.4 shows a mean wave V latency shift for the 4-kHz probe/masker plotted across forward-masker interval. The latency shift is relative to the no-forward-masking condition (within subject and probe/masker frequency). Circles represent the young-adult data, while the squares represent the old subjects' data. Few subjects showed reliable responses

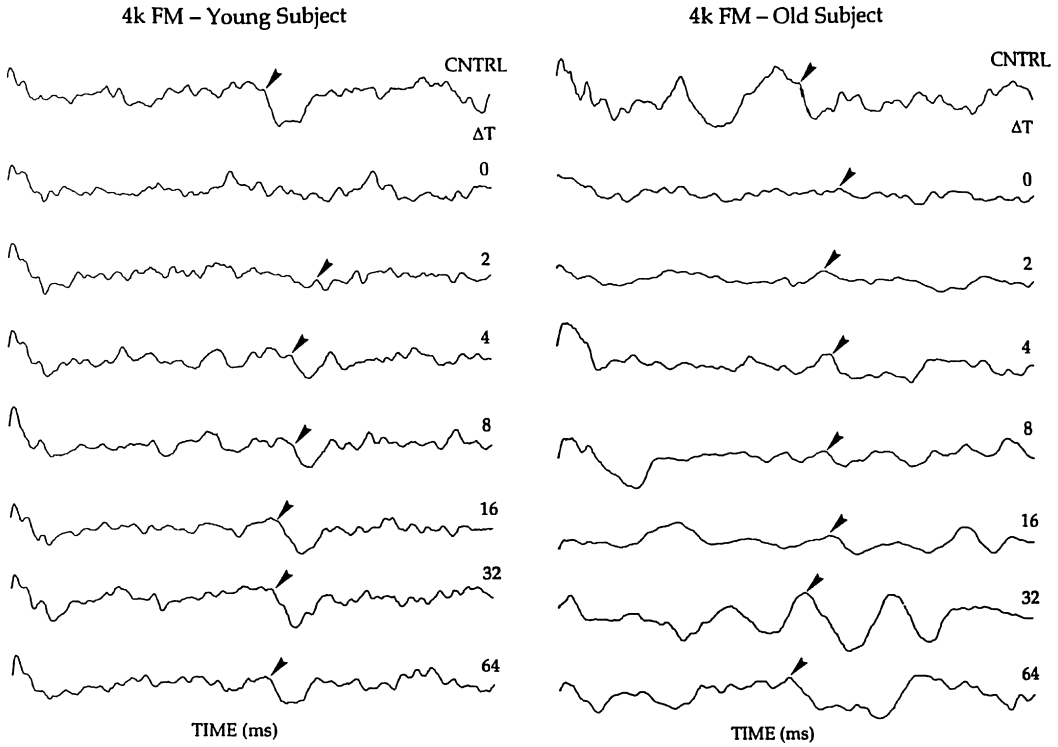

FIG. 39.3. ABRs across forward-masking interval (ΔT) for the 4 kHz masker-probe condition are shown for one young subject and one old subject. The unmasked, or control, response is shown as the top waveform for each subject, with the arrowheads denoting wave V latency. Note that for short ΔTs (2–8 msec) wave V latency in the young subject (24 years) has begun to recover to the control latency, while the wave V latency in the older subject (73 years) is still prolonged. ΔTs ranged from 0 to 64 msec and vertical calibration bar represent 0.2 nV while the horizontal bar represents 1 msec. (Reprinted from Walton *et al*., 1999, with permission from Elsevier Science.)

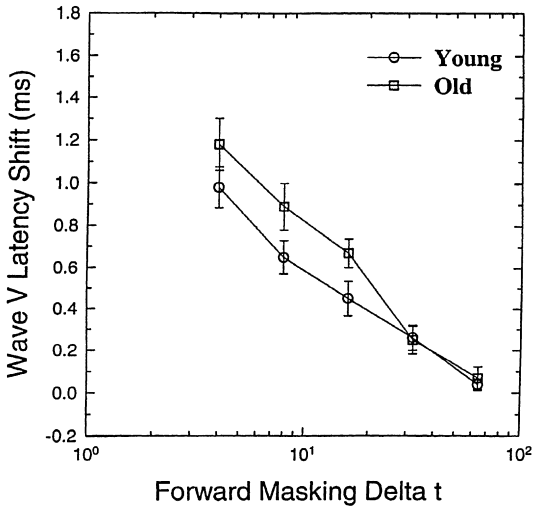

FIG. 39.4. Wave V latency shifts. The unmasked latencies are plotted across forward-masking interval for the 4 kHz probe for the young (◯) and old (□) subjects. Means are shown by symbols with standard deviation shown by vertical lines. The maximum prolongation of the mean wave V latency was approximately 0.9 msec for the young group and 1.2 msec for the old group. Again, means are based on 10 subjects. (Reprinted from Walton *et al*., 1999, with permission from Elsevier Science.)

for forward-masker intervals below 4 msec, and hence data are shown only for forward-masking intervals of 4 msec and above. Note that for young adults, wave V latency shift is less than that of the old adults, but only for forward-masking intervals of 16 msec and less. At longer intervals, at this tone-burst frequency, the two functions converge. It should be noted that a similar age effect emerged for the 8 kHz condition but that recovery functions for the young and old subjects were nearly identical for the 1 kHz condition.

Although it is difficult to explain the 1 kHz data, the 4 and 8 kHz data support the view that the old subjects showed a neurophysiological correlate of degraded temporal processing abilities, when compared to the young-adult subjects. Interestingly, this effect was most prominent at short forward-masking intervals. Specifically, at a given forward-masker interval the wave V latency shift was greater in old subjects, but both young and old groups showed complete (or near-complete) wave V recovery by 64 msec. This age-related degradation in temporal processing ability cannot be attributed to peripheral hearing loss, as all subjects had audiometric thresholds within normal limits.

D. Neuroimaging Brain Systems Underlying Speech Perception in Noise

We have begun initial hypothesis testing concerning the effects of background noise on cortical speech/language processing and how these systems are affected by age (Frisina *et al*., 1999). These studies have been made possible through the cooperation of the positron emission tomography (PET) center at Buffalo's Veterans Administration Medical Center of Western New York. Recent advances in the techniques of PET neuroimaging, coupled with more options for advanced statistical analyses of PET images, have made it possible for us to include this procedure in our study of presbycusis. Regional cerebral blood flow (rCBF) images are acquired by PET using ^{15}O-water as a tracer and indicator of brain activity (Posner *et al*., 1988). Differences in neural activity are reflected as differences in rCBF. The central thesis that underlies this strategy is the fact that the brain contains essentially no energy or metabolic reserves, and, as increments in neural activity are required to perform additional "brain work" there must be a corresponding increment in rCBF.

rCBF measurements were made as normal hearing young and old subjects listened to speech alone, speech in noise, and noise alone, i.e., tasks that were designed to activate brain functions and the corresponding neural systems that mediate the tasks. Measurements of these three conditions were compared to a resting state. Differences in rCBF associated with these listening tasks were analyzed using statistical parametric mapping. Statistical parametric mapping is a widely used statistical strategy for identifying increases in cerebral blood flow produced by stimuli presented to individuals (Friston and Frackowiak, 1991; Lockwood *et al*., 1993). We collected PET data on two groups of subjects, most of whom had completed the above-mentioned speech perception and temporal coding experiments, and thus were well characterized audiologically in terms of speech recognition and gap detection (Frisina and Frisina, 1997a,b). All young ($n=9$) and old ($n=7$) subjects had normal hearing as defined audiometrically, yet showed age-related differences in speech recognition and gap detection as reported above.

In our initial PET study, we measured the change in CBF produced by listening to context-positive sentences (Frisina *et al*., 1999). The subjects' task was to repeat aloud the last word in each sentence. Three conditions were used, with each condition being repeated for each subject to improve statistical power. The first condition consisted of listening to 30 sentences presented in quiet at a rate of one sentence every 4 sec at a level of 80 dB SPL. The second condition was identical to the first, except that the sentences were presented in the presence of their multitalker (babble) background noise. The noise was gated on/off 500 msec before and after each sentence. The noise intensity level was set individually for each subject so that each would achieve approximately 50% correct performance level (previous research with these subject groups prior to entering the PET study provided this information). The third condition consisted of presenting the noise stimuli without sentences. The subjects were instructed to give an answer to each sentence. In cases where they did not recognize the target word, they were instructed to say the word "nope" in order to help equilibrate the spoken word/motor portion of the task. The young (22–35 years of age) and elderly (60–71 years of age) normal listeners had rCBF measurements made at rest and while performing under the three conditions described above.

The PET data shown in Fig. 39.5 (see color insert) are for the young subjects only. They represent areas of activation for the speech stimuli presented in quiet, for all nine subjects combined. The background brain renderings showing lateral (top) and medial (bottom) views of the brain in MRI format are averages and do not represent any subject's specific brain of the present study. As indicated in the two top parts of the figure, rCBF maxima were found in the temporal lobes, consistent with earlier PET mapping studies of auditory cortex using speech-like stimuli in quiet. The two bottom portions of this

figure indicate that some activity also occurs in the auditory midbrain (inferior colliculus) and cerebellar portions of the brain stem involved in sound processing. Because of technical limitations that we recently surmounted, previous speech/language PET investigations have generally not been able to examine brain-stem nuclei. The effects of background noise on the brain activity occurring during this speech recognition task are shown in Fig. 39.6 (see color insert) for the young subjects. Temporal lobe activity is still present, as with the speech in quiet (top portions of Fig. 39.6). However, comparisons of the bottom portions of Fig. 39.5 and 39.6 indicate that there is a significant increase in the brain activity in the auditory midbrain and cerebellar regions when the speech was presented in background noise (Fig. 39.6). It may be that this significant increase in activity in the inferior colliculus and cerebellum is an auditory brain-stem-filtering requirement necessary for allowing perception of speech in the presence of a strong background noise. Similar results were obtained for the old subjects when comparing processing of speech in quiet and in the presence of significant background noise (Frisina *et al*., 1999; data not shown).

One representative finding concerning the effects of age on speech recognition in quiet and background noise is shown in Fig. 39.7 (see color insert) (Frisina *et al*., 1999). Here, activations of old subjects have been subtracted from young subjects for the speech-in-quiet speech recognition task. Results for the speech-in-noise task were similar (not shown). Note that the young subjects show more activity in the auditory midbrain on both sides (bottom portions of the figure). This may be a neural correlate of the young subjects' superior abilities of discriminating the speech from the background noise and thus having improved performance of speech recognition in noise. Additional increases in activity can be seen bilaterally in the visual cortex and on the left side of the brain in the anterior cingulate cortex for the young subjects. Earlier behavioral studies (Pichora-Fuller *et al*., 1995; Frisina and Frisina, 1997a,b) showed that old subjects with normal audiometric thresholds or peripheral hearing loss used context as well or better than young subjects when listening tasks were made difficult by the introduction of background noise. These behavioral comparisons suggested that increased cerebral resources of the old subjects were being used to overcome the deficit caused by the decline in auditory processing capacity in the brain-stem or inner ear hearing loss. Thus, increased rCBF to the cortical levels, as revealed in PET imaging, by old subjects with normal audiometric thresholds, would be consistent with their necessity to compensate for the brain-stem temporal resolution decline. Future studies will compare rCBF in young and old listeners with normal audiometric thresholds to rCBF in old subjects with different degrees of peripheral hearing loss. Such studies will help to elucidate further the neural bases of presbycusis.

III. Neurobiology of Temporal Processing—Animal Models

A. Neural Correlates of Acoustic Gap Detection

Initial investigations of the neural bases of auditory temporal processing were conducted in young adult CBA mice to determine whether any correspondence could be demonstrated between behavioral measures of temporal processing and single-nerve cell physiological data (Walton *et al*., 1997). The CBA mouse strain loses its hearing slowly in life, on a time frame similar to that of humans, when calibrated for the different absolute life spans of mice and men. This was one means for assessing the adequacy of the choice of the mouse as an animal model for correlation of behaviors involving hearing with underlying neurology. Our approach was to measure sensitivity to brief gaps in broadband noise using acoustic startle response paradigms. Then, utilizing the same strain of mouse, measure responses of single nerve cells in the auditory midbrain (inferior colliculus) for the same type of gap stimulus. More specifically, in this behavioral reflex modification procedure used to measure gap detection, stimuli are presented at brief intervals prior to the elicitation of a startle reflex by a noise burst. The startle response is inhibited depending upon the salience of the "warning" stimuli used. In this case, the warning sound were gaps ranging from 1 to 15 msec, placed in a continuous noise preceding the startle elicitor by 60 msec. The advantages of this method are that no prior training is required and that sensory thresholds in laboratory animals can readily be obtained in a matter of hours rather than days or months.

1. Behavioral Correlates of Acoustic Gap Detection

Temporal acuity was studied in about 200 CBA mice, ranging in age from 2 months to 29 months, using the behavioral technique of reflex modification audiometry (Young and Fechter, 1983). The mouse was placed in a small cage set on top of an accelerometer which measured the force of the acoustic startle response to a brief burst of white noise (115 dB SPL, 20 msec in duration), and this apparatus was placed in an anechoic chamber. The startle noise burst was given in the presence of a white noise background (70 dB SPL), either after 15 to 30 sec of continuous noise (since the preceding trial) or 60 msec after the occurrence of a brief silent period in the noise (a gap) that could be 1 to 15 msec in duration. The startle reflex is inhibited by gaps that briefly precede it and past research has shown that in a variety of species, including humans (Ison and Pinckney, 1983), the threshold for reflex inhibition approximates the threshold obtained by conventional psychophysical procedures. In addition to providing a behavioral measure of gap threshold, the methodology provides a measure of the effects of suprathreshold levels of stimulation, which can be assumed to be related to the general efficiency of temporal processing in the auditory system. The threshold was defined conventionally, as the first duration at which the strength of reflex inhibition was 50% of its maximum strength. This criterion avoided any measurement problem caused by the fact that the asymptotic levels of inhibition were affected by age.

The thresholds for gap detection varied with age, with the older mice being less sensitive to brief silent periods in noise: mice younger than 1 year of age ($n=109$) had a mean gap threshold value of 3.06 msec ($\pm$ 0.12, SEM); mice between 1 and 2 years of age ($n=42$) had a mean gap threshold of 3.76 ms ($\pm$ 0.40); and mice over 2 years of age ($n=67$) had a mean gap threshold of 4.02 msec ($\pm$ 0.45). These differ-

ences, while small, were significant, F (2,215) $=3.37$, $P < 0.05$, and the linear trend was also significant, $P = 0.01$. One very interesting aspect of these data is that the variance clearly increased in the older animals ($P < 0.001$), indicating that the decrement in auditory processing produced by age was far from uniform within the group. Many old mice had gap thresholds of 2 to 4 msec that were the equal of those obtained in the youngest mice. It may be noted that gap thresholds obtained in our mice were very similar to those obtained in human subjects under similar stimulus conditions (Snell, 1997): 2 msec in the youngest human listeners compared to 3 msec in the best of the oldest listeners. Again, as in the mice, this small difference was significant, and again, the variance in the old listeners was greater than that of the younger listeners.

In contrast to the small age effect on the detection thresholds for brief gaps, the behavioral effects of suprathreshold gaps were substantial. The longest gap, 15 msec in duration, produced 58% ($\pm$ 1.6%) inhibition in the youngest mice, 47% ($\pm$ 3.2%) in the middle group, and just 37% ($\pm$ 2.4%) in the oldest groups of mice. The analysis of these data showed that the age effect was highly significant, F (2,217) = 27.0, $P < 0.0001$, and in contrast to the effects of age on threshold, which accounted for but 3% of the variance, age accounted for 20% of the variance in asymptotic inhibition. In addition, while age substantially affected the variance of the threshold data, age had no effect on the variance of the inhibition data.

These data suggest that a major difference between young and old animals in their response to brief gaps in noise has to do with the behavioral effectiveness of the longer gap stimuli. As in humans, the ability to detect gaps presented at brief intervals is evidently maintained by many of the older animals, and their thresholds do not differ from those of the younger mice; in contrast, the behavioral effectiveness of suprathreshold gaps is substantially and more uniformly impaired in the older mice. These behavioral data are found to have many parallels in the neural activity of cells recorded in the auditory midbrain of a subgroup of these same mice. Together these data indicate that the time constant of the neural machinery required for tracking a rapidly changing acoustic event, characteristic of a speech signal, for example, is little affected by age, save in a small subgroup of aged listeners. In contrast, many aged listeners appear to be less successful than most young listeners in effectively responding to suprathreshold stimuli: measured either behaviorally or neurally, the aged auditory system is less able to generate a substantial response to a suprathreshold input.

2. Neurophysiological Correlates of Acoustic Gap Detection

Single unit studies were conducted on mildly tranquilized mice to determine if relations existed between the behavioral gap coding and the underlying neurology (Walton *et al*., 1997). At least 1 day prior to single neuron recording, a small threaded holding tube and indifferent electrode were attached to the skull with cyanoacrylate glue and dental acrylic under avertin anesthesia (0.02 ml/g body weight). Experiments were carried out in a heated (27–30°C) soundproofed booth. A small hole (approximately 500 mm diameter) was drilled over the surface of the inferior colliculus under methoxyflurane anesthesia. During experiments, mice were mildly tranquilized with taractan (5–12 mg/g body weight) and placed in a form-fitting plastic restraint attached to a custom-built stereotaxic frame. Metal tungsten (1–3 MΩ) or glass microelectrodes (8–12 MΩ) were advanced through the inferior colliculus using a remote-controlled micropositioner. Single units were isolated using conventional electrophysiological techniques and recording sites were verified to be in the central nucleus of the inferior colliculus using horseradish peroxidase histochemistry.

Stimulus generation and manipulation were computer-controlled using a digital signal processing platform (Tucker-Davis AP2) and custom software package. The amplified output was led to a high frequency leaf tweeter (Panasonic 100) having a frequency response ranging from 1 to 100 kHz and located at 30° contralateral azimuth. Noise was digitized from 2 to 60 kHz and normalized to within $\pm$ 2 dB by measuring the transfer function at the entrance of the external meatus (1/4″ B and K microphone). A second DAC channel synthesized continuous background noise which was attenuated independently. The noise carrier used for the gap stimulus and the background noise were ''fresh'': they were selected at random from a 7.2-sec-long normalized noise buffer at temporally different points for each of the 75 stimulus repetitions.

After a single unit was isolated, best frequency and minimum threshold were determined audiovisually. An automated gap series was then presented in quiet and in various signal-to-noise (S/N) ratios. Carrier intensity was typically 65 dB SPL with an overall duration of 150 msec and a cosine-shaped rise/fall time of 5 msec. A quiet gap of variable duration and 0.5 msec rise/fall time was inserted 100 msec into the noise carrier. The standard gap series consisted of gap durations of 1, 3, 6, 12, 24, 48, 96, and 0 (control) msec. The custom software time-stamped each spike arrival with a precision of 10 msec.

A neuron's ability to encode the gap is typically observed qualitatively as a difference in spike counts occurring either during the gap or in response to the second noise burst (correcting for neural latency). Composite histograms [summations of all the poststimulus time histograms (PSTHs)] were generated from the complete gap series in order to ''blind'' the analysis. The first composite histogram was simply the summation of all the PSTHs, and allows the offset latency to noise burst 1 to be determined. The second composite was constructed by shifting each gap file back in time by its appropriate gap duration, thus lining up the response to the second noise burst. Two time windows, quiescent and driven, were computed from the composites and the spike counts for each stimulus epoch was submitted to a Wilcoxon-signed rank test so that for each gap duration in a series a statistical comparison was made between the spike arrival times for control and gap stimuli. This analysis formed the bases for quantification of neural gap detection. The minimum gap threshold was defined as the shortest gap duration capable of producing a significant difference ($P < 0.05$) from the control, provided that the next largest gap stimulus also produced a significant effect.

In the case of startle inhibition, a decrease in the amplitude of the startle response is observed as gap duration increases. This is illustrated in Fig. 39.8 (adapted from Walton *et al*., 1997) where changes in the startle response, with increases

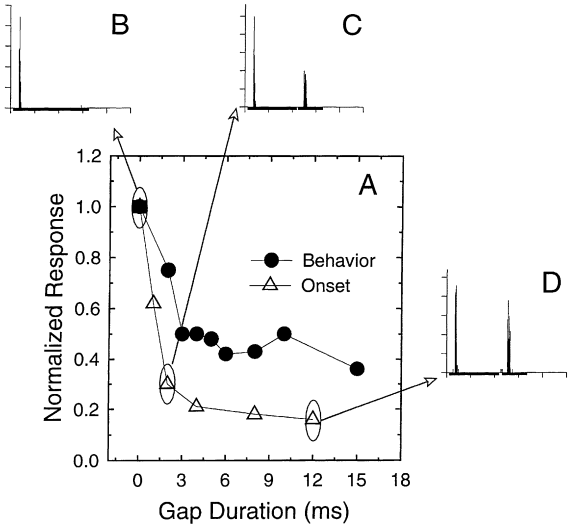

FIG. 39.8. Comparison of behavioral and neurophysiological measures of gap detection revealed the same functional relationships. (A) Mean acoustic startle results from five animals are plotted with normalized gap functions for the On ($n = 15$) units measured from the inferior colliculus in the same animals. Taking the spike counts in a control window (no response to the gap) and computing the relative change from the control value at each gap duration normalized the single unit data. (B) Single-unit PSTH showing normal onset response, with no gap. (C) PSTH showing response to a short gap (3 msec). (D) Response to a longer gap (12 msec.). (Adapted from Walton *et al*., 1997, with permission from Springer-Verlag.)

in gap duration, are plotted as filled circles (Fig. 39.8A). A normalized response of 1.0 represents the control condition, or no gap, and maximum startle amplitude. As the gap duration increases, the startle amplitude decreases and the function becomes asymptotic at about 6 msec. The neural data (open triangles) represent the mean change in discharge rate of 15 phasic type units located in the inferior colliculus. Phasic units comprise roughly 60–70% of the unit types encountered in the inferior colliculus. Phasic type neurons typically have very low background activity and discharge at the onset of each noise burst that marks the gap. The three PSTHs represent the response of a phasic unit to three different gap durations, 0 msec or the control condition (Fig. 39.8B), 3 msec (C), and 12 msec (D). To compare the startle data to the neural data, each unit's response magnitude was normalized by computing changes in spike counts relative to the control condition (Fig. 39.8B), which was represented by a value of 1.0. As the gap duration exceeded the gap threshold, phasic units exhibit rapid increases in the number of discharges evoked by the second noise burst (Figs. 39.8C and 39.8D). Although the absolute asymptote differs slightly between the behavioral and the neural data, the shape of the functions are remarkably alike. Both are characterized by a rapid change in response magnitude for gap widths up to 3 msec, and then asymptote for gaps longer than 4 msec in duration.

At the level of the inferior colliculus and auditory cortex, the majority of neurons display phasic type discharge patterns in response to noise bursts. The gap encoding ability of inferior colliculus neurons suggests that the neural code required for the perception of silent periods in ongoing sounds may be encoded by phasic type units. Considering the neural signal-to-noise ratios about acoustic signals that inferior colliculus neurons send rostrally to "central processors," phasic neurons would appear to be optimally suited for gap detection. Specifically, the targets of ON units would receive an unambiguous neural signal time locked to the second noise burst marking the gap. In addition, most ON units display rapid exponential increases in spike count as gap width increases from 1 to 10 msec (Fig. 39.8A). This results in a very high probability of discharge, which can approach >1 spike per stimulus. Correlation across many ON units could be used as a determinant of "gap detection," thus providing the neural mechanism for gap encoding. This investigation marked the first demonstration of possible neural correlates of gap detection in single neurons of the mammalian central auditory system

B. Aging Effects on Neurobiology of Temporal Processing: Animal Models

1. Neural Correlates of Acoustic Gap Detection: Single Cell Physiology

After demonstrating that nerve cells of the auditory midbrain can encode acoustic gaps in a manner similar to the behavior for the same species, investigations were undertaken to see whether this neural processing changed with age (Walton *et al*., 1998). It was hypothesized that there would be age-related degradations in the abilities of single nerve cells to encode gaps. To test this hypothesis, the same neurophysiological recording and analysis procedures were used as described in the previous section. Except here, not only were responses of nerve cells in young adult animals characterized, but also in aged animals of the CBA mouse strain. Based on extracellular recordings from 185 single nerve cells, it was discovered that the number of nerve cells capable of encoding very short duration acoustic gaps significantly declined in the old animals (Fig. 39.9) (Walton *et al*., 1998). This figure shows that about one-half of the nerve cells sampled from young animals can encode very short gaps (1 msec). Whereas only about 20% of the nerve cells of old animals can perform at this same level. In

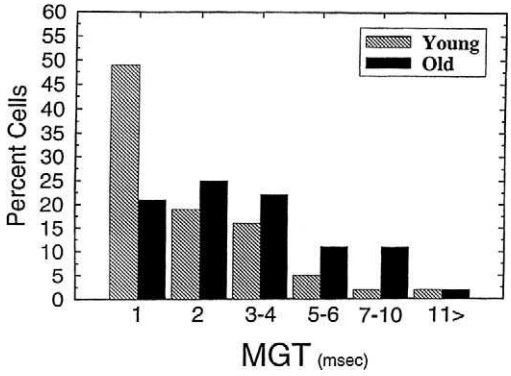

FIG. 39.9. Proportion of units having minimum gap thresholds (MGT) ranging from 1 to >11 msec for the young (hatched bars, n = 78) and old (filled bars, n = 108) mice. Note that the distribution favors considerably higher gap thresholds for the units from old animals. (From Walton *et al*., 1998, with permission from the Journal of Neuroscience.)

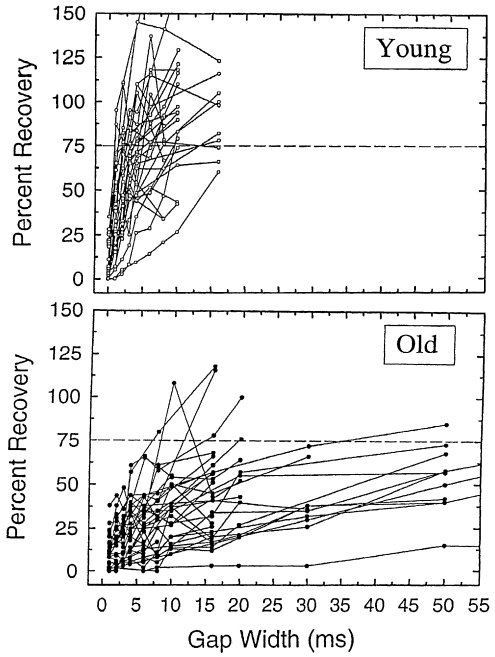

FIG. 39.10. Neural recovery functions plotted for 30 phasic units (ON and ONs types only) in young (top) and old (bottom) animals. Neural recovery was quantified by computing the number of spikes elicited by the second noiseburst (NB2) divided by the spike count to the first noiseburst (NB1) $\times$ 100. This was done for every histogram in the gap series. A recovery value of 100% would represent equal discharges to both NB1 and NB2. Dashed horizontal lines represent 75% recovery. Recovery to the 75% criterion is complete by $\leqslant$ 10–15 msec in nearly every neuron from the young animals, whereas most neurons from old animals do not reach this criterion for any of the gap durations tested. Note also that many neurons from young animals show facilitation; e. g., the response to NB2 is greater than NB1 for certain gap durations but this rarely occurred for neurons from old animals. (From Walton *et al*., 1998, with permission from the Journal of Neuroscience.)

addition, examination of this figure shows that the distribution of nerve cell gap encoding abilities shifted to longer gap durations in the old animals.

Not only was the sensitivity to gaps in sounds investigated, i.e., gap thresholds, but the strength of the neural responses to the gaps were also measured. This was done by comparing the number of neural action potentials, or "spikes," in response to the gap, relative to the response at the beginning of the noise burst that preceded the gap (Fig. 39.10) (Walton *et al*., 1998). In this figure, "Percent Recovery" is the proportion of action potentials in response to the gap, relative to the action potentials in response to the beginning of the noise burst that was presented before the gap. One hundred percent means that the response to the gap equaled that to the beginning of the stimulus. If a nerve cell showed a weaker response to the gap, the recovery would be less than 100%. If a nerve cell showed a response greater than 100%, the gap response was "facilitated." Comparing the data for the young (top) versus the old (bottom), two differences are quite striking. First, most old nerve cells did not recover to the 75% level, for even the longest gap durations tested, whereas most young nerve cells were fully recovered at relatively short gap durations (less than 15 msec). Second, many of the young nerve cells showed facilitated responses to the gap stimuli. Whereas this was virtually never seen in any of the nerve cells from old animals.

2. Neural Correlates of Acoustic Gap Detection: Nerve Cell Locations

At the conclusion of single-nerve cell recording experiments, as described in the previous two sections, injections of the marker and tracer horseradish peroxidase were made in the center of the regions from which the recordings were made. This marker/tracer performs three investigative functions. First, it marks the center of the anatomical regions from which the neurophysiological recordings were made. Because the same animals were used in the neurophysiological and histological experiments, direct comparisons were made between nerve cell sensitivity to sounds and their locations in the brain. In the present experiments on neural bases of gap coding, the region recorded from was identified to be the dorsomedial region of the auditory midbrain. This region included portions of the midbrain subdivisions known as the central nucleus, the dorsal cortex, and the commissural nucleus. Examples and details concerning these horseradish peroxidase injection sites can be found in Fig. 39.11 (Frisina *et al*., 1997). Second, the horseradish peroxidase is taken up by nerve cells that have cell bodies in the region of the injection site and is transported anterogradely down their axons to their terminal endings. For primary nerve cells that send axons to other parts of the brain, the presence of horseradish peroxidase in terminal endings indicates output regions of nerve cells from the injection site. Similarities and differences in outputs of the dorsomedial portions of the young adult CBA mouse auditory midbrain were reported as a foundation for subsequent aging experiments (Frisina *et al*., 1997). Third, horseradish peroxidase is also actively taken up by any nerve cells that send terminal endings into the effective area of the injection site. Determination of the inputs to the dorsomedial region of the auditory midbrain in young adult animals was accomplished, and their likenesses and differences with other mammals noted. Also, a point of comparison for studies in middle-aged and old CBA mice (Frisina *et al*., 1998) was charted. Major input regions were similar to other rodents, including strong inputs from the contralateral cochlear nucleus, the ipsilateral periolivary nucleus, and the ipsilateral ventral nucleus of the lateral lemniscus, as presented in Fig. 39.12 (Frisina *et al*., 1998).

3. Neural Correlates of Acoustic Gap Detection: Plasticity of Inputs

Investigations of the anatomical inputs to the region we functionally characterized as having an age-related temporal processing deficit were made by repeating the horseradish peroxidase tracing experiments, described in the previous section, in middle-aged and old CBA mice. We found that there were significant declines for some of the input regions to this portion of the auditory midbrain. For example, reductions in the number of horseradish peroxidase-labeled nerve cells were seen from all three divisions of the contralateral cochlear nucleus (Fig. 39.13) and from the ipsilateral anterolateral periolivary nucleus (Fig. 39.14). In contrast, it was discovered that there

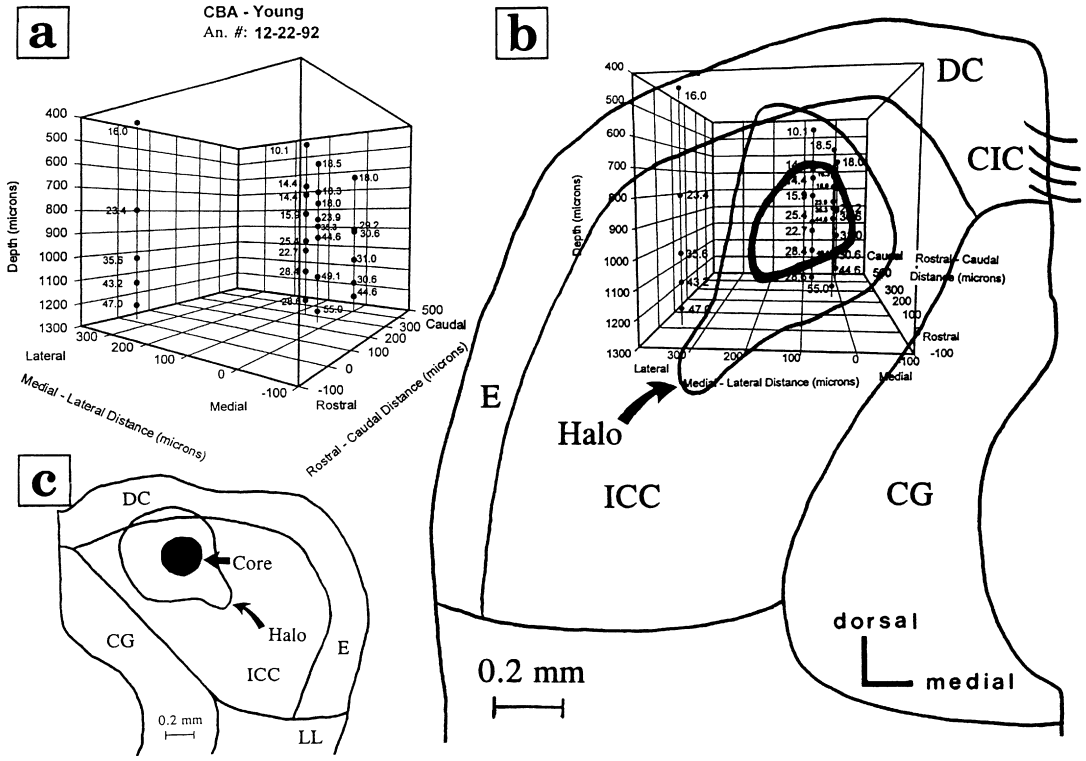

FIG. 39.11. Physiological recordings and iontophoretic injections of horseradish peroxidase were always centered in the 18 to 24 kHz region of the dorsomedial inferior colliculus, at about the halfway point rostrocaudally. Portions of the injection site halo and some of the recordings extended outside of this region as shown here. Boundaries between divisions within the inferior colliculus are based upon Willard and Ryugo (1983). (a) Example of a physiological map from one of the animals represented in a 3D graphical format. The best frequency of each single unit or multiunit cluster is given in kHz. The graph is rotated so as to provide a mediorostral vantage point. All axes are in arbitrary μm units. As expected, the best frequencies increase as one moves deeper in the inferior colliculus. An.; animal. (b) Correspondence of the physiological best frequency map (rotated to a paracoronal plane) and the neuroanatomical cytoarchitectonics. The dense core of the injection site has a bold border, and the halo a thin border (arrow). Injection sites were placed in dorsomedial central nucleus of the inferior colliculus (ICC) and halos sometimes extended into the dorsal cortex (DC). The 3D physiological data (a) are calibrated to the coronal, anatomical section at the 300 μm rostrocaudal plane, i.e., at the injection site center, both anatomically and physiologically. CG, central gray; CIC, commissure of inferior colliculus; E, external nucleus of inferior colliculus. (c) Camera lucida drawing of the dense core and halo of the center of the injection site in dorsomedial inferior colliculus from another animal (An. 12). Boundaries between ICC, DC, and E should be considered approximate since they are based upon Nissl-stained material (not Golgi). (Adapted from Frisina *et al*., 1997, with permission from the American Institute of Physics.)

was an age-related stability of inputs from the other regions of the auditory brain stem such as the rest of the superior olivary complex (Fig. 39.14) and nuclei of the lateral lemniscus (Fig. 39.15). It was noteworthy that behavioral measures of auditory temporal processing also started showing signs of age-related decline in middle-aged animals, correlated with the middle-aged declines in the inputs revealed here, despite the fact that middle-aged CBA mice have minimal changes in sensitivity to sounds (cochlear thresholds) (Frisina *et al*., 2000).

4. Neural Correlates of Acoustic Gap Detection: Immunocytochemistry Studies

The presence of calcium-binding proteins, involved in the intracellular regulation of calcium stores, was next examined in the same region of the auditory midbrain (dorsomedial inferior colliculus) that showed functional temporal processing declines with age as well as the reductions in neural inputs described in the previous section. Indicators of calcium regulation were examined since intracellular calcium imbalances are so intimately involved in cell function and death. Labeling with antibodies to calbindin and calretinin in young and old CBA mice were examined qualitatively and quantitatively. Quantitative analyses, including blind counts of immunostained cells, revealed a change in the presence of these calcium binding proteins with age (Zettel *et al*., 1997). As shown in Fig. 39.16 (Zettel *et al*., 1997), significant declines in the number of calbindin-immunoreactive nerve cells declined with age in the inferior colliculus region exhibiting the declines in auditory temporal processing. This reduction in the presence of calbindin appeared not to be related to the residual hearing of the CBA strain, because it also took place in the C57 strain that is profoundly deaf in old age. Age-related declines in calbindin immunoreactivity had previously been observed in other brain systems, so the reduction observed here was not unexpected.

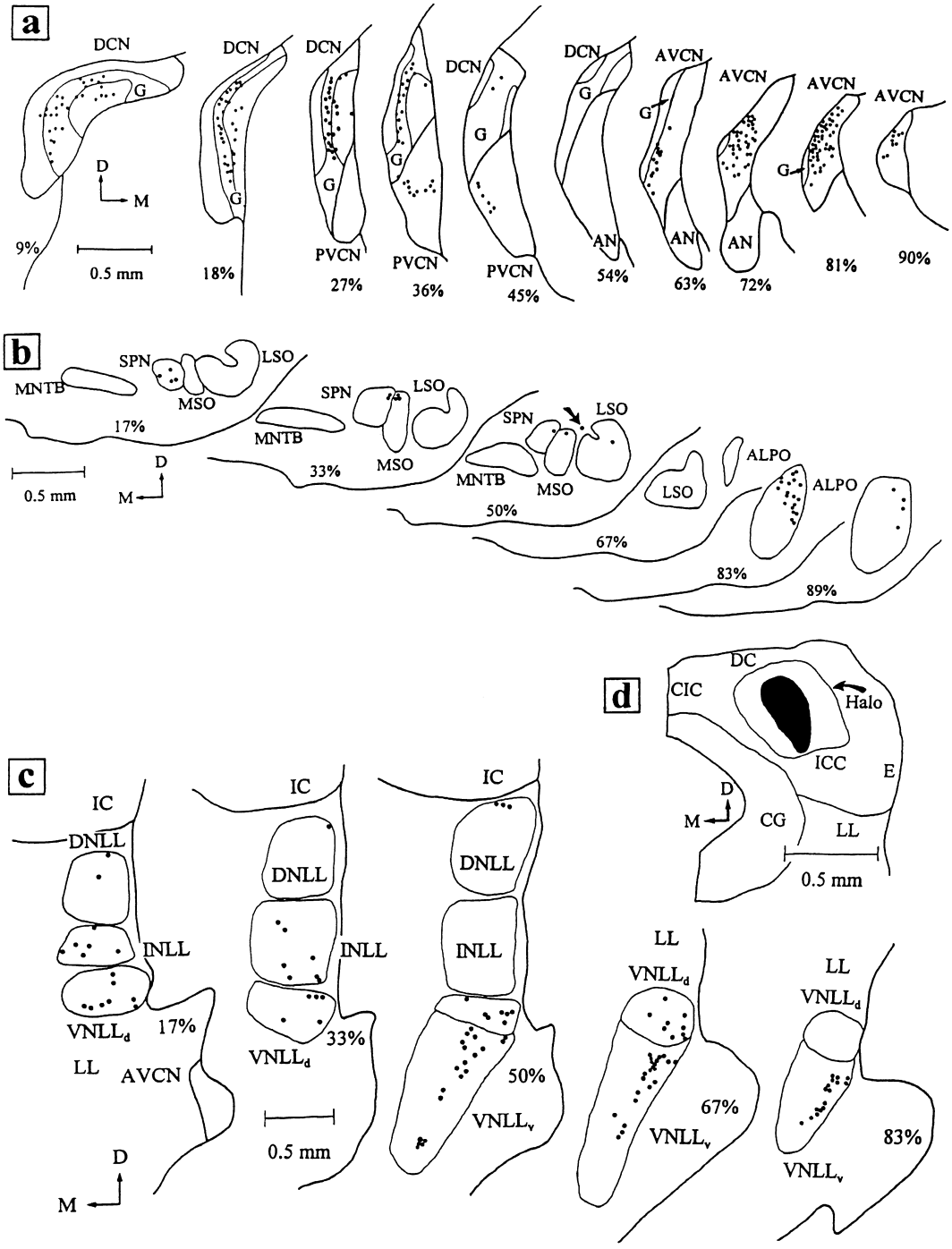

FIG. 39.12. Distribution of labeled neurons in serial coronal sections of contralateral cochlear nucleus (CN), ipsilateral superior olivary complex, and ipsilateral nucleus of the lateral lemniscus (NLL) from representative animals. (a) Camera lucida drawings showing spatial distribution of retrogradely labeled neurons in dorsal CN (DCN), posterior ventral CN (PVCN), and anterior ventral CN (AVCN). Percentages indicate distance from the caudal boundary of the CN. (b) Camera lucida drawings showing spatial distribution of retrogradely labeled neurons in the superior olivary complex. Arrow designates a labeled neuron in dorsolateral periolivary nucleus (DLPO). DNLL, dorsal nucleus of lateral lemniscus; ALPO, anterolateral periolivary nucleus; LSO, lateral superior olivary nucleus; MNTB, medial nucleus of the trapezoid body; MSO, medial superior olivary nucleus. Percentages indicate distance from the caudal boundary of the superior olivary complex. (c) Camera lucida drawings showing spatial distribution of retrogradely labeled neurons in the NLL. DNLL, dorsal nucleus of lateral lemniscus; INLL, intermediate nucleus of lateral lemniscus; VNLLd, ventral nucleus of lateral lemniscus, dorsal division; VNLLv, ventral nucleus of lateral lemniscus, ventral division. Percentages indicate distance from the caudal boundary of the NLL. (d) Camera lucida drawings showing spatial extent of the injection site in the dorsomedial inferior colliculus, centered in the 18 to 24 kHz physiological region. (D), dorsal; M, medial; CG, central gray; CIC: commissure of the inferior colliculus; DC, dorsal cortex of IC; E, external nucleus of inferior colliculus; ICC, central nucleus of inferior colliculus; LL, lateral lemniscus. (Reprinted from Frisina *et al.*, 1998, with permission from Elsevier Science.)

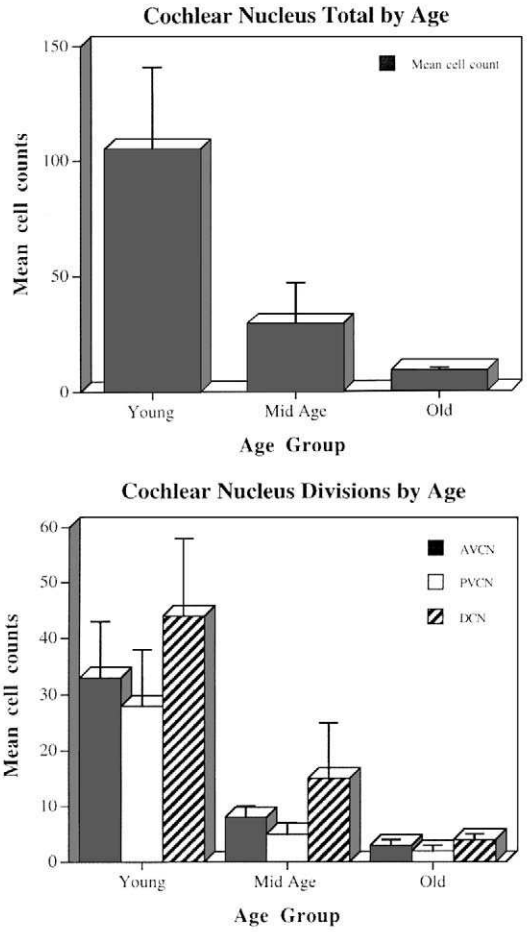

FIG. 39.13. Significant declines occurred for the inputs from the contralateral cochlear nucleus in the middle-aged and old animals relative to the young animals. (top) Large declines occurred for the total number of labeled neurons from all three divisions of the cochlear nucleus. The most dramatic decline was from the young to the middle-aged group. (bottom) Breakdown of mean cell counts for each division of the contralateral cochlear nucleus. As with the total number of cells (top), the most noticeable decline occurred in middle age. The relative declines were similar for all three divisions; i.e., the DCN always had the most labeled neurons, and the PVCN, the least. AVCN, anteroventral cochlear nucleus; DCN, dorsal cochlear nucleus; PVCN, posteroventral cochlear nucleus.

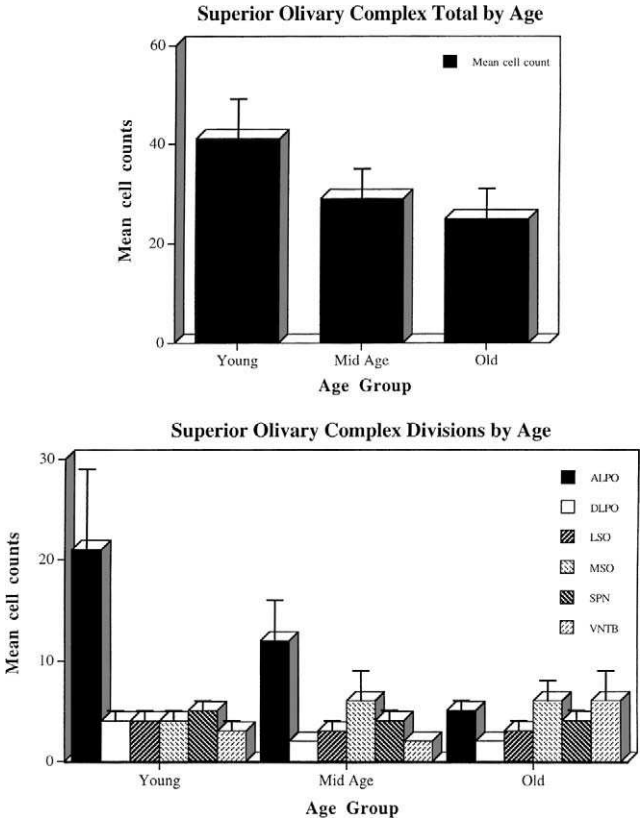

FIG. 39.14. Significant declines occurred for the inputs from the ipsilateral superior olivary complex in the middle-aged and old animals relative to the young animals. (top) Large declines occurred for the total number of labeled neurons summed over all the divisions of the olivary complex. (bottom) However, examination of the superior olivary complex nuclei that contained horseradish peroxidase-labeled neurons indicates that only in ALPO, which contains most stained cells in young animals, is there a significant age-related decline. ALPO, anterolateral periolivary nucleus; DLPO, dorsolateral periolivary nucleus; LSO, lateral superior olivary nucleus; MSO, medial superior olivary nucleus; SPN, superior paraolivary nucleus; VNTB, ventral nucleus of the trapezoid body.

What was surprising was that an upregulation of the presence of calretinin was discovered in this same region of the auditory midbrain for the CBA mouse strain. It may be that this increase was required for, or was in response to, the functional activity of these nerve cells in the old mice who could still hear, but who had reductions in their abilities to resolve timing aspects of sound. No age-related change in calretinin was observed in the C57 strain that is profoundly deaf in old age.

IV. Summary and Future Directions

In general, hearing loss in the aged has been attributed to ''presbycusis.'' However, to appreciate the specific problem that we addressed, it is necessary to understand the subtle but real distinction between ''hearing loss in the elderly'' and ''hearing loss due to biological aging.'' The former may result from a variety of acoustic insults that accumulate with time. For example, long-term exposure to noise and ototoxic substances, dietary factors, and circulatory deficiencies have been shown to exert deleterious effects on hearing due to hypoxia or glucose deprivation. It may be that the aging auditory system is more fragile and thus shows a greater effect of acoustic trauma. However, these insults are not inevitable and may be prevented in aging, for example, by appropriate changes in the noise condition of one's work and recreation, dietary habits, or early medical treatment. Alternatively it could be that physiological function is compromised and anatomical structure is degraded in the aged central and peripheral nervous systems even in the absence of explicit acoustic or metabolic threats. In order to make such distinctions our overall strategy was based upon two assumptions that could be tested in principle. The first assumption was that acoustic insults such as those described above have a specific affinity

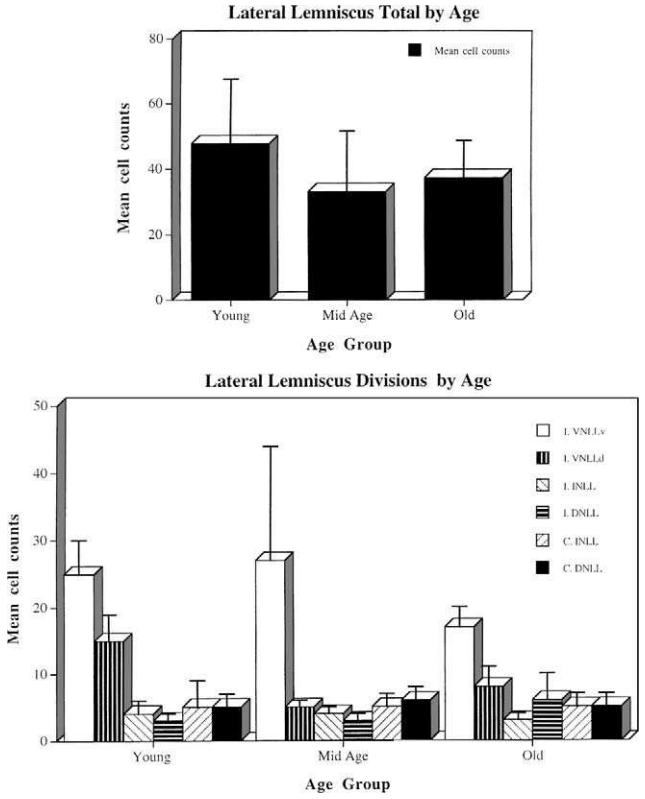

FIG. 39.15. In contrast to the cochlear nucleus and superior olivary complex, only modest declines (not statistically significant) were observed from the ipsilateral nuclei of the lateral lemniscus (NLL). (top) Overall declines in labeled neurons from NLL were small. (bottom) Ipsilateral VNLLv had the most labeled cells, and showed a small decline in the old animals. Ipsilateral VNLLd showed a modest decline in the middle-aged mice. The rest of the nuclei had low numbers of stained neurons that showed no age trends. C, contralateral; DNLL, dorsal nucleus of LL; I, ipsilateral; INLL, intermediate nucleus LL; LL, lateral lemniscus; VNLLd, ventral nucleus of LL, dorsal division; VNLLv, ventral nucleus of LL, ventral division.

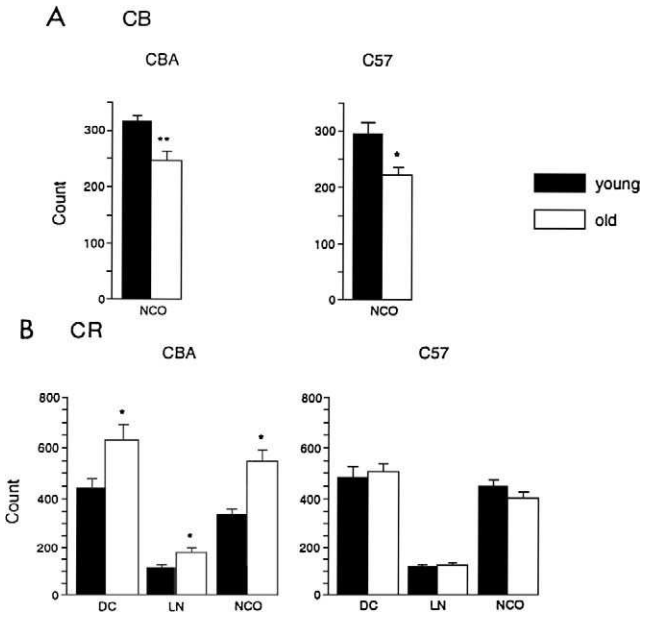

FIG. 39.16. (A) The average number of calbindin-immuneactive cells in the nucleus of the commisure (NCO) of the inferior colliculus (IC) of young (filled bars) vs old (unfilled bars) CBA and C57 mice. Error bars denote standard error of the mean. Calbinin-containing cells declined significantly in old mice of both strains. (B), The average number of calretinin-immuneactive cells in dorsal cortex (DC), lateral nucleus (LN), and NCO of young vs old CBA and C57 mice. Calretinin-containing cells increased significantly in all three areas in old CBA mice, but there were no significant differences between young and old C57 mice. ($^{*}P < 0.005$;$^{**}P < 0.01$). DC, dorsal cortex of inferior colliculus; LN, lateral nucleus of inferior colliculus; NCO, nucleus of the commisure of inferior colliculus. (From Zettel *et al*., 1997, reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

for peripheral locations within the auditory system, and thus persons with no apparent peripheral hearing loss may be assumed to be essentially free from traumatic insult. The second assumption was that animal models of presbycusis were available that captured the essential features of human presbycusis and would therefore allow us to differentiate central and peripheral neural effects on hearing in the aged.

Parallel human and animal multidisciplinary experiments on temporal processing were carried out to test these assumptions. Results from system-level experiments in humans suggested that reduced speech perception in background noise, characteristic of presbycusis, is likely caused by functional declines in the brain's temporal processing capacity. Further, manifestations of this decline were seen at the single-cell level in the auditory brain stem of experimental animals. In turn, associated structural and chemical changes in the same animals were found that could account for these functional declines. Future advances aimed at arresting, reversing, or preventing presbycusis are likely to rely upon pharmacological agents (medication or diet) and new developments in gene therapy.

Acknowledgments

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References

Amadeo, M., and Shagass, C. (1973). Brief latency click-evoked potentials during waking and sleeping in man. *Psychophysics* **10**, 244–250.

Burkard, R., and Hecox, K. (1983). The effect of broadband noise on the human brain-stem auditory evoked response. I. Rate and intensity effects. *J. Acoust. Soc. Am.* **74**, 1204–1213.

Burkard, R., and Hecox, K. (1987). The effect of broadband noise on the human brain-stem auditory evoked response. IV. Additivity of forward-masking and rate-induced wave V latency shift. *J. Acoust. Soc. Am.* **81**, 1064–1072.

Buus, S., and Florentine, M. (1985). Gap detection in normal and impaired listeners: The effect of level and frequency. *In* "Time Resolution in Auditory Systems" (A. Michelsen, ed.), pp. 159–179. Springer-Verlag, New York.

Committee on Hearing, Bioacoustics and Biomechanics (CHABA). (1988). Speech understanding and aging. *J. Acoust. Soc. Am.* **83**, 859–893.

Fitzgibbons, P. J., and Gordon-Salant, S. (1995). Age effects on duration discrimination with simple and complex stimuli. *J. Acoust. Soc. Am.* **98**, 3140–3145.

Frisina, D. R., and Frisina, R. D. (1997a). Speech recognition in noise and presbycusis: Relations to possible neural mechanisms. *Hear. Res.* **106**, 95–104.

Frisina, D. R., and Frisina, R. D. (1997b). Age alters free-field perception in noise for human listeners with normal audiometric thresholds. *Soc. Neurosci. Abstr.* **23**, 2069.

Frisina, R. D., Walton, J. P., Lynch-Armour, M. A., and Kotz, D. A. (1997). Efferent projections of a physiologically-characterized region of the inferior colliculus of the young adult CBA mouse. *J. Acoust. Soc. Am.* **101**, 2741–2753.

Frisina, R. D., Walton, J. P., Lynch-Armour, M. A., and Byrd, J. D. (1998). Inputs to a physiologically characterized region of the inferior colliculus of the young adult CBA mouse. *Hear. Res.* **115**, 61–81.

Frisina, R. D., Frisina, D. R., Karcich, K. J., Lockwood, A. H., and Salvi, R. (1999). Differential PET activation of brain regions of young and old subjects during speech recognition tasks in quiet and background noise. *Assoc. Res. Otolaryngol. Abstr.* **22**, 32.

Friston, K. J., and Frackowiak, R. S. J. (1991). Imaging functional anatomy. *In* ''Brain Work and Mental Activity'' (N. Lassen, D. Ingvar, M. Raichle, and L. Friberg, eds.), pp. 267–277. Munksgaard, Copenhagen.

Gates, G. A., Cooper, J. C., Kannel, W. B., and Miller, N. J. (1990). Hearing in the elderly: The Framingham Cohort, 1983-85. *Ear Hear.* **11**, 247–256.

Gordon-Salant, S. (1987). Age-related differences in speech recognition performance as a function of test format and paradigm. *Ear Hear.* **8**, 277–282.

Hecox, K., and Burkard, R. (1982). Developmental dependencies of the human brainstem auditory evoked response. *Ann. N.Y. Acad. Sci.* **388**, 538–556.

Ison, J. R., and Pinkney, L. A. (1983). Reflex inhibition in humans: Sensitivity to brief silent periods in white noise. *Percept. Psychophys.* **34**, 84–88.

Ison, J. R., Agrawal, P., and Frisina, D. R. (1998). An initial study of the beneficial effect of spatial separation of signal and noise on masking in the free field in the aged mouse, in comparison to that in the aged human listener. *Soc. Neurosci. Abstr.* **24**, 904.

Kalikow, D. N., Stevens, K. N., and Elliott, L. L. (1977). Development of a test of speech intelligibility in noise using sentence materials with controlled predictability. *J. Acoust. Soc. Am.* **61**, 1337–1351.

Lockwood, A. H., Murphy, B. W., Donnely, K. Z., Mahl, T. C., and Perini, S. (1993). Positron-emission tomographic localization of abnormalities of brain glucose metabolism in patients with minimal hepatic encephalography. *Hepatology* **18**, 1061–1068.

Møller, A. (1994). Neural generators of auditory evoked potentials. *In* ''Principles & Applications in Auditory Evoked Potentials'' (J. Jacobson, ed.), pp. 23–46. Allyn & Bacon; Boston.

Moore, B. C. J., and Glasberg, B. R. (1988). Gap detection with sinusoids and noise in normal, impaired and electrically stimulated ears. *J. Acoust. Soc. Am.* **83**, 1093–1101.

Moore, B. C. J., Peters, R. W., and Glasberg, B. R. (1992). Detection of temporal gaps in sinusoids by elderly subjects with and without hearing loss. *J. Acoust. Soc. Am.* **92**, 1923–1932.

Nilsson, M., Soli, S., and Sullivan, J. (1994). Development of the Hearing In Noise Test for the measurement of speech reception thresholds in quiet and in noise. *J. Acoust. Soc. Am.* **95**, 1085–1099.

Pichora-Fuller, M. K., Schneider, B. A., and Daneman, M. (1995). How young and old adults listen to and remember speech in noise. *J. Acoust. Soc. Am.* **97**, 593–608.

Picton, T., and Hillyard, S. (1974). Human auditory evoked potentials. II. Effects of attention. *Electroencephalogr. Clin. Neurophysiol.* **36,** 191–199.

Posner, M. I., Petersen, S. E., Fox, P. T., and Raichle, M. E. (1988). Localization of cognitive operations in the human brain. *Science* **240**, 1627–1631.

Schneider, B. A., Pichora-Fuller, M. K., Kowalchuk, D., and Lamb, M. (1994). Gap detection and the precedence effect in young and old adults. *J. Acoust. Soc. Am.* **95**, 980–991.

Sliwinski, M., Buschke, H., Kuslansky, G., Senior, G., and Scarisbrick, D. (1994). Proportional slowing and addition speed in old and young adults. *Psychol. Aging* **9**, 72–80.

Snell, K. B. (1997). Age-related changes in temporal gap detection. *J. Acoust. Soc. Am.* **101**, 2214–2220.

van Rooij, J. C. G. M., and Plomp, R. (1992). Auditive and cognitive factors in speech perception by elderly listeners. III. Additional data and final discussion. *J. Acoust. Soc. Am.* **91**, 1028–1033.

Walton, J. P., Frisina, R. D., Ison, J. R., and O'Neill, W. E. (1997). Neural correlates of behavioral gap detection in the inferior colliculus of the young CBA mouse. *J. Comp. Physiol. A* **181**, 161–176.

Walton, J. P., Frisina, R. D., and O'Neill, W. E. (1998). Age-related alteration in processing of temporal sound features in the auditory midbrain of the CBA mouse. *J. Neurosci.* **18**, 2764–2776.

Walton, J., Orlando, M., and Burkard, R. (1999). Auditory brainstem response forward masking recovery functions in older humans with normal hearing. *Hear. Res.* **127**, 86–94.

Willard, F. J., and Ryugo, D. K. (1983). Anatomy of the central nervous system. *In* ''The Auditory Psychobiology of the Mouse'' (J. F. Willott, ed.), pp. 201–304. Thomas, Springfield, IL.

Wingfield, A. (1996). Cognitive factors in auditory performance: Context, speed of processing, and constraints of memory. *J. Am. Acad. Audiol.* **7**, 175–182.

Young, L. S., and Fechter, L. D. (1983). Reflex inhibition procedures for animal audiometry: A technique for assessing ototoxicity. *J. Acoust. Soc. Am.* **73**, 1686–1693.

Zettel, M. L., Frisina, R. D., Haider, S., and O'Neill, W. E. (1997). Age-related changes in calbindin D-28k and calretinin immunoreactivity in the inferior colliculus of CBA/CaJ and C57BI/6 mice. *J. Comp. Neurol.* **386**, 92–110.

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Joseph P. Walton and Robert Burkard

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Neurophysiological Manifestations of Aging in the Peripheral and Central Auditory Nervous System

I. Introduction

The auditory system undergoes both age-related peripheral and central pathology. The former is obseved as a decline of sensitivity, while the latter is observed as alterations in neural processing in specific brain regions. Neurons can be selectively lost in a given brain region, and their interconnections reduced or severed. For example, large cortical neurons are lost and neuronal debris, plaques, and neurofibrillary tangles begin to appear. Synaptic connections, which provide the communication between neurons, are also degraded. These age-related structural changes certainly must affect the neural encoding of biologically relevant signals, including those involved in perception. In fact, one of the most devastating sensory deficits that accompany the aging process is the deterioration of auditory perception of biologically relevant sounds, clinically referred to as presbycusis.

Presbycusis is not only the most common communication problem among the elderly, it is also the most common auditory disorder in the entire population (Schoeborn and Marano, 1988). A common misconception is the belief that presbycusis solely involves dysfunction of the peripheral auditory system. Behavioral, neurophysiological and neuropharmacological evidence from human subjects and animal models of presbycusis indicate that there are age-related alterations in the central auditory system as well. This chapter explores the neurophysiological manifestations of aging in the peripheral and central auditory system at a microscopic and macroscopic level. Studies involving the recording from single brain neurons will provide the microscopic view, while auditory evoked potential (AEP) studies, which measure neural activity from populations of neurons, will provide macroscopic insight.

II. Animal Models of Presbycusis

One advantage of using AEPs to measure auditory function is that it enables investigators to compare data from animal models to data from human subjects using similar stimulus paradigms. In contrast, in order to investigate aging at the single neuron level one needs to incorporate animal models of presbycusis. Animal models are required because of the invasive nature of single nerve-cell electrophysiology. In addition, animal models offer several practical advantages to the study of age-related changes in the peripheral and central auditory systems, including short life spans and the ability to control extraneous variables that might alter auditory function, including noise exposure and susceptibility to middle-ear disease.

Not surprisingly, humans are not alone in suffering from age-related hearing impairment. All of the common small laboratory rodents, including rats (Krauter *et al*., 1981), mice (Henry and Chole, 1980; Henry, 1982), gerbils (Schmiedt *et al*., 1990), guinea pigs (Altman and Dittmer, 1972), and chinchillas (McFadden *et al*., 1999), show evidence of peripheral auditory dysfunction with age. The chinchilla and gerbil offer audiograms which are grossly similar to that of the human; however, both are outbred strains that exhibit wide variability in hearing loss and cochlear pathology as they age. In addition, the long life span of the chinchilla (12–20 years) makes it impractical for most age-related research (McFadden *et al*., 1999). Al-though the mouse has an audiogram that is displaced several octaves higher in frequency than the human, its lifespan of 2–3 years is optimal for aging research. Furthermore, where genetic control is required, more research devoted to the auditory system has been done on two strains of laboratory mouse, the CBA and C57Bl/6, than any other species. These two strains differ in the time course, severity, and cause of their age-related peripheral hearing loss (Willott, 1984, 1986; Willott *et al*., 1988).

The hearing of the CBA strain remains stable throughout much of its life and then gradually declines to a very mild loss by 24 months of age. The C57Bl/6 strain offers a unique control to the CBA strain in that it develops a rapid, genetically induced, progressive sensorineural hearing loss over the first 12 months of its life (Henry and Chole, 1980; Willott, 1986; Li and Borg, 1992). As is true for humans, the progressive

loss of sensitivity for high frequency tones in the middle-aged hearing-impaired C57BL/6 mouse can be ascribed to degeneration in the peripheral receptive end organ (Mikaelian, 1979). It is interesting that the severe high-frequency hearing loss seen in the middle-aged C57BL/6 mouse is accompanied by a reorganization of central afferent connectivity at the level of the auditory midbrain and cortex, as has been shown in a series of impressive studies (Willott, 1986; Willott *et al*., 1993). At present there is no evidence to suggest that a similar reorganization follows peripheral degeneration in the brain of old CBA mice (Willott *et al*., 1988; Walton *et al*., 1998).

In this chapter, we will first review what is known about age-related changes in the auditory system from single-unit studies. This, of course, will be limited to nonhuman animal models and it is also limited by the auditory areas studied. Specifically, the effects of age on single-unit processing have yet to be investigated in the cochlear nucleus, medial superior olive, all trapezoid body nuclei, medial geniculate body and auditory cortex. This will be followed by a review of aging effects on AEPs, which will include both human and animal studies.

III. Single Neuron Studies

A. Aging and Auditory Nerve Activity

Sound is converted from mechanical energy into neural impulses within the cochlea via a complex hydromechanical transduction mechanism. The central auditory system has available to it the spike discharge patterns of the entire population of auditory nerve fibers and sound features are encoded in the trains of auditory nerve fiber action potentials generated within the cochlea. An extensive body of work has shown that the entire population of auditory nerve fibers encode stimulus properties, both by variation in discharge rate and action potential timing. Therefore, any type of dysfunction in the normal cochlear transduction process will lead to changes in auditory nerve activity. For example, in the case of a cochlea that has a high frequency noise-induced sensorineural hearing loss, a region of the cochlear base is damaged and behavioral thresholds are elevated in the frequencies corresponding to the damaged area. When recording from single auditory nerve fibers that innervate this region, one finds that their thresholds at best frequency (BF; frequency at which a unit responds at the lowest intensity) are also elevated.

Nearly all of the work on the effects of aging on peripheral auditory function has been done by a group at the Medical University of South Carolina using the Mongolian gerbil as the animal model. To avoid problems with noise exposure from ambient environmental sources, animals were raised in a special noise abatement facility which limited the amount of external noise present in their cages. The South Carolina group's first studies with ''quiet-raised'' gerbils focused on the effects of age on intensity coding of auditory nerve fibers. Auditory nerve fibers encode the intensity of simple acoustic stimuli by systematically varying their discharge rate. Typically the change in discharge rate is plotted as a function of intensity to form a rate-level function. The slope of a rate-level function represents the degree of change in neural responsiveness that results from increases in stimulus intensity. Nearly all auditory nerve fiber rate-level functions asymptote at moderate to high sound intensities. Thus, a steep function indicates that a neuron is able to encode small changes in intensity very effectively. Hellström and Schmiedt (1991) collected auditory nerve fiber rate-level functions and whole-nerve cochlear action potential amplitude-level functions from young and old gerbils. The whole-nerve evoked response represents the population response of many auditory nerve fibers. They reported that the slopes of the functions of the whole-nerve amplitude versus stimulus intensity were shallower in aged gerbils. However, no age effect was found when they compared the slopes of auditory nerve fiber rate-level functions from young and old animals, with slopes ranging from 2 to 25 discharges/dB, regardless of an animal age or a fiber's BF.

Hellström and Schmiedt (1996) also found that minimal age-related changes occurred in the filtering properties of auditory nerve fibers recorded in quiet-aged gerbils. They compared the slopes and suppression areas of tuning curves from auditory nerve fibers in young and old gerbils and found that only the tip-to-tail ratios were smaller and that high-frequency selectivity decreases in units from aged animals. This is directly related to the high frequency loss being greater than the low frequency loss, resulting in a slight elevation in the tip of the tuning curve. No differences were found in the slopes of the tuning curves or in the shape or size of the suppression areas.

Auditory nerve fibers have relatively high spontaneous activity compared with other neurons in the central auditory system. A fiber's spontaneous rate (SR) is the discharge rate observed in the absence of external stimulation. A classification scheme used in many studies separates the auditory nerve fiber population into two classes: low (<18 spikes/sec) and high ($\geq$18 spikes/sec) SR fibers. Low and high SR fibers also differ in other important characteristics. High SR fibers tend to have low acoustic thresholds, steep slopes of rate-level functions, and saturating rate-level functions. Low SR fibers have higher thresholds, shallower rate-level slopes, and, most importantly, low SR fibers tend to encode amplitude modulation, or the rapid fluctuation in stimulus intensity, very well (Winter *et al*., 1990; Joris *et al*., 1994; Frisina *et al*., 1996). Therefore, it is thought that low SR fibers could play an important role in recovery from masking to high intensity maskers because of their expanded dynamic range and exquisite temporal processing abilities. Schmiedt *et al*. (1996) found an age-related decline in the proportion of auditory nerve fibers which had low spontaneous activity. Figure 40.1 shows the effects of age on the distribution of SRs in young and old gerbil auditory nerve fibers for two different best frequency regions, above and below 6 kHz. In young gerbils, more low SR fibers (dashed bars) have BFs above 6 kHz than below 6 kHz. In contrast, a similar proportion of high spontaneous rate auditory nerve fibers have BFs above and below 6 kHz, regardless of age. However, in old gerbils the proportion of low SR fibers with BFs above 6 kHz decreased by about 30%, which was significantly different when compared to that seen in young animals.

The South Carolina group's research findings concerning age-related changes in driven activity of auditory nerve fibers can be summarized as follows: (1) auditory nerve fiber responses in aged gerbils generally reflect changes in cochlear thresholds, (2) auditory nerve intensity and frequency coding are affected, but only mildly, and (3) there appears to be an

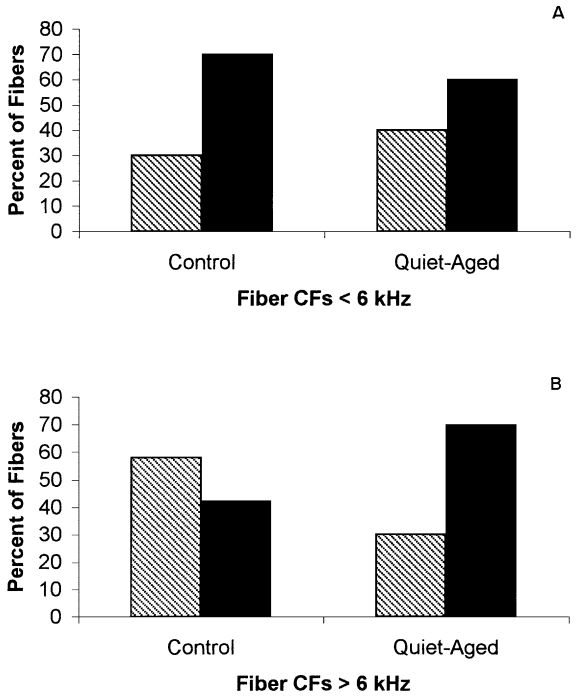

FIG. 40.1. Effects of age on spontaneous discharge rates (SRs) of auditory nerve fibers. The proportion of auditory nerve fibers encountered with low (<18 spikes/sec) and high (>18 spikes/sec) SRs for fibers with characteristic frequencies (CFs) below 6 kHz are shown in (A), while those with CFs above 6 kHz are shown in (B). Low SR fibers are indicated by the hatched bars and high SR fibers are indicated by the dark bars. For fibers with CFs less than 6 kHz the proportion of low and high SR fibers is similar. However, for fibers with CFs above 6 kHz the proportion of low SR fibers decreases and the number of high SR fibers increases in the aged animals. Adapted from Schmiedt *et al*. (1996), with permission of the author and publisher.

age-related decline in the number of auditory nerve fibers that have high thresholds and low spontaneous rates. An age-related factor in the maintenance of the endocochlear potential appears to be the underlying source of the deficits reported by the Schmiedt and coworkers. The endocochlear potential is a positive steady state potential which is generated in stria vascularis and provides the necessary driving potential for the transduction process. Numerous studies have shown that normal hearing depends on a normal endocochlear potential. Concurrently with the auditory nerve studies, Schulte and Schmiedt (1992) reported a reduction in the amplitude of the endocochlear potential with age and that the reduction is secondary to a decline in the ion transporting enzyme Na,K-ATPase located in stria vascularis (Gratton *et al*., 1995, 1996). Their data suggest that the drop in the endocochlear potential is directly related to decreased levels of Na,K-ATPase activity. They concluded that all of the findings related to auditory nerve activity could be explained by a systematic decrease in the endocochlear potential with age (Gratton *et al*., 1997).

B. Aging and the Superior Olivary Complex

The superior olivary complex is the first major convergence point for binaural information in the central auditory system. The major superior olivary complex nuclei involved in processing ascending information are the lateral superior olive (LSO), medial superior olive (MSO), and medial nucleus of the trapezoid body. Neurons in the LSO and MSO receive, and compare inputs from both ears following processing by the cochlear nuclei. In the LSO, when a sound arises from the ipsilateral ear the neural input is excitatory, and when the sound arises from the contralateral ear the input is inhibitory. Only one study has investigated age-related changes in single unit responses to binaural stimuli in the LSO (Finlayson and Caspary, 1993). Previous neurochemical studies had reported age-related alterations in inhibitory neurotransmitters in brain stem nuclei involved in binaural processing (Caspary *et al*., 1990, 1995; Milbrandt *et al*., 1994). Finlayson and Caspary (1993) hypothesized that this should lead to deficits in the ability of neurons to encode binaural signals, since neural processing of binaural inputs in the LSO is shaped by the interplay of excitatory and inhibitory circuits arising from each ear. To test this hypothesis, they recorded from single neurons in the LSO from young and old Fisher 344 rats and found that other than the characteristic age-related threshold shift nearly all other parameters studied remained stable with age. Although differences between young and old rats did not reach statistical significance, a greater proportion of neurons from old rats exhibited lower inhibitory drive when the contralateral ear was stimulated. This trend is supportive of the idea that a decline in inhibition may reduce the contrast in binaural signals, resulting in the perceptual deficits observed in elderly listeners.

C. Aging and the Auditory Midbrain

The auditory midbrain, or inferior colliculus, represents the first major convergence of both binaural and monaural neural information in the auditory system. The cytoarchitectonics of the central nucleus of the inferior colliculus (ICc) is remarkable, in that neurons form laminar sheets analogous to the layers of an onion. Each sheet is formed by the perikarya and processes of principal, or disc-shaped neurons whose dendrites extend outward in a circular fashion. A second type of ICc neuron is a stellate cell with dendrites that extend across the laminar sheets, an arrangement that is thought to allow for parallel processing within and across laminae. The tonotopic organization of the ICc is formed by the individual laminae, with each lamina representing a narrow range of frequencies. If an electrode is passed through the middle of the inferior colliculus and BFs are sampled, low frequencies are represented dorsally and high frequencies ventrally.

Sophisticated neural processing occurs within each lamina. For example, Langner and Schreiner (1988) found that amplitude modulation was mapped in a systematic fashion onto individual laminae. A similar systematic map is also found for sound intensity, where the most sensitive neurons (lowest thresholds) are located near the center of an isofrequency lamina and neurons with increasingly higher thresholds fall on concentric isointensity contours around the center (Stiebler and Ehret, 1985). Thus, the characteristics of the neuronal architecture within each lamina could provide a basis for parallel processing of complex acoustic signals, so that multiple sound features (e.g., frequency, intensity, amplitude modulation, etc.) could be analyzed simultaneously.

Willott was the first to examine age-related changes in basic response properties of inferior colliculus neurons in the CBA mouse model of presbycusis (Willott *et al*., 1988). Willott observed only minor age-related changes in responses properties of 2-year-old CBA/J mice. Multiple-unit recordings revealed a slight reduction in sensitivity across all frequencies, and a maintenance of the ICc's tonotopic organization and of the Q_{10}s (sharpness of threshold tuning curves) in 22-month-old mice. In old mice, although single units in the ICc showed no alterations in their temporal response patterns to tonal stimuli and were generally responsive to suprathreshold sounds, there were several signs of more subtle aging dysfunction. There was an increase in the number of so-called "sluggish" units, or neurons which responded with fewer than 50 action potentials for the entire recording period, regardless of sound intensity. Sluggish neurons were found to increase with age, comprising 3% of the neuronal population in the young inferior colliculus, but increasing to 22% in the old inferior colliculus.

Willott and colleagues also discovered the connection between altered neural processing in aged inferior colliculus neurons and their topographic location. Specifically, while no age-related change was noted in spontaneous activity across the whole inferior colliculus, when the dorsal (low frequency) and ventral (high frequency) regions of the inferior colliculus were analyzed separately, an age-related difference emerged. In the dorsal inferior colliculus the proportion of neurons which were spontaneously active decreased with age, while in the ventral inferior colliculus the number of units increased with age. Several parameters of single-unit response areas were measured, including BF, upper and lower frequencies to which a unit was responsive at 80 dB SPL and BF for rate (the frequency to which the unit exhibited the greatest number of spikes). Figure 40.2 summarizes these results and shows that there was a decrease in the upper frequency to which units from old mice would respond. This implies that the frequency selectivity of these units had been altered without affecting threshold or rate BF.

With respect to binaural processing in the inferior colliculus, the initial combination of signal processing by the two ears occurs at the level of the SOC, and further processing takes place in the nuclei of the lateral lemniscus (NLL). The targets of SOC and NLL neurons project to inferior colliculus neurons with similar BFs. Palombi and Caspary (1996a) measured binaural response properties for inferior colliculus neurons in young and old rats. Because binaural responses are shaped by the summation of excitatory and inhibitory inputs, Palombi and Caspary hypothesized that if there were an age-related alteration in inhibitory neurotransmitters, binaural processing would be affected. Surprisingly, they found no statistically significant differences in any of the binaural responses properties, for units from young and old rats. The authors did report a trend toward encountering fewer E/I units in the aged rat inferior colliculus than in the young rat inferior colliculus.

These same authors published a follow-up study that investigated the effects of age on monaural response properties to simple sinusoidal stimuli in the Fisher 344 rat (Palombi and Caspary, 1996b). The temporal response patterns of neurons located in the central nucleus and external nucleus of the inferior colliculus were classified into four groups: onset, pause, sustained, and inhibitory (Fig. 40.3). In the central nucleus

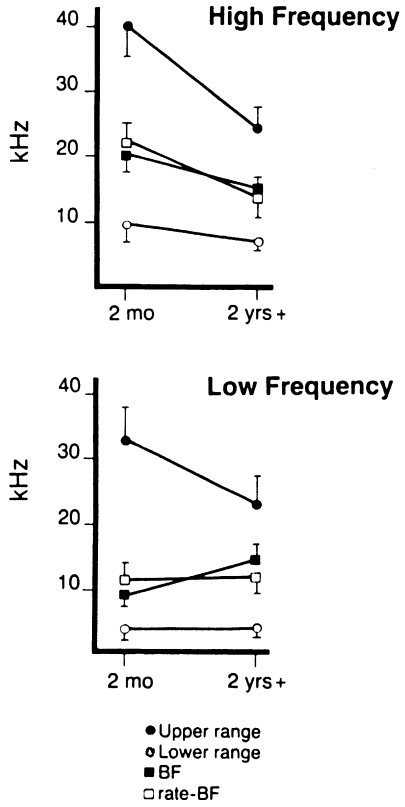

FIG. 40.2. Characteristics of excitatory tone response areas in young (2-month-old) and old (>24-month-old) CBA mice for neurons encountered in the dorsal (low-frequency) and ventral (high-frequency) regions of the central nucleus of the inferior colliculus. The upper (•) and lower boundaries (○) represent the edges of the low- and high-frequency sides of the response area. The best frequency, (BF, ■) represents the frequency–intensity combination at threshold. The rate-BF (□) is the frequency at which the strongest response occurred. Note that in both the high- and the low-frequency regions of the inferior colliculus the upper boundary of the excitatory response area significantly decreases in the aged animal. Error bars represent standard error of the mean. From Willott *et al*. (1988), with permission of the author and publisher.

there was a decline in the proportion of units with onset type patterns with advancing age, and this appeared to be compensated for by a slight increase in pause and sustained patterns. Overall, aging did not appear to affect the proportion of the different types of temporal response patterns encountered in the external nucleus. They also found no effect of age on the spontaneous rates and no change in the mean absolute first spike latency of inferior colliculus neurons from old rats when compared to young rats. Aged neurons did appear to be affected in the manner in which stimulus intensity was encoded. Specifically, there was a decline in the percentage of units displaying nonmonotonic rate-level functions. The mean maximum discharge was significantly lower in aged neurons, decreasing over 11%. The authors conclude that these age-related changes reflect a subtle, but real, deficit in the dynamic range of many neurons recorded from old rats. They postulate that the alteration in dynamic range might be due to a loss of inhibition, especially at higher intensities.

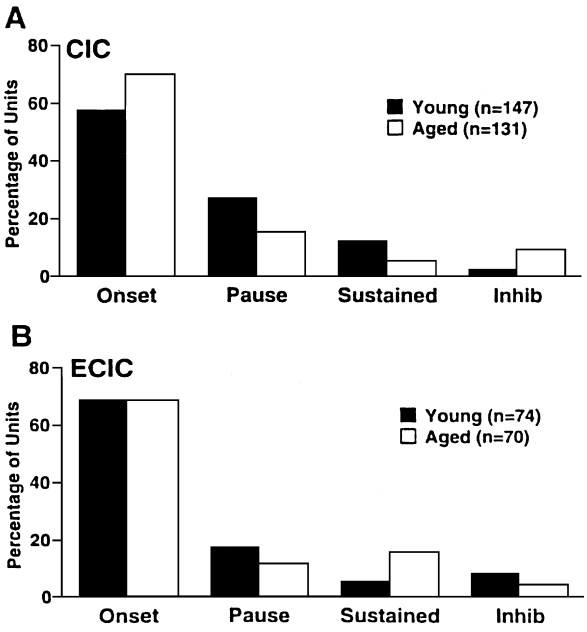

FIG. 40.3. Proportion of inferior colliculus neurons which were classified into the four response patterns: onset, pause, sustained, and inhibitory, in young and old Fisher 344 rats. The data are derived from the central (top) and external (bottom) nuclei of the inferior colliculus. The number of units from young and old rats are shown in each panel. There was a slight increase in proportion of onset units in the aged rat, which was offset by a slight decrease in pausers and sustained units. From Palombi and Caspary (1996b), with permission of the author and publisher.

D. Age-Related Deficits in Neurophysiological Correlates of Temporal Processing

Birren and Fisher (1995) characterized aging as a generalized slowing in the speed of behavior. The speed at which the auditory system processes incoming acoustic signals can be measured by several temporal processing tasks, one of which is gap detection. Gap detection requires the listener to detect brief silent intervals in an ongoing sound, usually a wideband noise (Plomp, 1964; Green and Forrest, 1989). Since one of the key factors contributing to poor speech recognition in elderly listeners is a deficit in temporal resolution, neurophysiological correlates of gap detection may provide evidence of a central auditory temporal processing component (Schneider *et al*., 1994; Fitzgibbons and Gordon-Salant, 1996; Snell, 1997). Walton *et al*. (1997) explored the relation between behavioral gap detection and neural correlates of gap detection in IC neurons recorded from the same young-adult mice, as a preface to investigating temporal processing in aged mice. This study addressed a common question in research which aims to compare behavioral and neurophysiological data: That is, can the neurophysiological representation of a given stimulus underlie the behavioral response?

In the Walton *et al*. (1997) study, behavioral gap detection was measured using the startle inhibition paradigm (see Chapter 39). This was followed by single-neuron recordings in the inferior colliculus using a similar gap detection paradigm. Behaviorally, increasing the gap duration results in an increase in the strength of startle inhibition, i.e., the perceptual salience of the gap increases. This is illustrated by the filled circles in Fig. 40.4, which represent the magnitude of startle inhibition plotted as a function of gap duration. A value of 1.0 represents the startle amplitude when there is no gap preceding the startle stimulus. As the gap duration increases, reflex inhibition increases and the startle amplitude decreases, with the function reaching an asymptote at about 6 msec. In order to compare the neural data to the behavioral data, two types of units which differ in their temporal response pattern were analyzed: onset and primary-like. Onset type neurons are characterized by their robust response to the onset of a stimulus, after which they either cease responding or show only a slight response for the duration of the stimulus. In contrast, primary-like units respond strongly to the onset and continue to respond vigorously throughout the remainder of the stimulus. Onset units encode gaps in ongoing stimuli by their onset response to the second noise burst, while primary-like units encode gaps by a complete cessation of activity during the gap. The neural data were normalized by computing the change in response strength evoked by the gap to the response of a control stimulus (no gap). The mean change in normalized discharge rate to

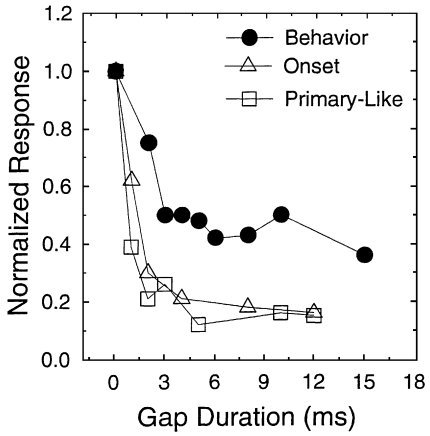

FIG. 40.4. Data comparing behavioral gap detection and neural encoding of gaps by inferior colliculus neurons in young CBA mice. Behavioral gap detection was measured using the startle inhibition paradigm. A value of 1.0 for the normalized response represents the control condition, or no gap, and maximum startle amplitude. As the gap duration increases the startle amplitude decreases and the function becomes asymptotic at about 6 msec. Neural encoding of gaps is shown for two different unit types, onset and primary-like. Phasic type neurons typically have very low background activity and discharge at the onset of each noise burst that marks the gap, while primary-like units discharge continuously to both noise bursts which mark the gap and stop discharging during the gap. In order to compare the startle data to the neural data, each unit's response magnitude was normalized by computing changes in spike counts elicited in a control condition, which is represented by a value of 1.0. As the gap duration exceeds the gap threshold, phasic units exhibit rapid increases in the number of discharges evoked by the second noise burst. Although the absolute asymptotes differ slightly between the behavioral and neural data, the time course of the behavioral and neural response are remarkably similar. All are characterized by a rapid change in response magnitude for gap widths up to 3 msec, and then asymptote for gaps longer than 4 msec in duration. From Walton *et al.* (1996), with permission of the author and publisher.

gaps at various durations is shown for onset units (triangles) and primary-like units (squares) for gap durations of up to 15 msec. Although the absolute asymptote differs slightly for the behavioral and neural data, the shapes of all three functions are remarkably alike. All three functions are characterized by a rapid change in response magnitude for gap widths up to 3 msec, and then asymptote for gaps longer than about 4 msec in duration.

The major difference in terms of gap encoding between onset and primary-like neurons is the background signal-to-noise level from which they operate in order to signal the presence of the gap. Onset neurons typically have very low spontaneous or ongoing activity and discharge at the onset of each noise burst marks the gap. In contrast, primary-like units respond vigorously before and after the gap. It is important to note that at the level of the inferior colliculus and auditory cortex the majority of neurons display onset discharge patterns in response to noise bursts (Irvine, 1986). Onset neurons, with their low spontaneous and sustained discharge rates, appear to be optimally suited for gap detection. The central targets of onset units would receive an unambiguous neural signal, time locked to the onset of the second noise burst marking the gap. In addition, most onset units display a rapid exponential increase in spikes as gap width increases from 1 to 10 msec (Fig. 40.4). This results in a very high probability of discharge, which can approach >1 spike per stimulus. If correlation across many onset units could be used for gap detection, then this would provide one central neuronal population mechanism for gap encoding. In summary, neurophysiological correlates of an auditory temporal processing task (gap detection), which appears to be related to presbycusic deficits, are present in the discharge patterns of the majority of inferior colliculus neurons in the CBA mouse.

Walton *et al*. (1998) systematically compared response properties and neural gap detection ability in inferior colliculus neurons of young and old CBA mice. Two questions concerning age-related changes in temporal acuity were asked. First, is the ability of the auditory midbrain to encode temporal features of sound (e.g., gaps) affected by age? Second, are there concomitant age-related changes in other single-unit response properties that could underlie behavioral gap detection? Neurons were studied using stimuli that were 20 dB or more above the specific units' threshold, and no correlation was found between threshold to tone or noise and gap threshold. The data in answer to the first question are shown in Fig. 40.5, indicating that aging does have an affect on neural gap detection. The frequency distributions shown in Fig. 40.5 represent single unit minimal gap thresholds of neurons from young and old CBA mice. Although short gap thresholds of 1 msec were observed in units from both young and old animals, the frequency of occurrence of thresholds of 1 msec was much lower in old CBA mice. More specifically, roughly 50% fewer neurons had gap thresholds of 2 msec or less in the inferior colliculus of old CBA mice relative to units from young mice. This magnitude of neuronal decline in temporal acuity parallels the behavioral deficit observed in gap detection studies in old mice (see Chapter 39).

Walton *et al*. (1998) also noted a second, more striking age-related difference, which is illustrated in the gap recovery functions of Fig. 40.6. They found that the majority of neurons

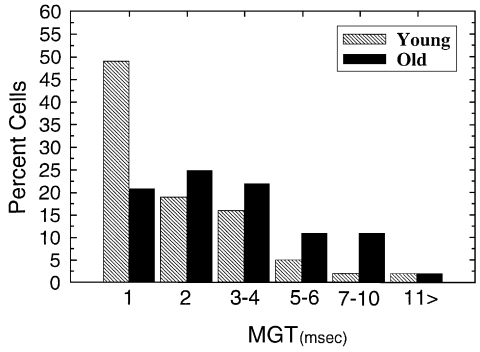

FIG. 40.5. Comparison of neural gap thresholds from IC units in young ($n = 78$) and old ($n = 108$) CBA mice. The proportion of units with the shortest gap thresholds decreases by about 50% in the old mice. This is offset by an increase in the number of units having longer gap thresholds of 2 msec or more in the old animals. From Walton *et al*. (1998), with permission of the author and publisher.

with onset response patterns displayed a deficit in neural recovery. Neural recovery can be quantified by comparing the spike counts to the first and second stimuli in the gap-detection paradigm. In Fig. 40.6 (top), gap recovery functions from young mice display rapid recovery, that is, the majority of units increase their spike counts to near control values (1.0) for gaps of about 10 msec. In contrast, in many neurons from old animals, the responses typically failed to recover to 75% of control values by 10 msec. It is also worthwhile to note that many units from young mice show enhancement or facilitation, where the response to the second stimulus exceeds the control response (response greater than 100%). This rarely occurred in neurons from old mice.

Another metric of neural temporal resolution is the mean first-spike latency, or the time it takes to elicit the first spike for each stimulus presentation. In the young CBA mouse inferior colliculus, the distribution of first-spike latencies is rather broad, spanning from several milliseconds to over 50 msec. The latency gradient runs along the tonotopic axis, with neurons having long first-spike latencies lying dorsally and those units with the shortest latencies located ventrally. Therefore units with low BFs should have long first-spike latencies and high BF units should have short first-spike latencies (Stiebler and Ehret, 1985; Willott, 1986; Walton *et al*., 1998). Mean first-spike latency was computed for the control stimulus for both young and old mice and is plotted as a function of BF in Fig. 40.7. As expected, response latency for units from young mice (top) declines with increases in BF. In contrast, for neurons from aged mice (bottom) the dependence of mean first-spike latency on BF is much less apparent, even though the distributions of first-spike latencies are similar (two-sample t test, $P > 0.05$). Furthermore, note that the gradient of shortest response latencies across BF is readily apparent in units from young mice (top, dotted line). That is, the shortest latencies are associated with the highest BFs. This gradient appears to be absent in the first-spike distribution for the old mice. Neurons with short first-spike latencies occur for both low (<10 kHz) and high BF units. This does not appear to be due to the lengthening of latencies in high-frequency units as one might expect, but rather to the shortening of laten-

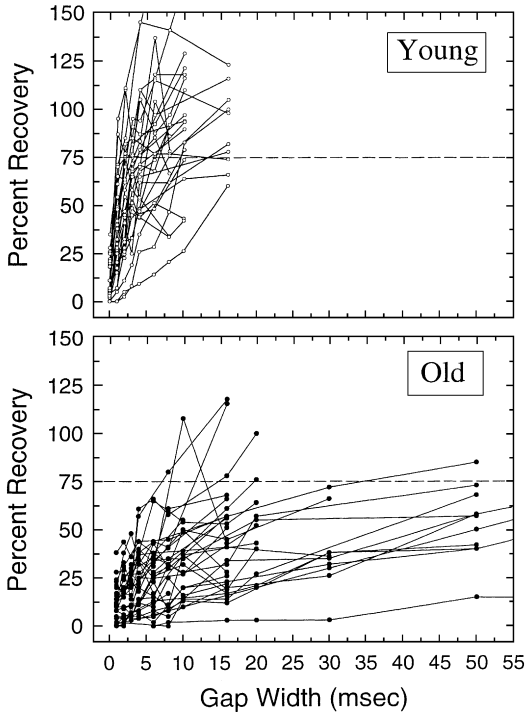

FIG. 40.6. Neural recovery functions derived from the response to gaps of various durations plotted for 30 onset units in young (top) and old (bottom) CBA mice. Neural recovery was quantified by computing the number of spikes elicited by the second noise burst marking the gap and then dividing that number by the number of spikes elicited by the first noise burst $\times 100$. Therefore, a recovery value of 100% would represent equal discharges to both the first and the second noise-burst markers. This calculation was completed for every histogram in the gap series. The dashed horizontal lines represent 75% recovery. Note that recovery to the 75% criterion is complete for gaps of 10–15 msec or shorter in nearly every neuron from the young animals, whereas most neurons from old animals do not reach this criterion even for gap durations longer than 20 msec. Also, facilitation (gap response is greater than control response) was discovered for units in young animals, but was rarely observed in units from old animals. From Walton *et al.* (1998), with permission of the author and publisher.

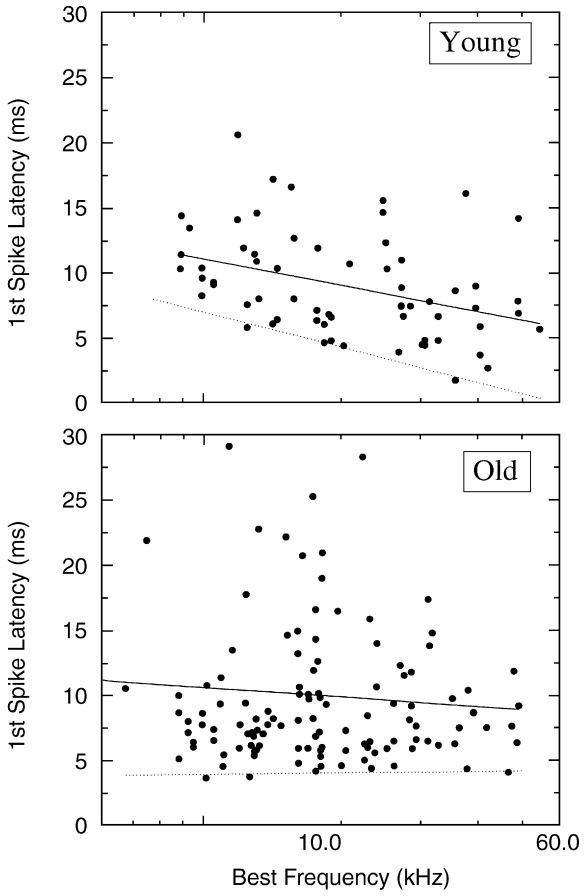

FIG. 40.7. Comparison of the mean first spike latency distributions and regression analyses plotted as a function of BF for young (top) and old (bottom) units. Response latency measures were derived from the control gap condition, a noise burst presented at 65 dB SPL. In order to measure only spikes evoked by the signal, the analysis period was restricted to the first 25 msec following response onset for onset units. The measurement window was increased to 50 msec for sustained and primary-like units. Response latencies ranged from 3 to 22 msec in the young distribution and 3.3 to 28 msec in the old distribution. The results of the linear regression analysis are shown by the solid lines, whereas the dotted lines (fit by eye) highlights the gradient of shortest first spike latencies in both young and old distributions. See text for additional discussion. From Walton *et al.* (1998), with permission of the author and publisher.

cies in low frequency units. The spread of latencies among neurons with similar BFs either remained unchanged (high BFs) or slightly expanded with age (low BFs). Shorter first-spike latencies in neurons from old CBA mice is consistent with reduced inhibition in old CBA mice.

The finding that aging differentially affects the representation of first-spike latencies (presumably) along the tonotopic axis, but not the spread of latencies within isofrequency slabs, suggests that there are at least two mechanisms influencing latency in the inferior colliculus. One mechanism establishes a latency gradient for determining the shortest latencies along the tonotopic axis, and it is this factor that is affected by age. The second factor establishes a latency spread within each isofrequency sheet, and this representation of response latency appears to be preserved with age, at least for high BF units. One other metric of response latency, the variance of the first-spike latency, was analyzed and remained stable with age. Previous reports indicate that variance in first-spike latency from inferior colliculus units should increase with the mean first-spike latency (Langner and Schreiner, 1988). Walton *et al.* also found that this relationship holds true for units from both young ($r^2 = 0.35$), $P < 0.001$) and old ($r^2 = 0.52$), $P < 0.001$) animals.

To summarize results from auditory midbrain studies, key findings from single cell recordings across multiple laboratories suggest that both intensity and temporal processing are disrupted in the inferior colliculus of the old rodent. Although Caspary's group found no significant decline in inferior colliculus binaural response properties with age, they did find subtle changes in other response measures, such as spontaneous activity and changes in the ratio of monotonicity. Walton's group has demonstrated the occurrence of an upward shift in the distribution of gap thresholds with age, increased recov-

ery from prior stimulation, as evidenced in increased recovery times in neurons from old mice, and alterations in the normal topographic map of first-spike latency in old mice. What are the implications of these findings? First, it must be reiterated that the temporal coding deficits noted above are not associated with global changes in all inferior colliculus neurons in aged animals; i.e., some neurons are spared. Whatever mechanisms are responsible for the alteration in temporal coding, they are acting on specific neurons. Second, the magnitude of the age-related deficits by single units using the gap detection paradigm are on the order of those reported in human listeners. Schneider *et al.* (1994), for example, found that mean gap thresholds were 1 to 1.5 msec greater in older subjects, with near-normal audiometric thresholds when compared to that seen in the young adult control group. Finally, many of the neurophysiological observations fit within the framework of the loss of inhibition model. Previous work has shown that both inferior colliculus single-unit recovery functions and topographic latency gradient present in the inferior colliculus can be modified by blocking GABAergic and glycinergic circuits (Park and Pollak, 1993a,b). Age-related alterations in single neuron recovery and the map of latency in single neuron recordings could be a reflection of an age-related imbalance in excitation and inhibition required for normal cellular function.

E. Summary of Single-Unit Studies

The challenge of exploring the effects of aging on auditory neural processing is made difficult because of the auditory system's enormous complexity. For the most part, the mechanisms which alter neural processing in the majority of nuclei of the central auditory nervous system remain unknown. Yet we believe that both degradations in the central nervous system and the sensorineural hearing loss that accompanies the aging process are responsible for the decrements in speech perception of the elderly listener in quiet and background noise. If therapeutic interventions are to be developed, we must continue to investigate how auditory processing at the cellular level is affected by age. The results presented in this portion of the chapter indicate that many age-related alterations in auditory processing are present in neurophysiological measures. In future studies, neurophysiological investigations must be complemented by neuroanatomical, pharmacological, and molecular approaches in order to elucidate the cellular machinery responsible for the behavioral deficits related to presbycusis.

IV. Effects of Aging on Auditory Evoked Potentials

A. Introduction

Unlike the single-unit electrophysiology measures summarized earlier in this chapter, auditory evoked potentials (AEPs) can be recorded noninvasively and thus can be recorded in both humans and in nonhuman mammals. The advantage of human studies concerning the physiological manifestations of aging is that the results are directly relevant to human aging. Furthermore, they provide a physiologic measure of auditory function that can be compared to perceptual measures of auditory function, ranging from tonal thresholds to the processing of speech in a noise background. The study of human AEPs, in isolation, does have several disadvantages. First, it is not typically possible to perform parallel physiologic and anatomical studies. Thus, we cannot verify, for example, hair cell loss at the time that the physiologic measures were obtained. Second, humans can live upward of 100 years, and hence longitudinal studies of aging are generally impractical, and cross-sectional studies are required. For these cross-sectional studies, we need to match for myriad factors, the most important of which are gender and hearing loss. Third, humans do not exist in a controlled environment, and hence many uncontrollable factors influence the effects of aging on the auditory system (e.g., noise exposure, administration of ototoxic agents, diet). The study of animal AEPs allows combined physiologic and anatomic study. In addition, many species of rodents have life spans of about 3–5 years, thus allowing longitudinal studies if these are deemed optimal for a specific experiment. The use of animals allows much greater control of both genetic and environmental factors than is possible in human studies. Finally, one can use animal models for physiologic studies using both invasive (e.g., single unit measures) and noninvasive (e.g., AEP measures). Once the relationships, between AEP and single-unit changes in the aging animal model are understood, it becomes possible to study human AEP in aging. If the animal and human AEP changes with aging are similar, then we can generalize the results of the invasive animal measures to the human aging auditory system.

In the present review of aging and AEPs, we will present both human and nonhuman work, as these parallel bodies of work are complementary. When the human and animal studies show similar changes with aging, we can conclude that the particular animal species is a good animal model for the aging human auditory system. Where possible, we will present the hearing status of the animals, as audiometric threshold in young adults can influence AEPs, and we would like to separate the effects of sensorineural hearing loss from age-related changes, per se. This process is difficult for several reasons. First, the hearing status of humans/animals in these studies is investigated or reported with varying degrees of rigor. Some studies simply report that they have ''normal hearing'' or reported ''no hearing difficulties.'' Others closely match the hearing thresholds of young and old subjects. The latter approach may seem optimal, but it appears that substantial inner hair cell loss can occur in the presence of normal hearing thresholds (Trautwein *et al.*, 1996; Qiu *et al.*, 2000). In addition, distortion product otoacoustic emissions (DPOAEs) are not only within the normal range, but may actually be larger than normal in ears with inner hair cell lesions (Jock *et al.*, 1998). Although normal threshold does not guarantee a completely normal auditory periphery, threshold does remain the primary clinical measurement of hearing.

B. Overview of Auditory Evoked Responses

Following presentation of an acoustic stimulus, a group of potentials can be recorded that reflect excitation from cochlear hair cells, auditory nerve fibers, and the nuclei and tracts of the auditory nervous system. If one places an electrode in or near the cochlea of the inner ear, one can record a series of poten-

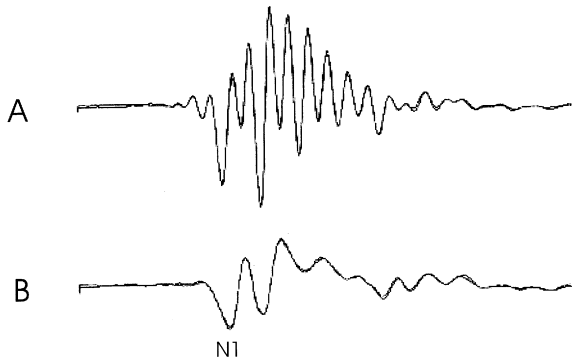

FIG. 40.8. Responses recorded with a round window electrode in the chinchilla. (A) An 80 dB pSPL fixed-phase 2 kHz tone burst is the stimulus, and the cochlear microphonic (CM) is seen as a series of potentials following the stimulus microstructure. (B) An 80 dB pSPL alternating-phase tone burst is the stimulus, and the CM has been cancelled, leaving the compound action potential. In each waveform, the time base is 12.8 msec. Tone-burst envelope is 2 msec rise time, (cosine^2), 1 msec plateau, presented at 21 Hz. Each response is the average of 100 stimulus presentations, and two waveforms are superimposed for each condition.

tials that are generated by the hair cells and auditory nerve fibers. One such recording, obtained from a round window electrode of a chinchilla, is shown in Fig. 40.8. Three potentials can be recorded. One is called the cochlear microphonic (CM), which is thought to primarily reflect outer hair cell function (Trautwein *et al*., 1996). The CM has the same frequency as the activating stimulus (in the case of Fig. 40.8A, this frequency is 2000 Hz). The summating potential (SP) is a DC potential that follows the envelope of the stimulus and appears to represent both inner and outer hair cell activity (Durrant *et al*., 1998). Otoacoustic emissions (OAEs) are also responses that appear to come predominantly from outer hair cells (Trautwein *et al*., 1996) and are recorded noninvasively from the ear canal with a sensitive microphone. Finally, the eighth nerve response, as recorded from the round window, can be seen as a series of one or more negative deflections and is called either the compound action potential (CAP) or the whole-nerve action potential (WNAP). The first negative peak of the CAP or WNAP is typically labeled N_1, as shown in Fig. 40.8B. Using scalp electrodes, a series of up to perhaps seven peaks can be measured within 10 or so msec following the onset of a moderate to high level click stimulus. This response is called (among other names) the ABR. As the name indicates, the generators of this response are (mostly) located in the auditory brain stem. The vertex-positive peaks are typically labeled with capital Roman numerals. An example of a human ABR is shown in Fig. 40.9, with several of the more prominent peaks appropriately labeled.

Middle latency responses (MLRs) are a series of potentials whose latencies extend out to approximately 70–80 msec. The peaks are labeled by a P or an N (indicating whether they are vertex positive or vertex negative), subscripted by a, b, or c (although several authors have reported a positive wave occurring before P_a, and label it P_0). An example of MLRs obtained from a human subject is shown in Fig. 40.10. A series of potentials with latencies extending up to 500 msec or more are referred to as event-related potentials (ERPs). These potentials

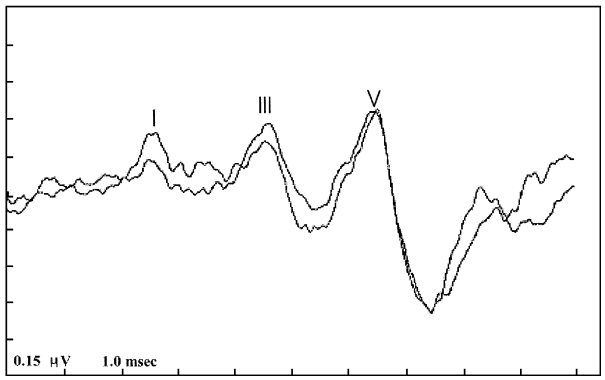

FIG. 40.9. The auditory brain-stem response (ABR) from one human subject is shown. Each response is the averaged response to 2000 presentations of an 80 dB nHL alternating-polarity click stimulus presented at a rate of 21 Hz. The time base is 10 msec and two responses are superimposed.

as a group are dependent on both the stimuli used (e.g., frequency, level, rate) and on subject state (e.g., attention and arousal). The slow vertex potential (SVP) is a series of peaks that can be recorded to a simple transient stimulus, and consists of a series of positive and negative peaks (labeled by "P" or "N"), and subscripted with either increasing peak number (e.g., N_2) or by the average latency of the peak (e.g., N_{200}). An example of the SVP is shown in Fig. 40.11A. Other peaks (such as the P_3 or the mismatch negativity, MMN) must be obtained by more complex stimulus paradigms, such as the oddball paradigm. In the oddball paradigm, a frequent or standard stimulus is presented most of the time (say, 85%), while a rare or deviant target stimulus is randomly presented occasionally (e.g., 15%). The responses to the frequent and rare stimuli are averaged separately. In the example shown in Fig. 40.11, the subject was asked to count the rare stimuli, and a response is seen in the "rare" condition (Fig. 40.11B) that is not seen in the "frequent" condition (Fig. 40.11A) which is labeled the P_3. In the sections that follow, we will review age-related changes in OAEs, CAPs, ABR, MLRs, and ERPs.

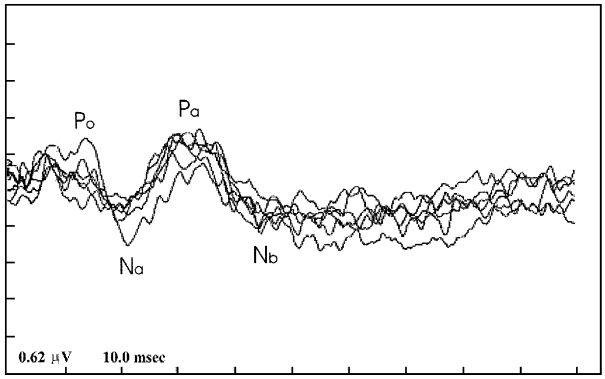

FIG. 40.10. The middle-latency response (MLR) from one human subject is shown. Each response is the averaged response to 1000 presentations of an 80 dB nHL, alternating-phase click stimulus presented at a rate of 9.1 Hz. The time base is 100 msec.

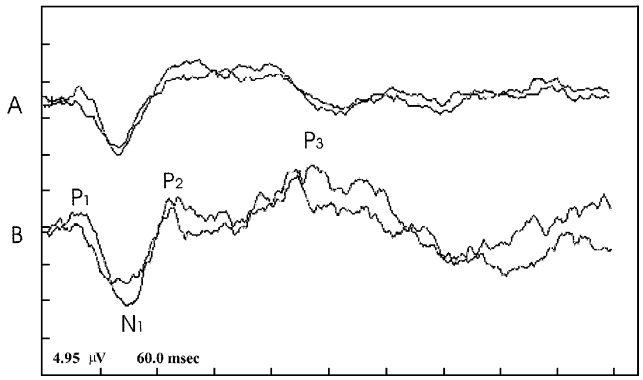

FIG. 40.11. The event-related potential (ERP) obtained using the oddball paradigm. Frequent stimuli were 2 kHz tone bursts, and rare stimuli were 1 kHz tone bursts. Rare stimuli were presented 20% of the time. The time base is 600 msec, and each set of responses (frequent plus rare) was to 200 stimulus presentations. Two responses are superimposed for each condition. Responses to frequent stimuli are shown in (A), while responses to rare stimuli are shown in (B).

C. Otoacoustic Emissions

Bonfils *et al*. (1988) investigated click-evoked OAEs (COAEs) across age in 151 ears of human subjects whose age ranged from 2 to 88 years. Mean audiometric thresholds increased with increasing age. In addition, COAE threshold increased with increasing age. The increase in COAE threshold paralleled the increase in perceptual click threshold with increasing age. Stover and Norton (1993) evaluated COAEs, tone-burst evoked transient OAEs (TBOAEs), spontaneous OAEs (SOAEs), stimulus frequency OAEs (SFOAEs), and distortion product OAEs (DPOAEs) in normal hearing subjects ranging in age from 20 to 80 years. With increasing age, there was an increase in mean audiometric threshold. Fewer SOAEs were observed in the older age groups. The older age groups showed smaller amplitude COAEs and TBOAEs than did the younger groups. SFOAEs were present in all subjects in the younger age groups, but present in only half of the subjects in their 60s and 70s. No clear patterns of DPOAE changes with aging emerged. Despite the observed changes in OAEs with age, statistical analyses revealed that these age-related changes were not independent of hearing thresholds; i.e., age-related changes in OAEs are confounded by changes in audiometric threshold across groups. Karzon *et al*. (1994) investigated DPOAEs in elderly subjects. Elderly subjects, on average, had higher audiometric thresholds than did the young adults. Mean DPOAE amplitudes (to 75 dB SPL primary tones) were reduced in the older subjects. When they compared the normal hearing young adult ears to the subset of elderly ears with normal hearing (20 dB HL or less from 250 to 8000 Hz), the mean DPOAE amplitude of the elderly ears was lower than that of the young ears, with the largest differences below 2 kHz and above 4 kHz. Kimberley *et al*. (1994) evaluated DPOAEs across age and hearing loss. DPOAE amplitude decreased with increasing (worsening) threshold. In the subset of ears with normal threshold, there was a systematic decrease in DPOAE amplitude with increasing age. It appears that normal hearing elderly ears have reduced DPOAE amplitudes, suggesting that OHCs may not be functioning normally in the aged ear with normal audiometric thresholds.

Parham (1997) investigated C57BL/6 mice across age. C57 BL/6s exhibit an adult-onset, progressive hearing loss, starting in the high frequencies. He found progressive elevation of ABR thresholds with increasing age, starting in the high frequencies, and progressing towards lower frequencies. Evaluating high frequency DPOAEs, using f2 frequencies between 8 and 16 kHz, he found a progressive decrease in response amplitude with aging. It appears that progressive hair cell damage results in elevation of ABR threshold and decreases of DPOAE amplitude.

D. Electrocochleography

Nozawa *et al*. (1996) found a gradual elevation in guinea pig click-evoked CAP threshold with advancing age. Hellström and Schmiedt (1990) evaluated CM and CAP input/output functions in young-adult (4- to 7-month) and old (35- to 37-month) gerbils. Mean CAP thresholds were higher in the older gerbils, and there was much greater variability in thresholds in the old gerbils, as compared to young-adult gerbils. The maximum CAP amplitude at each frequency in each animal was determined, and a mean ''maximum'' amplitude was compared across age groups. On average, there was a substantial (roughly 50%) decrease in maximum CAP amplitude with advanced age. Maximum CM amplitudes, evaluated in a similar manner, were smaller in the old gerbils at 500 Hz, similar in young and old gerbils at 1 kHz, and actually somewhat larger in older gerbils at 2 and 4 kHz. The reduction in amplitude of the auditory nerve response (i.e., decrease in CAP amplitude) does not appear to be entirely the result of threshold elevation; otherwise CM amplitude decrement (reflecting outer hair cell function) would parallel CAP changes.

E. Aging Effects on the Auditory Brain-Stem Response

1. Effects on Peak Latencies and Interwave Intervals

In humans, there is much controversy over the effects of aging on the ABR peak latencies across age. Jerger and Johnson (1988) evaluated the latency of ABR wave V to high-level click stimuli across gender, age, and hearing loss. There was an increase in wave V latency with increasing high-frequency hearing loss. When separated by age (those less than 50 years, those greater than 50 years) and grouped according to their 4 kHz threshold, the young females had shorter wave V latency than young males, and older females had shorter wave V latencies than older males. When compared across age (and within gender), older females had longer wave V latencies than younger females, but there was little effect of age on wave V latency for the males. These data reveal complex interaction of gender, age, and hearing loss on wave V latency. Elberling and Parbo (1987) evaluated the ABR interwave intervals across gender, age, and high-frequency hearing loss using multiple-regression analysis. The I–III and I–V intervals were smaller in females than in males. In addition, these interwave intervals were found to decrease with increasing age and increase with increasing hearing loss. Costa *et al*. (1990) found increasing wave I latency and decreasing wave III and V latency with

increasing age in normal hearing subjects. Thus, they found a decrease in the I–V interval (''central conduction time'') with aging. Rowe (1978) evaluated young and old subjects with no history of hearing loss. He found an increase in ABR peak latencies and an increase in the I–III interval but not the III–V interval with increasing age. Chu (1985) investigated normal-hearing young and old subjects, and found an increase in ABR peak latencies with increasing age. There was little change in the I–III interval across age, but a small increase in the III–V and I–V intervals with age. Even when older subjects were limited to those with ''normal hearing,'' there is little consensus as to the effects of aging on ABR peak latencies and interwave intervals. The sparse description of audiometric data in some of these studies suggests that one cause of the across-study variability regarding aging and ABR peak latency shifts could be the varied criteria for ''normal hearing.''

A number of animal studies have investigated the effects of aging on ABR peak latencies. Backoff and Caspary (1994) investigated ABR peak latencies in young-adult (3- to 6-month) and old (20 to 23 months) rats. To click stimuli, ABR thresholds were increased for the older rats. In addition, ABR peak latencies and interwave intervals were increased in the older rats. Nowaza *et al*. (1996) investigated guinea pig ABR peak latencies and interwave intervals to click stimuli. CAP and ABR thresholds increased with advancing age (and hence serve as a confounding factor in this study). ABR peak latencies increased with increasing age, but there was no change in interpeak intervals with increasing age. Ingham *et al*. (1998) evaluated ABR changes in guinea pigs to click stimuli, and found an increase in ABR threshold with advancing age and a trend toward increasing ABR peak latencies with advancing age that failed to reach significance. In addition, there was no change in interwave intervals with advancing age. Hunter and Willott (1987) investigated ABR changes with aging in C57BL/6J and CBA/J mice. To click stimuli, there is an increase in latency of the ABR peaks in C57 BL/6 mice, but only when threshold elevation is substantial. CBA mice ABR peak latencies did not show any systematic changes with aging. Cooper *et al*. (1990) investigated changes in ABRs in young and aged rats to tone-burst frequencies of 3, 8, and 40 kHz. Thresholds increased with increasing age, which was most pronounced for the higher frequencies. ABR peak latencies increased with increasing age, with the smallest age-related latency shifts seen at 8 kHz. In addition, there were increases in the interwave intervals with increasing age.

Perhaps the most systematic series of aging studies of the ABR has been performed by Mills and his colleagues. Mills *et al*. (1990) evaluated ABR threshold with advancing age in gerbils, and found that the tone-burst thresholds of 6- to 8-month-old gerbils increased on average by roughly 10 dB when compared to young animals and by 36 months increased by roughly 20–35 dB. The gerbil is a somewhat unique animal model of presbycusis, in light of the fact that age-related hair cell loss is comparatively modest, and age-related changes in the stria vascularis are quite pronounced (Schulte and Schmiedt, 1992). Boettcher *et al*. (1993b) compared ABR peak latencies in young adult and aged gerbils. They grouped aged gerbils by audiometric threshold. Aged gerbils with near-normal thresholds showed similar or somewhat shorter latencies than young adult gerbils. Aged gerbils with mild threshold elevation (compared to young adult gerbils) showed increased peak latencies at low tone-burst levels, with normal latencies at higher tone-burst intensities. In aged gerbils with greater threshold elevation (compared to young adults), ABR peak latencies were typically increased at all tone-burst levels. These data appear to support the view that threshold elevation is a confounding variable when evaluating ABR peak latency shifts with advanced age.

2. Effects on Peak Amplitudes

ABR peak amplitudes have been reported to decrease with increasing age in humans (Costa *et al*., 1990; Psatta and Matei, 1988). Psatta and Matei (1988) reported an increase in the V/I amplitude ratio with advancing age, which suggests a greater age-related change in wave I amplitude than wave V amplitude. Rowe (1978) found little change in wave V amplitude across age for 60 dB nHL clicks presented at 30 Hz. Mean wave V amplitude was slightly greater for the old subjects to 60 dB nHL clicks presented at 10 Hz, while wave V amplitude was substantially less in old adults than young adults to 30 dB nHL clicks presented at 30 Hz. Thus, amplitude changes with aging may depend, in part, on the acoustic stimuli used. Although not universal, generally speaking it appears that human ABR peak amplitudes decrease with increasing age.

In animal studies, ABR peak amplitudes also appear to decrease with advancing age. In Fischer 344 rats, Backoff and Caspary (1994) found a general decrease in ABR peak amplitudes to click stimuli in old (20 to 23 months) rats as compared to young adult (3 to 6 months) rats. Hunter and Willott (1987) found that in aging C57 BL/6 and CBA mice, ABR peak amplitudes decreased with increasing age. Ingham *et al*. (1998) found a decrease in guinea pig ABR peak amplitudes to click stimuli with advancing age. Boettcher *et al*. (1993a) evaluated ABR peak amplitudes to tone-burst stimuli in young (6 to 10 months) and aged (36 months) gerbils. Even for aged animals with near normal thresholds, ABR peak amplitudes were reduced, compared to young adult gerbils. This age-related amplitude decrease was particularly evident at higher tone-burst levels. This age-related decrease in ABR peak amplitude tended to increase with increasing hearing loss to high frequency tone-bursts for ABR wave III, and for all tone-burst frequencies except 16 kHz for ABR wave IV. In general, animal studies tend to show a reduction in ABR peak amplitudes with advancing age.

3. Temporal Processing and Masking in the ABR across Age

A number of AEP studies have investigated temporal processing and masking effects across age in humans and other mammals. Walton *et al*. (1999) investigated temporal processing in normal hearing young and old adults using a forward masking paradigm, where a tone-burst masker was presented prior to the presentation of a same frequency tone-burst stimulus. The time between masker offset and stimulus onset was varied. For high-frequency toneburst stimuli (but not low frequency tone-bursts), there was a substantial increase in wave V latency shift (compared to no masker control condition) in the

normal hearing older subjects. These data suggest a degradation in temporal processing in older subjects.

McFadden *et al*. (1997a,b) also demonstrated age-related changes in several suprathreshold auditory measures in the aged chinchilla. Consistent with the observations in gerbils and other rodents, they reported a 20 to 30 dB decline in peripheral sensitivity in old chinchillas. In addition, they recorded near field evoked responses from the inferior colliculus using a variety of masking paradigms. McFadden and colleagues found that old chinchillas displayed abnormal peripheral masking patterns, in that tone-on-tone masking produced greater threshold shifts in old chinchillas relative to young animals. Aging effects on temporal resolution were not observed, as forward masking recovery functions were comparable across age. They concluded that the cochlea of the aged chinchilla displays many of the functional deficits found in aged humans and that aging of the brain, in and of itself, may be the major factor in the perceptual deficits related to presbycusis.

Backoff and Caspary (1994) investigated ABR peak latencies and interwave intervals across click rate and age (3 to 6 months, and 20 to 23 months) in Fischer 344 rats. An evaluation of ABR peak latency shifts when increasing rate from 10 to 40 Hz showed greater latency shifts of waves 4 and 5 in the older rats (compared to young adult rats). Boettcher *et al*. (1996) investigated gap detection by CAP and ABR in gerbils. They found reduced CAP and ABR amplitudes with increasing age, essentially replicating earlier findings from their lab (Hellström and Schmiedt, 1990; Mills *et al*., 1990; Boettcher *et al*., 1993). At brief noise gaps, there was a substantial prolongation of ABR wave IV latency (but not CAP latency) in the aged animals. These data support the notion of a central component to age-related auditory changes in temporal processing. In one study of masking effects across subject age, Boettcher *et al*. (1995) investigated masked ABR thresholds in young and old gerbils using a 1 kHz low-pass and an 8 kHz high-pass masker. They found higher thresholds in the old gerbils to the low-pass noise for 2 and 4 kHz tone bursts, but not to the high-pass masker. These data are best interpreted as excess upward spread of masking in the aged animals, and hence the result of reduced peripheral frequency specificity, rather than the result of any central change.

F. Middle Latency Responses and Aging

Woods and Clayworth (1986) investigated MLR peak latencies and amplitudes to click stimuli in young and old adults. The older subjects showed higher audiometric thresholds than the young adults, with mean differences of 12.3 dB at 500 Hz, increasing to a mean difference of 49.6 dB at 8 kHz. In elderly subjects, Pa was prolonged as compared to that seen in young adults. Peak-to-peak amplitudes that included Pa were larger in the elderly subjects than in the young adults. Amenado and Diaz (1998a) evaluated MLR peak latencies and amplitudes in adults ranging in age from 20 to 86 years. Binaural clicks were presented at 60 dB above threshold. As perceptual thresholds to click stimuli increased with increasing age, click level was higher in the older subjects in this study. MLR peak latencies showed no consistent change across age group, but MLR peak amplitudes typically increased with advancing age. Kelly-Ballweber and Dobie (1984) investigated binaural interaction in the ABR and MLR of young and older males. They matched average audiograms for both age groups, such that both groups showed normal low-frequency sensitivity, and a moderate high-frequency hearing loss. Clicks were presented monaurally or binaurally at 99 dB peSPL. Evaluating mean data to binaural stimulation, ABR wave V and MLR waves Pa and Pb were prolonged in the older subjects. Most interestingly, mean peak amplitudes to binaural stimulation were larger for wave V, Na–Pa, and Pa–Nb. Lenzi *et al*. (1989) investigated MLRs in normal hearing (thresholds of 20 dB HL or less from 250 to 2000 Hz) young and aged adults. ABR peak latencies increased with advancing age in response to 50-dB SL clicks. There was a general increase in the latency of MLR peaks Na, Pa, and Nb with increasing age, to both clicks and 1 kHz tone burst stimuli. Although they did not show any data, the authors also reported a general decrease in MLR amplitude in the elderly subjects.

G. Event-Related Potentials and Aging

Spink *et al*. (1979) investigated ERPs to 1 kHz tone bursts in young (all 22 years old) and older (61–64 years old) adults. Tone-burst level was varied and ERPs collected in response to 64 tone-burst presentations at each level. For both age groups, N1/P2 amplitude increased with increasing amplitude up to 45 dB HL, with no change in amplitude for higher stimulus levels. They report no difference in ''relative amplitude'' across age group. For both age groups, ERP peak latencies decrease with increasing tone-burst level. There were no significant differences across age in the latencies of P1 or N1. In contrast, P2 latency was significantly shorter in older subjects for high-level tone bursts, and similar to young adults at lower tone-burst levels. Goodin *et al*. (1978) evaluated ERPs in young and old subjects whose hearing status was unspecified, using the oddball paradigm. After age 20, there is an increase in the latency of N1, P2, N2, and P3 with increasing age. This latency shift (by linear regression) was 0.1, 0.7, 0.8, and 1.8 msec/year for N1, P2, N2, and P3, respectively. They also reported a decrease in N1–P2 and N2–P3 amplitude with increasing age. Smith *et al*. (1980) investigated the effects of subject age on ERPs, again using the oddball paradigm. All subjects were women (young, 18–33 years old; aged, 65–80 years old), and half of the subjects in each age group were instructed to ignore the tones while reading, while the other half were instructed to count the tones. Three recording channels were utilized, with the noninverting leads located at Fz, Cz, and Pz. In comparison to young adult subjects, old subjects showed significantly greater P1 amplitude, significantly smaller P2 amplitude and statistically identical N1 amplitude. Young adult subjects showed larger P3 amplitudes than the older subjects, for both the reading and the counting tasks. Amenado and Diaz (1998b) evaluated ERPs to an oddball paradigm in subjects ranging in age from 20 to 86 years. In these subjects, three age groups were constructed: young adults (20–39 years old), middle-aged adults (40–59 years old), and older adults (60–86 years old). Stimuli were presented at 90 dB SPL. Target stimuli were 2 kHz tone bursts, while nontarget stimuli were 1 kHz tone bursts. Audiometric data were not reported. N1 and P2 latencies did not differ significantly across age groups. To nontarget stimuli, P2 ampli-

tude at Fz increased with increasing age. P3 amplitude decreased with increasing age, but only in males. Amenedo and Diaz (1998c) evaluated N2b and the MMN in young (23 to 29 years old), middle-aged (41 to 59 years old), and older (63 to 77 years old) adults to 90 dB SPL 1 kHz (standard tones) and 1500 Hz (deviant tones) presented dichotically. The audiometric thresholds of these subjects were not specified. N2b latency was longer in the middle-aged and older adults than in the young adults. N2b amplitude did not vary significantly across age group. MMN latency and amplitude did not vary across age group.

Puce *et al*. (1989) evaluated P3 as recorded from both the scalp and the intracranial electrodes in patients being investigated for focal seizures. Using the oddball paradigm, the scalp-recorded P3 latency increased and its amplitude decreased with increasing age in a group of control subjects. In the patient population, both intracranial and surface P3 recording showed increasing P3 latency with increasing age. In contrast to the control subjects, in the patients with seizures, there was no systematic change in either intracranial- or surface-recorded P3 amplitude across age. Pfefferbaum *et al*. (1980a,b) evaluated auditory ERPs in young adult (mean age, 22.5 years old) and elderly (mean age, 78.6 years old) women using an oddball paradigm composed of frequent stimuli (1 kHz tone bursts), and two infrequent stimuli (500 and 2000 Hz). The subjects were instructed to press a key to one of two infrequent stimuli (called the target) and performed no motor task for the other infrequent stimulus (the nontarget infrequent stimulus). Responses to both infrequent stimuli were analyzed off-line, both by averaging and by using single-response analysis procedures. Young and old subjects did not differ in terms of correct responses, false alarms, or reaction time. N1 latency did not differ across age groups, while P2 latency was significantly prolonged in older subjects. N1/P2 amplitude was significantly larger in the older subjects. Evaluating N1 and P2 amplitudes (relative to baseline), both showed mean amplitudes that were greater in the older subjects, but only P2 amplitude differences reached significance. P2 amplitude distribution across the scalp varied with age, with P2 amplitude largest at Cz in the younger subjects, but equally large at Fz and Cz in the older adults. To both target and nontarget infrequent stimuli, P3 latency was prolonged in the older subjects. P3 amplitude distribution differed across age, with the young adults showing larger P3 amplitudes in more frontal electrode locations. In contrast, older subjects showed similar P3 amplitudes for all electrode leads. Finally, slow wave activity (a negativity following P3 at more frontal electrodes) was decreased in the older subjects. In an evaluation of both visual and auditory P3, Pfefferbaum *et al*. (1984) found increasing P3 latency with increasing age to both auditory and visual stimuli. For both modalities, age-related P3 amplitude changes appeared as a change in scalp topography: Young adult subjects showed P3 amplitudes that were largest posteriorly (e.g., as Pz), while older subjects showed a more uniform distribution (i.e., amplitudes were similar at Fz, Cz, and Pz).

Swartz *et al*. (1994) investigated P3 latency and amplitude to a variety of auditory discrimination tasks using musical stimuli, in both young (18–37 years old) and older (65–85 years old) adults. Audiometric data were not obtained, but subjects reported that they could follow conversation without the use of a hearing aid. Although mean P3 amplitude was smaller in the older subjects for several of the discrimination tasks, this effect failed to reach statistical significance. In contrast, P3 latency was significantly longer in a subset of these discrimination tasks. Bellis *et al*. (2000) investigated ERP amplitudes to an oddball paradigm in a group of children (8–11 years old), young adults (20–25 years old), and older adults (greater than 55 years old). Hearing thresholds were less than 25 dB HL for the octave frequencies ranging from 500 to 8000 Hz. Deviant stimuli were synthetic /da/ syllables, and standard stimuli were synthesized /ga/ syllables. All stimuli were presented to the right ear, and responses recorded over the left and right temporal lobes were compared. P1–N1 amplitude was larger in children than in the adults, but there was no difference in P1–N1 amplitude for the young and older adults groups. In the children and young adults, responses recorded over the left (contralateral) hemisphere were significantly larger than those recorded over the right (ipsilateral) hemisphere. This asymmetry was not observed in the older adults. In contrast, the MMN did not significantly differ over the right and left temporal lobes in any age group.

H. Summary of AEP Studies

The most consistently reported age-related change in evoked responses is a reduction in response amplitude from responses emanating from the auditory periphery and brain stem. This has been reported for all time epoch of responses, including OAEs, CAP, and ABR. Less consistently, there are reports of age-related changes in AEP peak latencies and interwave intervals. Most interestingly, there have been several reports showing response enhancement of MLRs. Paradigms involving temporal processing (forward masking or gap detection) show greater ABR peak latency shifts at short forward-masking intervals or brief gaps in aged subjects. AEP studies that stress the temporal processing capabilities of the auditory system appear to be promising paradigms for the study of the aging auditory system. A recurring theme throughout this literature review is that even mild threshold elevations across age groups appears to confound the interpretation of age-related changes. This highlights the importance of careful documentation or control of audiometric thresholds when evaluating AEP changes across age.

V. Conclusions

Aging is frequently accompanied by a decline in sensory perception, clinically referred to as presbycusis. Until recently, the underlying neural mechanisms responsible for the perceptual decline in the aged auditory system remained poorly understood largely due to the lack of research. This chapter has presented a review of peripheral and central auditory processing from single cells and from populations of cells using auditory evoked potentials as it relates to neural presbycusis. Studies which have investigated neural processing to simple stimuli found no evidence which suggests an age-related deterioration in neural function. However, when more complex stimuli or masking paradigms are used age-related alteration in neural processing begin to emerge. A complete understand-

ing of the neurological bases of presbycusis will be unraveled only by using acoustic signals which model the types of listening situations where elderly listeners have difficulty. The final objective is to correlate age-related deficits in neural processing to biological changes in auditory neurons so that potential therapies can be formulated which could slow or alleviate presbycusis.

References

Altman, P., and Dittmer, D. (1972). ''Biology Data Book,'' Vol. 1, Federation of the American Societies for Experimental Biology, Bethesda, MD.

Amenedo, E., and Diaz, F. (1998a). Effects of aging on middle-latency auditory evoked potentials: A cross-sectional study. *Biol. Psychol*. **43**, 210–219.

Amenedo, E., and Diaz, F. (1998b). Aging-related changes in processing of non-target and target stimuli during an auditory oddball task. *Biol. Psychol*. **48**, 235–267.

Amenedo, E., and Diaz, F. (1998c). Automatic and effortful processes in auditory memory reflected by event-related potentials. Age-related findings. *Electroencephalogr. Clin. Neurophysiol*. **108**, 361–369.

Backoff, P., and Caspary, D. (1994). Age-related changes in auditory brainstem responses in Fischer 344 rats: Effects of rate and intensity. *Hear. Res*. **73**, 163–172.

Bellis, T., Nicol, T., and Kraus, N. (2000). Aging affects hemispheric asymmetry in the neural representation of speech sounds. *J. Neurosci* **20**, 791–797.

Birren, J. E., and Fisher, L. M. (1995). Aging and speed of behavior: Possible consequences for psychological functioning. *Annu. Rev. Psychol*. **46**, 329–353.

Boettcher, F., Mills, J., and Norton, B. (1993a). Age-related changes in auditory evoked potentials of gerbils. I. Response amplitudes. *Hear. Res*. **71**, 137–145.

Boettcher, F., Mills, J., Norton, B., and Schmiedt, R. (1993b). Age-related changes in auditory evoked potentials of gerbils. II. Response latencies. *Hear. Res*. **71**, 146–156.

Boettcher, F., Mills, J., Dubno, J., and Schmiedt, R. (1995). Masking of auditory brainstem responses in young and aged gerbils. *Hear. Res.* **89**, 1–13.

Boettcher, F., Mills, J., Swerdloff, J., and Holley, B. (1996). Auditory evoked potentials in aged gerbils: Responses elicited by noises separated by a silent gap. *Hear. Res*. **102**, 167–178.

Bonfils, P., Bertrand, Y., and Uziel, A. (1988). Evoked otoacoustic emissions: Normative data and presbycusis. *Audiology* **27**, 27–35.

Caspary, D. M., Raza, A., Lawhorn, Armour, B. A., Pippin, J., and Arneric, S. P. (1990). Immunocytochemical and neurochemical evidence for age-related loss of GABA in the inferior colliculus: Implications for neural presbycusis. *J. Neurosci*. **10**, 2363–2372.

Caspary, D. M., Milbrandt, J. C., and Helfert, R. H. (1995). Central auditory aging: GABA changes in the inferior colliculus. *Exp. Gerontol*. **30**, 349–360.

Chu, N. (1985). Age-related latency changes in the brain-stem auditory evoked potentials. *Electroencephalogr. Clin. Neurophysiol*. **62**, 431–436.

Cooper, W., Coleman, J., and Newton, E. (1990). Auditory brainstem responses to tonal stimuli in young and aging rats. *Hear. Res*. **43**, 171–180.

Costa, P., Benna, P., Bianco, C., Ferrero, P., and Bergamasco, B. (1990). Aging effects on brainstem auditory evoked potentials. *Electromyogr. Clin. Neurophysiol*. **30**, 495–500.

Durrant, J., Wang, J., Ding, D., and Salvi, R. (1998). Are inner or outer hair cells the source of summating potentials recorded from the round window. *J. Acoust. Soc. Am*. **104**, 370–377.

Elberling, C., and Parbo, J. (1987). Reference data for ABRs in retrocochlear disease. *Scand. Audiol*. **16**, 49–55.

Finlayson, P. G., and Caspary, D. M. (1993). Response properties in young and old Fischer-344 rat lateral superior olive neurons: A quantitative approach. *Neurobiol. Aging* **14**, 127–139.

Fitzgibbons, P. J., and Gordon-Salant, S. (1996). Auditory temporal processing in elderly listeners. *J. Am. Acad. Audiol*. **7**, 183–189.

Frisina, R. D., Karcich, K. J., Tracy, T. C., Sullivan, D. M., and Walton, J. P. (1996). Preservation of amplitude modulation coding in the presence of background noise by chinchilla auditory-nerve fibers. *J. Acoust. Soc. Am*. **99**, 475–490.

Goodin, D., Squires, K., Henderson, B., and Starr, A. (1978). Age-related variations in evoked potentials to auditory stimuli in normal human subjects. *Electroencephalogr. Clin. Neurophysiol*. **44**, 447–458.

Gratton, M. A., Smyth, B. J., Schulter, B. A., and Vincent, D. A., Jr. (1995). Na, K-ATPase activity decreases in the cochlear lateral wall of quiet-aged gerbils. *Hear. Res*. **83**, 43–50.

Gratton, M. A., Schmiedt, R. A., and Schulter, B. A. (1996). Age-related decreases in endocochlear potential are associated with vascular abnormalities in the stria vascularis. *Hear. Res*. **94**, 116–124.

Gratton, M. A., Smyth, B. J., Lam, C. F., Boettcher, F. A., and Schmiedt, R.A. (1997). Decline in the endocochlear potential corresponds to decreased Na, K-ATPase activity in the lateral wall of quiet-aged gerbils. *Hear. Res*. **108**, 9–16.

Green, D. M., and Forrest, T. G. (1989). Temporal gaps in noise and sinusiods. *J. Acoust. Soc. Am*. **86**, 961–970.

Hellström, L., and Schmiedt, R. (1990). Compound action potential input/output functions in young and quiet-aged gerbils. *Hear. Res*. **50**, 163–174.

Hellström, L. I., and Schmiedt, R. A. (1991). Rate/level functions of auditory-nerve fibers in young and quiet-aged gerbils. *Hear. Res*. **53**, 217–222.

Hellström, L. I., and Schmiedt, R. A. (1996). Measures of tuning and suppression in single fibers and whole-nerve responses in young and quiet-aged gerbils. *J. Acoust. Soc. Am.* **100**, 3275–3285.

Henry, K. R. (1982). Age-related auditory loss and genetics: An electrocochleographic comparison of six inbred strains of mice. *J. Gerontol*. **37**, 275–282.

Henry, K. R., and Chole, R. A. (1980). Genotypic differences in behavioral, physiological and anatomical expressions of age-related hearing loss in the laboratory mouse. *Audiology* **19**, 369–383.

Hunter, K. P., and Willott, J. F. (1987). Aging and the auditory brainstem response in mice with severe or minimal presbycusis. *Hear. Res.* **30**, 207–218.

Ingham, N., Thornton, S., Comis, S., and Withington, D. (1998). The auditory brainstem response of aged guinea pigs. *Acta Oto-Laryngol*. **118**, 673–680.

Irvine, D. R. F. (1986). ''The Auditory Brainstem: A Review of the Structure and Function of Auditory Processing Mechanisms.'' Springer, Berlin.

Jerger, J., and Johnson, K. (1988). Interactions of age, gender and sensorineural hearing loss on ABR latency. *Ear Hear.* **9**, 168–176.

Jock, B. M., Hamernik, R. P., Aldrich, L. G., Ahroon, W. A., Petriello, K. L., and Johnson, A. R. (1998). Evoked-potential thresholds and cubic distortion product otoacoustic emissions in the chinchilla following carboplatin treatment and noise exposure. *Hear. Res.* **96**, 179–190.

Joris, P. X. and Yin, T. C. (1992). Responses to amplitude-modulated tones in the auditory nerve of the cat. *J. Acoust. Soc. Am.* **91**, 215–232.

Karzon, R., Garcia, P., Peterein, J., and Gates, G. (1994). Distortion product otoacoustic emissions in the elderly. *Am. J. Otol*. **15**, 596–605.

Kelly-Ballweber, D., and Dobie, R. (1984). Binaural interaction measured behaviorally and electrophysiologically in young and old adults. *Audiology.* **23**, 181–194.

Kimberly, B., Hernadi, I., Lee, A., and Brown, D. (1994). Predicting pure tone thresholds in normal and hearing impaired ears with distortion product otoacoustic emissions. *Ear Hear.* **15**, 199–209.

Krauter, E. E., Wallace, J. E., and Campbell, B. A. (1981). Sensory-motor function in the aging rat. *Behav. Neural Biol*. **31**, 367–392.

Langner, G., and Schreiner, C. E. (1988). Periodicity coding in the inferior colliculus of the cat. I. Neuronal mechanisms. *J. Neurophysiol*. **60**, 1799–1822.

Lenzi, A., Chiarelli, G., and Sambataro, G. (1989). Comparative study of middle-latency responses and auditory brainstem responses in elderly subjects. *Audiology* **28**, 144–151.

Li, H., and Borg, E. (1992). Influence of genotype and age on acute acoustic trauma and recovery in CBA/Ca and C57BL/6J. *Acta Oto-Laryngol*. **112**, 956–967.

McFadden, S. L., Campo, P., Quaranta, N., and Henderson, D. (1997a). Age-related decline of auditory function in the chinchilla *(Chinchilla laniger). Hear. Res*. **111**, 114–126.

McFadden, S. L., Quaranta, N., and Henderson, D. (1997b). Suprathreshold measures of auditory function in the aging chinchilla. *Hear. Res*. **111**, 127–135.

Mikaelian, D. (1979). Development and degeneration of hearing in the C57/bl6 mouse: Relation between electrophysiological responses from the round window and cochlear nucleus to cochlear anatomy and behavioral responses. *Laryngoscope* **89**, 1–15.

Milbrandt, J. C., Albin, R. L., and Caspary, D. M (1994). Age-related decrease in $GABA_B$ receptor binding in the Fischer 344 rat inferior colliculus. *Neurobiol. Aging* **15**, 699–703.

Mills, J., Schmiedt, R., and Kulish, L. (1990). Age-related changes in auditory evoked potentials of Mongolian gerbil. *Hear. Res*. **46**, 201–210.

Nozawa, I., Imamura, S., Fujimori, I., Hashimoto, K., Shimomura, S., Hisamatsu, K., and Murakami, Y. (1996). Age-related alterations in the auditory brainstem responses and the compound action potentials in guinea pigs. *Laryngoscope* **106**, 1034–1039.

Palombi, P. S., and Caspary, D. M. (1996a). Responses of young and aged Fischer 344 rat inferior colliculus neurons to binaural tonal stimuli. *Hear. Res*. **100**, 59–67.

Palombi, P. S., and Caspary, D. M. (1996b). Physiology of the aged Fischer 344 rat inferior colliculus: Responses to contralateral monaural stimuli. *J. Neurophysiol.* **5**, 3114–3121.

Parham, K. (1997). Distortion product otoacoustic emissions in the C57BL/6J mouse model of age-related hearing loss. *Hear. Res*. **112**, 216–234.

Park, T. J., and Pollak, G. D. (1993a). GABA shapes sensitivity to interaural intensity disparities in the mustache bat's inferior colliculus: Implications for encoding sound location. *J. Neurosci*. **13**, 2050–2067.

Park, T. J., and Pollak, G. D. (1993b). GABA shapes a topographic organization of response latency in the mustached bat's inferior colliculus. *J. Neurosci.* **13**, 5172–5187.

Pfefferbaum, A., Ford, J., Roth, W., and Kopell, B. (1980a). Age differences in P3-reaction time associations. *Electroencephalogr. Clin. Neurophysiol*. **49**, 257–265.

Pfefferbaum, A., Ford, J., Roth, W., and Kopell, B. (1980b). Age-related changes in auditory event-related potentials. *Electroencephalogr. Clin. Neurophysiol*. **49**, 266–276.

Pfefferbaum, A., Ford, J., Wenegrat, B., Roth, W., and Kopell, B. (1984). Clinical application of the P3 component of event-related potentials. I. Normal aging. *Electroencephalogr. Clin. Neurophysiol*. **59**, 85–103.

Plomp, R. (1964). Rate of decay of auditory sensation. *J. Acoust. Soc. Am*. **36**, 277–282.

Psatta, D., and Matei, M. (1988). Age-dependent amplitude variation of brain-stem auditory evoked potentials. *Electroencephalogr. Clin. Neurophysiol*. **71**, 27–32.

Puce, A., Donnan, G., and Bladin, P. (1989). Comparative effects of age on limbic and scalp P3. *Electroencephalogr. Clin. Neurophysiol*. **74**, 385–393.

Qiu, C. X., Salvi, R., Ding, D., and Burkard, R. (2000). Inner hair cell loss leads to enhanced response amplitudes in auditory cortex of unanesthetized chinchillas: Evidence for increased system gain *Hear. Res*. **139**, 153–171.

Rowe, J. (1978). Normal variability of the brain-stem auditory evoked response in young and old adult subjects. *Electroencephalogr. Clin. Neurophysiol*. **44**, 459–470.

Schmiedt, R. A., Mills, J. H, and Adams, J. C. (1990). Tuning and suppression in auditory nerve fibers of aged gerbils raised in quiet or noise. *Hear. Res*. **45**, 221–236.

Schmiedt, R. A., Mills, J. H., and Boettcher, F. A. (1996). Age-related loss of activity of auditory-nerve fibers. *J. Neurophysiol*. **76**, 2799–2803.

Schneider, B. A., Pichora-Fuller, M. K., Kowalchuk, D., and Lamb, M. (1994). Gap detection and the precedence effect in young and old subjects. *J. Acoust. Soc. Am.* **95**, 980–991.

Schoeborn, C. A., and Marano, M. (1988). Current estimates from the National Health Interview Survey: United States 1987. *In* "Vital Health and Statistics," Series 10, No. 166. Washington DC.

Schulte, B. A., and Schmiedt, R. A. (1992). Lateral wall Na, K-ATPase and endocochlear potentials decline with age in quiet-reared animals. *Hear. Res*. **61**, 35–46.

Smith D. B., Michalewski, H. J., Brent, G. A., and Thompson, L. W. (1980). Auditory averaged evoked potentials and aging: Factors of stimulus, task and topography. *Biol. Psych.* **11**, 135–151.

Snell, K. B. (1997). Age related changes in temporal gap detection. *J. Acoust. Soc. Am*. **101**, 2214–2220.

Spink, U., Johannsen, H. S., and Pirsig, W. (1979). Acoustically evoked potential: Dependence upon age. *Scandinavian Audiol.* **8**, 11–14.

Stiebler, I., and Ehret, G. (1985). Inferior colliculus of the house mouse. I. A quantitative study of tonotopic organization, frequency representation and tone-threshold distribution. *J. Comp. Neurol*. **238**, 65–76.

Stover, L., and Norton, S. (1993). The effects of aging on otoacoustic emissions. *J. Acoust. Soc. Am*. **94**, 2670–2681.

Swartz, K., Walton, J., Hantz, E., Goldhammer, E., Crummer, G., and Frisina, R. (1994). P3 event-related potentials and performance of young and old subjects for music perception tasks. *Int. J. Neurosci*. **78**, 223–239.

Trautwein, P., Hofstetter, P., Wang, J., Salvi, R., and Nostrant, A. (1996). Selective inner hair cell loss does not alter distortion product otoacoustic emissions. *Hear. Res*. **96**, 71–82.

Walton, J. P., Frisina, R. D., Ison, J. R., and O'Neill, W. E. (1997). Neural correlates of gap detection in the inferior colliculus of the young CBA mouse. *J. Comp. Physiol*. A **119**, 263–278.

Walton, J. P., Frisina, R. D., and O'Neill, W. E. (1998). Age-related alteration in neural processing of temporal sound features in the inferior colliculus in the CBA mouse model of presbycusis. *J. Neurosci*. **267**, 2117–2130.

Walton, J., Orlando, M., and Burkard, R. (1999). Auditory brainstem response forward masking recovery functions are delayed in older humans with normal hearing. *Hear. Res*. **127**, 86–94.

Willott, J. F. (1984). Changes in frequency representation in the auditory system of mice with age-related hearing impairment. *Brain Res*. **309**, 159–162.

Willott, J. F. (1986). Effects of aging, hearing loss and anatomical location on thresholds of inferior colliculus neurons in C57BL/6 and CBA mice. *J. Neurophysiol*. **56**, 391–408.

Willott, J. F., Parham, K., and Hunter, K. P. (1988). Response properties of inferior colliculus neurons in young and old CBA/J mice. *Hear. Res*. **37**, 1–14.

Willott, J. F., Aitkin, L. M., and McFadden, S. L. (1993). Plasticity of auditory cortex associated with sensorineural hearing loss in adult C57BL/6J mice. *J. Comp. Neurol*. **329**, 402–411.

Winter, I. M., Robertson, D., and Yates, G. K. (1990). Diversity of characteristic frequency rate-intensity functions in guinea pig auditory nerve fibers. *Hear. Res*. **45**, 191–202.

Woods, D., and Clayworth, C. (1986). Age-related changes in human middle latency auditory evoked potentials. *Electroencephalogr. Clin. Neurophysiol*. **65**, 297–303.

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Sandra L. McFadden

41

Genetics and Age-Related Hearing Loss

One of the important challenges confronting modern molecular genetics is identifying the genes responsible for presbycusis, or age-related hearing loss. The genetic causes of presbycusis and other nonsyndromic types of hearing impairment are largely unexplored. However, clues about presbycusis genes are available from studies of animals (particularly inbred and transgenic strains of mice), humans with other forms of progressive, late-onset hearing loss, and mitochondrial diseases. This chapter provides an overview of basic genetic concepts as a background for understanding advances in the rapidly changing field of molecular genetics of hearing loss and age-related diseases. In addition, it offers insight into the possible genetic basis of presbycusis, with a focus on the potential contributions of mitochondrial dysfunction.

I. Introduction

Age-related hearing loss (AHL), or presbycusis, affects 30–35% of the population ages 65–75 years and 40% of the population over 75 years of age. Most of the changes in auditory perception that occur with age can be attributed to pathological changes in the cochlea, particularly the loss of sensory hair cells and degeneration of the stria vascularis. The causes of AHL are unknown, but several factors are likely to play a role. As summarized in Fig. 41.1, etiological agents may include environmental factors such as noise, ototoxic drugs, and environmental toxins and genetic defects, both inherited and acquired. A substantial proportion of AHL cases are likely to arise from interactions between genetic and environmental factors. Individuals with certain genetic predispositions may be more sensitive to environmental insults that produce hearing loss over time. In addition, disease states and the genetic background of an individual can exacerbate the effects of deafness-causing genes.

Even though the field of molecular genetics is advancing rapidly, it is still in its infancy with respect to identifying genes causing various forms of hearing loss in humans, including presbycusis. The purpose of this chapter is twofold. The first aim is to provide the reader with an overview of basic genetic concepts, to help establish the background necessary for keeping abreast of developments in the rapidly expanding field of molecular genetics. The second aim is to present some thoughts about potential genetic mechanisms that could contribute to human presbycusis. It is important to note that genes for presbycusis in humans have not yet been identified. Nevertheless, we have several clues about potential genes from studies of mice and other animals, humans with other forms of genetic late-onset hearing loss, and mitochondrial diseases.

II. Genetic Mutations and Disease

Currently there are about 4000 known human genetic diseases, and more than 10% of these include hearing loss as a component. Some genetic conditions are caused by changes in the normal number of chromosomes, as in Down syndrome. Mutations in chromosome number are relatively frequent, occurring in approximately 20% of human conceptions. However, they are usually lethal early in development, and are therefore seen in fewer than 1% of human births. The more common type of mutation in surviving individuals involves a single gene or a single pair of genes. Genetic hearing loss arising from mutations of nuclear and mitochondrial genes will be the primary focus of this chapter.

III. Classification of Genetic Hearing Impairment

Hearing impairment can be acquired (e.g., the result of acoustic trauma, disease, or ototoxicity), inherited, or due to unknown causes. As shown in Fig. 41.2, heredity accounts for the largest proportion (20–60%) of hearing impairment in humans.

In classical genetics, it is important to know how a genetic condition is transmitted from one generation to the next (if at all) and whether the genetic condition has single or multiple effects. This leads to a classification of hereditary hearing impairment into two categories based on physical manifestations (phenotypes) and four categories based on inheritance (see Fig. 41.2). When hearing loss is accompanied by other clinical manifestations, it is said to be syndromic. When it occurs alone, as in presbycusis, it is nonsyndromic.

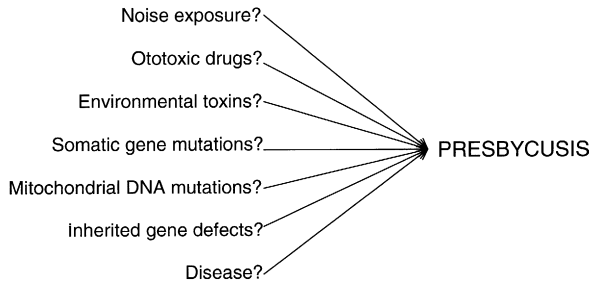

FIG. 41.1. Potential etiological factors for presbycusis. Etiological factors include environmental agents, spontaneous and inherited genetic mutations, and interactions between environmental agents and the individual's genetic background.

There are more than 200 forms of syndromic hereditary hearing impairment. However, the largest proportion (70–95%) of hereditary deafness is nonsyndromic. It is estimated that autosomal recessive defects account for about 80% of cases of nonsyndromic hearing impairment, autosomal dominant forms for about 15%, and X-linked forms for 1–3%. There is currently no way to estimate the proportion of cases of genetic hearing impairment due to mitochondrial and polygenic inheritance. However, evidence for the role of mitochondrial inheritance and mitochondrial mutations in causing hearing loss directly or indirectly (e.g., by increasing susceptibility to environmental agents) is growing.

A second way of classifying genetic hearing impairments is according to their underlying pathology. Steel and her colleagues (e.g., Steel *et al*., 1983; Steel and Brown, 1994) classify gene mutations into three categories: (1) *morphogenetic* abnormalities, arising from mutations affecting development of the auditory placode; (2) *cochleosaccular* defects, arising from disturbances in the stria vascularis and typically associated with pigmentation defects; and (3) *neuroepithelial* defects, which involve primary abnormalities of the organ of Corti in the cochlea, the maculae of the sacculus and utricule and the cristae of the semicircular canals. Neuroepithelial defects are associated with nonsyndromal hearing loss and are often recessive. They appear to be the most common type of cochlear pathology in humans. The recent mapping and cloning (sequencing) of genes responsible for neuroepithelial defects represent major steps toward solving the genetic puzzle of presbycusis.

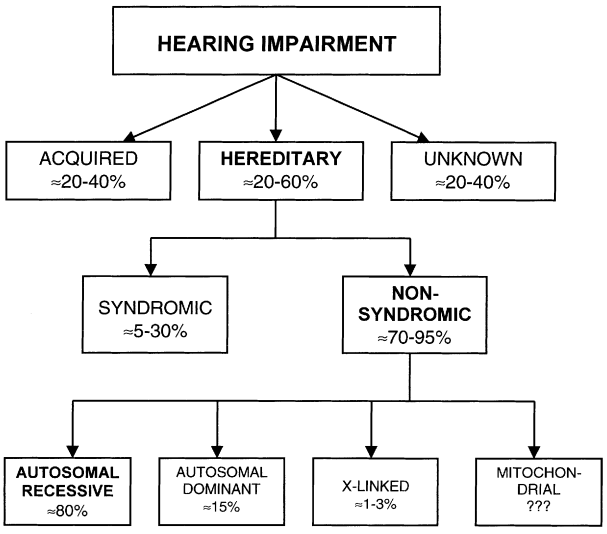

FIG. 41.2. Classification of genetic hearing impairment based on clinical manifestations and mode of inheritance.

IV. Mapping and Sequencing Genes

Mapping a gene refers to the process of localizing the gene to a particular section (locus) of DNA on a specific chromosome. This provides clues as to the gene identity and function. Sequencing a gene refers to the process of identifying the order of bases on the gene that direct the coding of a protein. The entire human genome contains approximately three billion bases. It has been estimated that the information from sequencing the entire human genome will fill the equivalent of 14 sets of the Encyclopedia Britannica, or 200 volumes the size of a Manhattan phone book. Sequencing the entire human genome is the goal of a program supported by the Department of Energy and the National Institutes of Health called the Human Genome Project.

As of this writing, the Hereditary Hearing Loss Home Page (Van Camp and Smith, 1999) lists 31 autosomal dominant deafness loci (DFNA1–31), 28 autosomal recessive deafness loci (DFNB1–28), 6 X-linked deafness loci (DFN1–4; DFN 6; DFN8), and 2 mitochondrial gene mutations. More than 50 genes causing nonsyndromic hearing impairment have been localized to specific chromosomes, and in 19 cases, the gene has been sequenced or cloned. Cloned genes now include 10 autosomal dominant genes, 6 autosomal recessive genes, 1 X-linked gene, and 2 mitochondrial genes. Most of these genes are associated with neuroepithelial defects. The identification of so many loci for deafness (DFN) genes illustrates that many genes control hearing function and that hearing impairment is heterogeneous. All of the known DFN genes produce sensori-neural hearing loss, and most forms of DFNA and DFNB deafness are progressive. These genes may therefore provide clues about genes responsible for presbycusis. A brief overview of basic genetic concepts is provided in the Appendix at the end of this volume.

V. Clues for Presbycusis Genes

Although a genetic basis for some forms of AHL has been suspected for many years (e.g., Schuknecht, 1974), nothing is known about the mode of inheritance of AHL, the number of genes that might be involved, or how the genes might act. One of the challenges for molecular genetics in the 21st century is to map and sequence the genes responsible for AHL. This is a daunting proposition for several reasons. First, there is no single clinical phenotype that defines presbycusis. The classic symptoms associated with AHL are a bilaterally symmetrical elevation of high-frequency thresholds, and decreased ability to understand speech, especially in the presence of background noise. However, other phenotypes are frequently observed, and there is controversy over the predominant cochlear pathology

in presbycusis. Is the loss of sensory hair cells the most common form of histopathology, or is strial dysfunction more prevalent? The diversity of AHL phenotypes will complicate the search for underlying genes. Second, the various phenotypes of genetic AHL are likely to be due to the actions of many genes, each with allelic variants, or alternate forms, and both direct and indirect effects. Third, the fact that different genetic conditions can result in identical clinical findings will make it difficult to use gene linkage to identify AHL genes.

Before concluding that these difficulties will make it impossible to uncover genes for presbycusis, one should consider other recent successes that seemed impossible only a decade ago. Until recently, mapping genes responsible for any autosomal recessive hearing loss was considered too difficult because of the extreme genetic heterogeneity and the wide range of audiometric patterns which affected individuals exhibit. Now, several DFN genes have actually been cloned or sequenced. The identification of genes for human presbycusis may not be far behind.

Many of the clues for presbycusis genes come from genetic studies of animals, particularly mice. Presbycusis is one of a number of conditions that occur in both mouse and human in which hearing loss develops as an inconsistent and late-onset feature. Of the thousands of gene loci that have been mapped in mice and humans, more than 400 are homologous between the two species. Fortunately, there is a strong tendency for gene loci order and groupings to be conserved between humans and mice. Thus, when genes for hearing loss are mapped in the mouse, reasonable predictions can be made regarding the location of homologous genes in the human. Human homologs and mutations can be sought in conserved areas of the human genome.

A. Clues from Genes Causing Neuroepithelial Defects in Mice and Humans

1. Genes for Unconventional Myosins

The *myo7a* gene codes for myosin VIIA, an unconventional myosin expressed in hair cells of the inner ear. A mutation of *myo7a* was found to be responsible for the *sh1* mutation in the shaker-1 mouse (Gibson *et al*., 1995). The *sh1* mutation was first reported in 1929 by Lord and Gates (see Ruben, 1991) and has since served as a valuable model for progressive hearing loss. Shaker-1 mice have profound hearing loss associated with degeneration of the organ of Corti, along with the shaker–waltzer type behavior that is typical of mice with vestibular defects.

Since 1995, mutations in the homologous human gene, *MYO7A*, have been shown to cause three distinct forms of hearing loss. One mutation produces Usher's syndrome type 1b, a recessively inherited syndromic disease characterized by congenital deafness, vestibular dysfunction, and retinitis pigmentosa (Weil *et al*., 1995). Another mutation, corresponding to the *sh-1* mutation in mice, gives rise to DFNB2 (Liu *et al*., 1997; Weil *et al*., 1997), and a third mutation has been found to cause DFNA11 (Liu *et al*., 1997).

Discovery of *myo7a* was an extremely important event in molecular genetics for three reasons. *Myo7a* was the first gene found that affects the sensory hair cells directly. The discovery of *myo7a* in the mouse led to identification of the gene causing Usher's syndrome type 1b in the human. Importantly, this is the first gene found where mutations cause both dominant and recessive, and syndromic and nonsyndromic forms of hearing loss.

A second myosin gene mutation causing a neuroepithelial defect was found shortly after *myo7a*. This mutation was found in the gene coding for myosin VI, responsible for the Snell's waltzer mouse mutation (Avraham *et al*., 1995). In shaker-1 and Snell's waltzer mice, the earliest gross abnormalities involve the actin-rich stereocilia of the sensory hair cells. Shaker-1 mice show disorganization of the stereocilia bundle, and Snell's waltzer mice have stereocilia that are fused.

Myosin VI and VII are two members of a class of unconventional (non-muscle-like) myosins. The precise roles of the unconventional myosins in the cochlea are unknown, but two functions are likely (Steel and Brown, 1994). One function of the unconventional myosins may be in adjusting the tension of the tip links that connect adjacent stereocilia. The tip links are involved in opening and closing the hair cell transduction channels, thereby increasing or decreasing the flow of potassium ions through the apical surface of the hair cell, and mutations may interfere with this process. A second role of the unconventional myosins is in the movement of vesicles and other elements around the hair cell, using actin as a substrate. Interestingly, a mutation that affects another protein that may be involved in the trafficking of membrane vesicles has been shown to cause DFNB9. This protein, called otoferlin, is coded by a gene (*OTOF*) that is expressed in the inner hair cells of the organ of Corti and in vestibular type I sensory cells.

A third myosin gene mutation is responsible for DFNB3 in the human and the *sh-2* mutation in the mouse. This gene codes for myosin XV, a unique myosin protein that appears to be necessary for actin organization in hair cells. The hair cells of shaker-2 mice have very short stereocilia, and a long actin-containing bundle protrudes from the basal end.

Mutations affecting myosin VII, myosin VI, myosin XV, and otoferlin point to the importance of vesicle trafficking, regulation of current flow, and actin organization in normal hair cell function. Each of the mutations produces a different nonsyndromic recessive hearing impairment.

2. Genes for Connexin

GJB2 is the gene that codes for the beta-2 gap junction protein, connexin 26 (CX26). CX26 is one of six connexin protein subunits that form gap junctions–plasma membrane channels that allow small molecules to pass from one cell to another. In the cochlea, CX26 is found in the epithelial supporting cells surrounding the sensory hair cells and in fibrocytes lining the cochlear duct. Gap junctions between the supporting cells and fibrocytes provide a route whereby potassium ions that pass through the base of the hair cell can be returned to the endolymph above the hair cell.

The *GJB2* gene is small, and the entire protein-coding sequence is located in a single exon. This makes the gene relatively easy to screen for mutations. More than 20 different mutations of *GJB2* have been found in families with autosomal recessive nonsyndromic hearing loss. The most common mutation, called the 30delG or 35delG mutation because it arises

from a deletion of one base in a sequence of 6 guanine residues at position 30–35 in the CX26 DNA sequence, causes DFNB1. The 30delG mutation appears to have arisen several times independently in many human populations and may be carried by nearly 1 in every 30 people, making it one of the most common disease-causing mutations identified so far. Interestingly, individuals who are homozygous for the 30delG mutation show wide phenotypic variation, with hearing impairment ranging from mild to profound. Two carriers of *GJB2* mutations, one for the 30delG mutation and the other for another *GJB2* mutation causing hearing loss, 167delT (deletion of a thymine residue starting at position 167), have moderate hearing impairment themselves. It is intriguing to speculate that some cases of AHL could be associated with carrier status for a *GJB2* mutation (Steel, 1998).

Identification of genes causing neuroepithelial pathology and nonsyndromic recessive deafness in mice and humans is an important advance. Mutations involving myosins and connexin point to vulnerable elements in the cochlea and show how small changes in channel function can profoundly disrupt normal hair cell function. Could the cochlear pathology leading to presbycusis involve similar genes?

B. Clues from Genetic Studies with Gerbils

Next to the mouse, the gerbil is the most frequently used animal model of human presbycusis. The primary age-related pathological change in the gerbil inner ear is degeneration of the stria vascularis, resulting in ''metabolic'' presbycusis, characterized by a relatively flat loss of sensitivity across frequencies. Researchers at the Medical University of South Carolina are using a molecular biology technique known as mRNA differential display–polymerase chain reaction (DD-PCR) to investigate age-related changes in cochlear gene expression (LaMarche *et al*., 1999). Eleven differentially expressed PCR products have been cloned and sequenced. Five of them match known genes. In aged gerbils, upregulation was seen in the partial transcripts for the mitochondrial genes cytochrome C oxidase and 16S rRNA and a gene coding for an actin monomer binding protein that is a cell suicide protein. Downregulation was observed in partial transcripts for a gene coding for a transporter protein involved in assembly and turnover of cell membranes and extracellular matrix constituents, and the gene that codes for the receptor for activated C-kinase. These findings are significant, because they are the first to show evidence of altered gene expression in aged animals with hearing loss. Ultimately, studies will localize the differentially expressed transcripts to specific cell types through *in situ* hybridization or immunocytochemistry.

C. Clues from Genes Producing Dominant Progressive Hearing Loss in Humans

Dominant progressive hearing loss (DPHL) in humans resembles presbycusis in several ways. It is a nonsyndromic form of progressive sensori-neural hearing loss. Some individuals lose the ability to hear high tones or frequencies first, while others may lose the ability to hear the low or middle frequencies first. The biggest difference between DPHL and AHL is in age of onset. In some families, DPHL is first detected in early childhood. In other families, hearing loss may not be evident until early or middle adulthood. However, all known forms of DPHL have onset prior to the third decade of life.

The onset, rate of progression, initial frequencies involved, and ultimate severity of hearing loss vary among families, but are fairly consistent within families. The variability of phenotypic expression suggests that more than one gene can cause DPHL. Indeed, several genes have now been identified. Given the phenotypic similarities between DPHL and AHL, it is likely that an understanding of how the DPHL genes act will provide insights into the mechanisms leading to presbycusis.

D. Clues from Studies of AHL in Mice

A series of studies using inbred, F1 hybrid, and BXD recombinant inbred strains of mice (Erway *et al*., 1993; Johnson *et al*., 1997; Willott and Erway, 1998) has implicated three separate genes for AHL. One gene, responsible for the AHL trait in C57BL/6J mice, has been mapped through linkage analysis to a region of chromosome 10 (Johnson *et al*., 1997). Classical genetic analyses have shown that *Ahl* is inherited as a recessive trait (Erway *et al*., 1993). The phenotype produced by *Ahl* resembles human presbycusis in several ways. It is progressive, primarily involves the sensory hair cells and spiral ganglion cells of the cochlea, and produces a hearing loss that is initially high frequency in nature.

The location of the *Ahl* gene on chromosome 10 is depicted in Fig. 41.3. It is interesting that many other genes for hearing loss map to the same region on the mouse chromosome or to homologous regions of human chromosomes. In mice, waltzer (*v*), Jackson circler (*jc*), Ames waltzer (*av*), and deaf waddler (*mdfw*) mutations map to the same region. It is possible that the AHL trait in C57 mice is a less severe, allelic form of one of these four genes. The *Ahl* locus also has positional homology with human DFN genes DFNA10, DFNB8, and DFNB12, and the Usher syndrome type ID gene, USH1D. Each of these genes is a candidate for *Ahl*. Other candidates for *Ahl* based on positional homology are *Gja,* which codes for an alpha-1 gap junction protein similar to CX26, and various collagen genes. Three defective human collagen genes are responsible for syndromes with late-onset hearing loss, and other atypical collagens have been shown to influence structural integrity of the inner ear matrix (Slepecky *et al*., 1992).

E. Could Mitochondrial Dysfunction Be the Key to AHL?

It is easy to speculate that mtDNA mutations could play a role in presbycusis. MtDNA has a high mutation rate, and somatic mtDNA defects tend to accumulate with age, particularly in tissues with high metabolic rate and low mitotic activity (Lestienne and Bataille, 1994; Sherratt *et al*., 1997; Suomalainen, 1997). Many studies have documented decreased oxidative phosphorylation activity in senescent and degenerative tissues, particularly in tissues with high metabolic rates. It has been postulated that the progressive decline in oxidative phosphorylation and the accumulation of mtDNA mutations results from damage by ROS (Sherratt *et al*., 1997; Suomalainen, 1997). All of these observations suggest that the metabolically active cells of the inner ear, particularly cells of the stria

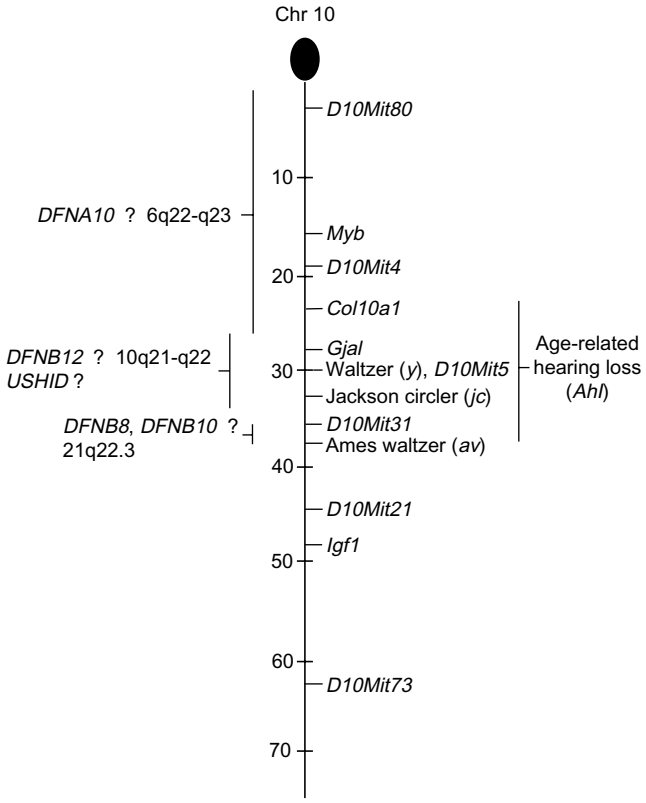

FIG. 41.3. Location of *Ahl* on mouse chromosome 10. There are possible homologies with human chromosomal regions containing nonsyndromal deafness genes and the Usher syndrome type ID gene. (From Johnson *et al*., 1997, with permission from Elsevier.)

vascularis and postmitotic hair cells, would be prime targets for mitochondrial dysfunction.

Investigations of the auditory system in aged animal models have uncovered mtDNA deletions (Seidman *et al*., 1997; Ueda *et al*., 1997) and evidence of impaired energy metabolism (Ding *et al*., 1999). Recent studies with C57BL/6J mice (Ueda *et al*., 1997) and human patients with presbycusis (Fischel-Ghodsian *et al*., 1997) support a relationship between acquired mtDNA defects and presbycusis. Mitochondrial mutations in the peripheral auditory system increase with age in both C57BL/6J mice and humans with presbycusis.

One mutation of mitochondrial rRNA associated with nonsyndromic hearing loss (not presbycusis) has been identified. This is a point mutation (A to G transition) of the 12S rRNA gene at nucleotide 1555 (1555A > G), associated with maternally inherited nonsyndromic deafness (Prezant *et al*., 1993). In some families with this mutation, deafness occurs only after use of aminoglycosides. This is because aminoglycosides affect translational fidelity by binding to the mRNA decoding region and stabilizing mismatched tRNAs (Lestienne and Bataille, 1994). This is an instance where the mtDNA mutation is not pathogenic itself, but causes maternally inherited sensitivity to specific environmental factors.

Because cells usually contain mitochondria in excess of basal needs, clinical features of mtDNA mutations may appear only if a large proportion of the mitochondria is affected or if the energy demands of the cell are extreme. The number of mitochondria found in a particular cell tends to reflect the cell's energy requirements. Hepatocytes may contain several thousand mitochondria, whereas resting lymphocytes may have only a few, and mature erythrocytes have no mitochondria at all (Sherratt *et al*., 1997). Mitochondria are constantly changing shape and positions within a cell, and they tend to accumulate in regions of the cell where energy requirements are high. In the normal inner ear, mitochondria numbers are highest in the cells of the stria vascularis and the spiral ganglion neurons. These cells also express the greatest amounts of Na,K-ATPase, an enzyme whose function depends on the ATP produced by mitochondria. The large numbers of mitochondria and the high energy requirements of the stria vascularis cells and spiral ganglion neurons leads to the hypothesis that these cells might be particularly vulnerable to degeneration during aging.

Recently, Seidman *et al*. (1997) examined tissue samples from brain, auditory nerve and stria vascularis of Fischer rats for evidence of a common mtDNA deletion involving 4834 base pairs. The 4834-base-pair deletion was absent from cochleas of young rats but present in 50% of the stria vascularis samples and in 29% of the auditory nerve samples from animals over 18 months of age.

The region of human mtDNA that is equivalent to the 4834-base-pair deletion in rat mtDNA corresponds to a 4977-base-pair deletion in humans. Bai *et al*. (1997) looked for 4977-base-pair deletions in mtDNA obtained from archived temporal bones. They found the common deletion in 14 of 17 samples from individuals who had presbycusis when they died. However, 8 of 17 aged individuals with no hearing loss also showed the deletion. In another study, Fischel-Ghodsian *et al*. (1997) found a high frequency of mtDNA mutations in temporal bones of aged individuals, but there was no apparent relationship between the amount of hearing loss and the number of sequence changes in mtDNA samples. Thus, while these studies show a strong positive correlation between aging and the common 4977-mtDNA deletion, the link between hearing loss and this particular deletion is weak. However, there are many reasons to continue the search for a link between other mtDNA mutations and presbycusis.

VI. Interactions between Genetic Background and Environment

Environmental agents can cause hearing loss only when the exposed individual is genetically susceptible. Differences in the degree of susceptibility among individuals may also have genetic causes (see Spillman, 1994). An example of this is seen with SOD1 mice created at Cephalon, Inc. Knockout mice of the strain have targeted deletions of *sod1*, the gene that codes for copper–zinc superoxide dismutase (Cu/Zn SOD). Cu/Zn SOD is an antioxidant enzyme found in the cytoplasm of cells. Its role is to convert the superoxide radical to a less reactive molecule. Wild type and knockout SOD1 mice show progressive high-frequency hearing loss and basal cochlear pathology as they age. However, knockout mice lacking Cu/Zn SOD show far greater hearing loss and cochlear pathology than normal. The effects of partial (50%—heterozygous knockout mice) and total (100%—homozygous knockout mice) elimination

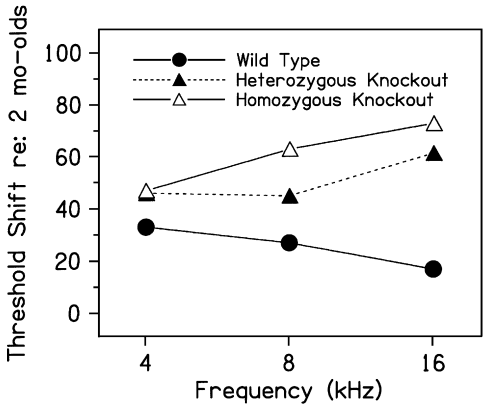

FIG. 41.4. Age-related threshold shifts of SOD1 mice at 13 months of age. Threshold shifts were estimated by comparing auditory brain-stem response thresholds of 13-month-old mice to 2-month-old mice. Wild type mice with normal levels of Cu/Zn SOD had less hearing loss than mice that partially or totally lacked Cu/Zn SOD (heterozygous knockout and homozygous knockout mice, respectively).

of Cu/Zn SOD on age-related hearing loss in SOD1 mice are shown in Fig. 41.4. Compared to young (2-month-old) SOD1 mice, aged (13-month-old) SOD1 mice of all three genotypes had substantial threshold shifts at all frequencies. However, threshold shifts were greatest in the aged mice lacking Cu/Zn SOD, particularly at high frequencies. It is clear that the *sod1* gene does not cause AHL. However, absence of its normal gene product, Cu/Zn SOD, exacerbates genetic AHL (McFadden *et al*., 1999a,b).

VII. Looking to the Future

The information coming from molecular genetics research is likely to influence the field of presbycusis research in two distinct ways. First, molecular techniques may soon aid in the diagnosis of presbycusis. Second, individuals identified as being at risk for presbycusis may one day have the option of choosing gene therapy to prevent hearing loss. Conceivably, gene therapy could involve replacing the defective gene with a functional copy, using carrier vectors such as adenoviruses and herpes simplex I viruses. Alternatively, genes could be inserted that would encode therapeutic agents such as neurotrophins and growth factors to reverse damage in early stages. The cochlea is a good candidate for gene transfer therapies, because of its large fluid spaces and its immunological isolation.

VIII. Conclusions

The role of genetic factors in the etiology of AHL is not known. However, rapid progress in the field of molecular genetics and the availability of appropriate animal models of presbycusis hold promise for unraveling the genetics of AHL in the early part of the 21st century. Clues for human presbycusis genes come from studies with animals, particularly inbred strains of mice, humans with other forms of nonsyndromic hearing loss, and mitochondrial disorders. These studies suggest that it may be most fruitful to concentrate initial research efforts on genes that influence myosin, connexin, actin, and collagen in the inner ear and on mitochondrial mutations.

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References

Avraham, K. B., Hasson, T., Steel, K. P., Kingsley, D. M., Russell, L. B., Mooseker, M. S., Copeland, N. G., and Jenkins, N. A. (1995). The mouse snell's waltzer deafness gene encodes an unconventional myosin required for structural integrity of inner ear hair cells. *Nat. Genet*. **11**, 369–375.

Bai, U., Seidman, M. D., Hinojosa, R., and Quirk, W. S. (1997). Mitochondrial DNA deletions associated with aging and possibly presbycusis: A human archival temporal bone study. *Am. J. Otol.* **18**, 449–453.

Ding, D. L., McFadden, S. L., Wang, J., Hu, B. H., and Salvi, R. J. (1999) Age- and strain-related differences in dehydrogenase activity and glycogen levels in CBA and C57 mouse cochleas. *Audiol. Neuro-Otol.* **4**, 55–63.

Erway, L. C., Willott, J. F., Archer, J. R., and Harrison, D. E. (1993). Genetics of age-related hearing loss in mice. I. Inbred and F1 hybrid strains. *Hear. Res*. **65**, 125–132.

Fischel-Ghodsian, N., Bykhovskaya, Y., Taylor, K., Kahen, T., Cantor, R., Ehrenman, K., Smith, R., and Keithley, E. M. (1997). Temporal bone analysis of patients with presbyacusis reveals high frequency of mitochondrial mutations. *Hear. Res*. **110**, 147–154.

Gibson, F., Walsh, J., Mburu, P., Vareia, A., Brown, K. A., Antonio, M., Beisel, K. W., Steel, K. P., and Brown, S. D. M. (1995). A type VII myosin encoded by the mouse deafness gene *shaker-1*. *Nature (London)* **374**, 62–64.

Johnson, K. R., Erway, L. C., Cook, S. A., Willott, J. F., and Zheng, Q. Y. (1997). A major gene affecting age-related hearing loss in C57BL/6J mice. *Hear. Res.* **114**, 83–92.

LaMarche, M. D., Niedzielski, A. S., and Schulte, B. A. (1999). Age-related changes in gene expression in the gerbil inner ear. *Assoc. Res. Otolaryngol. Abstr.* **643**, 163.

Lestienne, P., and Bataille, N. (1994). Mitochondrial DNA alterations and genetic diseases: A review. *Biomed. Pharmacother.* **48**, 199–214.

Liu, X. Z., Walsh, J., Mburu, P., Kendrick-Jones, J., Cope, M. J., Steel, K. P., and Brown, S. D. (1997). Mutations in the myosin VIIA gene cause non-syndromic recessive deafness. *Nat. Genet.* **16**, 188–190.

Martini, A., Mazzoli, M., and Kimberling, W. (1991). An introduction to the genetics of normal and defective hearing. *Ann. N. Y. Acad. Sci.* **630**, 361–374.

McFadden, S. L., Ding, D. L., Burkard, R. J., Jiang, H., Salvi, R. J., and Flood, D. G. (1999a). Cu/Zn SOD deficiency potentiates hearing loss and cochlear pathology in aged 129,CD-1 mice. *J. Comp. Neurol*. **413**, 101–112.

McFadden, S. L., Ding, D. L., Reaume, A. G., Flood, D. G., and Salvi, R. J. (1999b). Age-related cochlear hair cell loss is enhanced in mice lacking copper/zinc superoxide dismutase. *Neurobiol. Aging* **20**, 1–8.

Prezant, T. R., Agapian, J. V., Bohlman, M. C., Bu, X., Oztas, S., Qiu, W. Q., Arnos, K. S., Cortopassi, G. A., Jaber, L., Rotter, J. I., *et al*. (1993). Mitochondrial ribosomal RNA mutation associated with both antibiotic- induced and nonsyndromic deafness. *Nat. Genet.* **4,** 289–294.

Ruben, R. J. (1991). The history of the genetics of hearing impairment. *Ann. N.Y. Acad. Sci.* **630**, 6–15.

Schuknecht, H. F. (1974). "Pathology of the Ear." Harvard University Press, Cambridge, MA.

Seidman, M. D., Bai, U., Khan, M. J., and Quirk, W. S. (1997). Mitochondrial DNA deletions associated with aging and presbycusis. *Arch. Otolaryngol. Head Neck Surg.* **123**, 1039–1045.

Sherratt, E. J., Thomas, A. W., and Alcolado, J. C. (1997). Mitochondrial DNA defects: A widening clinical spectrum of disorders. *Clin. Sci*. **92**, 225–235.

Slepecky, N. B., Savage, J. E., and Yoo, T. J. (1992). Localization of type II, IX, and V collagen in the inner ear. *Acta Oto-Laryngol*. **112**, 611–617.

Spillmann, T. (1994). Genetic diseases of hearing. *Curr. Opin. Neurol.* **7**, 81–87.

Steel, K. P. (1998). A new era in the genetics of deafness. *N. Engl. J. Med.* **339**, 1545–1547.

Steel, K. P., and Brown, S. D. (1994). Genetics of deafness. *Curr. Opin. Neurobiol.* **6**, 520–525.

Steel, K. P., Niaussat, M. M., and Bock, G. R. (1983). The genetics of hearing. *In* "The Auditory Psychobiology of the Mouse" (J.F. Willott, ed.), pp. 34–394. Thomas, Springfield, IL.

Suomalainen, A. (1997). Mitochondrial DNA and disease. *Ann. Med.* **29**, 235–246.

Ueda, N., Oshima, T., Ikeda, K., and Takasaka, T. (1997). Mitochondrial DNA deletion in the aged mouse cochlea. *Assoc. Res. Otolaryngol. Abstr.* **877**, 220.

Van Camp G., and Smith, R. J. H. (1999). Hereditary Hearing Loss Homepage. World Wide Web *URL: http://dnalab-www.uia.ac.be/dnalab/hhh*

Weil, D., Blanchard, S., Kaplan, J., Guilford, P., Gibson, F., Walsh, J., Mburu, P., Varela, A., Levilliers, J., Weston, M. D., Kelley, P. M., Kimberling, W. J., Wagenaar, M., Levi-Acobas, F., Larget-Piet, D., Munnich, A., Steel, K. P., Brown, S. D. M., and Petit, C. (1995). Defective myosin VIIA gene responsible for Usher syndrome type 1B. *Nature (London)* **374**, 60–61.

Weil, D., Kussel, P., Blanchard, S., Levy, G., Levi-Acobas, F., Drira, M., Ayadi, H., and Petit, C. (1997). The autosomal recessive isolated deafness, DFNB2, and the Usher 1B syndrome are allelic defects of the myosin-VIIA gene. *Nat. Genet.* **16**, 191–193.

Willott, J. F., and Erway, L. C. (1998). Genetics of age-related hearing loss in mice. IV. Cochlear pathology and hearing loss in 25 BXD recombinant inbred mouse strains. *Hear. Res*. **119**, 17–36.

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James F. Willott

42

Animal Models of Presbycusis and the Aging Auditory System

A variety of animal models are available for the study of presbycusis and the aging auditory system. Most widely used are mice, gerbils, rats, chinchillas, and guinea pigs. The rate and severity of presbycusis ranges from mild to severe in various animals, paralleling the range observed in humans.

I. Introduction

One of the most important ways that animal models contribute to presbycusis research is by showing what types of processes and events can and do occur in the aging mammalian ear. All of these may not be shared by humans, and one should never assume that they necessarily are. Nevertheless, the information obtained from animal research can be used to formulate credible, testable hypotheses about mechanisms underlying presbycusis or to suggest new clinical approaches to the human condition. The study of animals also speaks to the ubiquity of certain changes—their generality within the mammalian family. Understanding the properties of presbycusis in various animal species contributes to understanding presbycusis in humans.

The major advantage of using animal models, of course, is the ability to overcome the ethical and practical limitations inherent in the use of human subjects. These limitations include the following: (1) With respect to histological studies, it is usually impossible to obtain rapid and thorough fixation of postmortem neural and cochlear tissue in humans, increasing the likelihood of autolytic artifacts while decreasing the quality and validity of histological specimens. (2) Notwithstanding the rapid evolution of modern imaging technology, it is still the case that most modern *in vivo* methods, required to reveal the anatomical and physiological details of neurons, cannot be used with human subjects. (3) Most elderly people exhibit some degree sensorineural cochlear pathology which can cause secondary morphological and physiological changes in the central auditory system. This would result in a confounding of the influences of peripheral impairment and biological aging, as discussed below. (4) Environmental and genetic influences may interact with age-related biological events to affect the auditory system of older individuals in unknown ways, making it difficult to determine the role of each. The cochlea is especially vulnerable to noise-induced damage, and it is likely that many—if not most—individuals who spend decades of their life in industrialized settings have some degree of noise-induced hearing loss. These problems limit the usefulness of much data obtained from human subjects. The use of animal models can mitigate at least some of the problems, particularly those of a technical nature.

II. Research Considerations in Choosing Animal Models

A. Peripheral Hearing Loss

Perhaps the most important consideration in choosing a particular animal model for gerontological auditory research is the rate and magnitude of age-related hearing loss. Individual humans exhibit very large differences in this regard, with some fortunate people maintaining near-normal hearing well into old age, whereas others begin to experience high-frequency loss during young middle age or earlier. Thus, animal models are needed to represent a range of severity of presbycusis. Hearing loss can be manipulated experimentally in animals using intense noise exposure, cochlear surgery, ototoxic drugs, or other means. However, these procedures can be problematic in gerontological contexts because they typically produce sudden cochlear damage, whereas presbycusis develops gradually over a significant segment of the life span. In addition, mechanically induced lesions tend to be anatomically well delineated, whereas age-related cochlear pathology is not likely to have a discrete "border" between damaged and healthy regions, and drug and noise effects tend to exhibit a good deal of variability across individual animals, even when conditions are tightly controlled. Fortunately, various animal models exhibit age-related hearing loss ranging from minimal to profound, without the necessity of experimental manipulation. These are discussed below.

B. Aging and the Central Auditory System

Hearing is accomplished by the brain and its ability to take the trains of electrical impulses traversing the auditory nerve fibers and transform them into auditory sensations and perceptions. The neuroanatomical ''hardware'' required for this daunting task is incredibly complex, subtle, and, like the cochlea, subject to negative age effects. Indeed, the CAS is threatened by two concomitants of aging. First, changes in the structure or function of the CAS can occur as an aspect of biological aging, as is the case for many other brain regions discussed throughout this book (see also Willott, 1999). With respect to the auditory system, these central effects of biological aging can occur whether or not significant cochlear pathology accompanies aging. However, the cochlear pathology typically associated with aging results in the partial removal or attenuation of neural input from the ear to the brain. The central effects of peripheral pathology can include a variety of changes in the central auditory system secondary to cochlear damage (Willott, 1991). Central effects of peripheral pathology can be superimposed upon and/or interact with central effects of biological aging and it is clear that, for a full understanding of the fate of the central auditory system and the perceptual deficits that accompany aging, both types of central effects must be addressed.

In trying to differentiate between central effects of biological aging and central effects of peripheral pathology the use of animal models becomes extremely important. The majority of older people suffer some loss of hearing and, at the same time, a number of other central age effects. Thus, central effects of biological aging and central effects of peripheral pathology tend to coexist in older humans, making it difficult to interpret the nature of their hearing deficits, particularly those involving the perception of speech and other suprathreshold sounds. In animal models, however, it may be possible to differentiate central effects of biological aging and central effects of peripheral pathology to some extent. This is particularly true with inbred mice, as discussed later.

III. Methods for Evaluating the Functioning Auditory System in Animals

Numerous neurobiological and other methods can be used to study the aging nervous system of animal models, as indicated throughout this book. In order to understand the significance of such changes in the auditory system it is useful to know whether an aging animal exhibits presbycusis; and if so, how severe are the hearing deficits? One of the obvious limitations of animal models is the inability of subjects to indicate directly whether, how well, and what they hear. Thresholds and suprathreshold perception must be inferred from physiological measures and/or behavioral performance. Fortunately, an adequate arsenal of techniques is available.

A. Physiological Approaches

The logic of physiological measures of auditory function is straightforward: use acoustic stimuli to evoke responses in the auditory system and then vary the stimuli while observing changes in the responses. Whereas evoked responses of various types can be obtained easily from animals, there are some gerontological issues that must be addressed. To use these methods with animals usually requires anesthesia or tranquilization. If dose–response curves and/or tolerance of the drug were to differ in old animals (as is often the case), evaluation of auditory function could be confounded. Also, it is important to maintain body temperature in anesthetized animals because hypothermia can alter neural activity. Size, body fat, and temperature regulation mechanisms might be different in old animals, so care should be taken to maintain body temperature with a heating pad or appropriate device. Another issue is the ''endurance'' of old animals in experiments that require long periods of time, such as single-unit recordings.

One method that can deal with these sorts of concerns is the averaged auditory brain-stem response (ABR), perhaps the most widely used measure of auditory sensitivity in animal models. The ABR is easily obtained using commercially available hardware and software and is capable of determining thresholds as well as some suprathreshold parameters (e.g., response latency and amplitude). ABR thresholds are not strongly influenced by anesthesia, the procedure is relatively brief, and body temperature can be maintained by placing the animal on a heating pad or blanket. ABRs can also be used in masking paradigms to provide additional information about auditory functioning. For example, Walton *et al.* (1995) showed that, in mice with age-related hearing impairment, forward masking was evident in the auditory brain stem—more so than in the cochlea. Other event-related potentials, such as middle-latency responses and other cortical responses have been used less frequently with aging animals, but have promise for evaluation of suprathreshold auditory function.

Otoacoustic emissions (OAEs) are finding increasing use in gerontological studies. The OAEs reflect outer hair cell function, and outer hair cell are usually the most vulnerable of cochlear cells with respect to age-related degeneration. An example of the use of OAEs in chimpanzees is shown later.

The use of single- and multiple-unit recordings to obtain more sophisticated information about auditory processing has resulted in some important findings. Issues of anesthetic effects and experiment duration are more cogent for this type of work, especially when recordings are obtained from cortex. However, despite these potential problems a number of important findings have emerged. Some examples are mentioned later in this chapter, such as the discovery of central reorganization of frequency maps, sluggish neurons, deficits in gap detection responses, and indications of diminished inhibition in older animals.

B. Behavioral Approaches

While we can describe the physiological changes in animals' auditory systems, it is difficult to fully appreciate their significance without the use of behavioral and psychophysical methods. Whereas auditory behavioral studies are presently part of the gerontological research landscape, a great deal more effort is need in future research.

Auditory sensation and perception are inferred from behavioral performance (e.g., the animal is trained to respond to a sound). Because both learning ability and performance

may be affected by age-related factors (Willott, 1999; other chapters in this book), great care must be taken not to misinterpret a behavioral deficit as being due to hearing loss, rather than more general learning/performance changes. The best way to do so is by demonstrating that the old animals are capable of performing the behavioral task using nonauditory stimuli and/or using some auditory stimuli that can still be heard well. For example, if an animal fails to respond to a high-frequency tone but still responds well to a low-frequency tone, one can assume a high-frequency hearing deficit, rather than general performance failure.

A variety of auditory behaviors are amenable to gerontological studies in animals. These include the following.

1. The Acoustic Startle Response

The acoustic startle response is reliably elicited by bursts of noise or tones having sound pressure levels (SPLs; re 20 μPa) of 80–90 dB and greater. The jerk-like motor reflex is easily measured by commercially available movement-sensitive (startle) devices, and acoustic startle response latency, amplitude, and threshold are readily obtained. Inability or difficulty in evoking acoustic startle response is diagnostic of either severe hearing loss (Parham and Willott, 1988) or a brain-stem neurological deficit. Figure 42.1 shows an example of how the amplitude of acoustic startle response evoked by 100 dB SPL tones changes with age in two strains of mice differing in severity of presbycusis. As C57 mice lose high-frequency sensitivity with age, acoustic startle response can no longer be obtained with 16-kHz tones, whereas amplitudes decline moderately with 4-kHz stimuli. By comparison, acoustic startle response amplitudes remain large as CBA mice age.

2. Prepulse Inhibition

Prepulse inhibition is a behavioral method that utilizes the startle response as a behavioral "probe" to determine the effectiveness or salience of sounds that modulate the startle. Prepulse inhibition is highly reliable, requires no training, and is readily observable in rodents and humans (Hoffman and Ison, 1980). Prepulse inhibition occurs when a moderately intense (nonreflexogenic) tone "prepulse" (S1) is presented

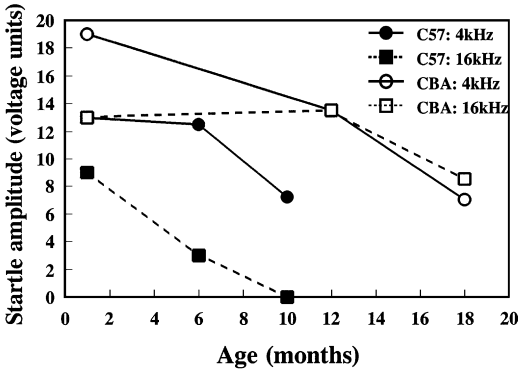

FIG. 42.1. Acoustic startle amplitude in C57 and CBA mice as a function of age. Amplitudes decline rapidly in C57 mice, particularly for 16 kHz tones. CBA mice maintain good acoustic startle response amplitudes as they age. Data were obtained from Parham and Willott (1988).

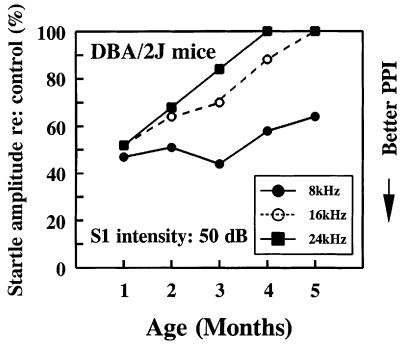

FIG. 42.2. Prepulse inhibition used to show age-related hearing loss. As DBA mice lose high-frequency cochlear sensitivity, prepulse inhibition is no longer produced by 24 kHz tone prepulses. However, lower frequency prepulses maintain their ability to produce prepulse inhibition. Data were obtained from Willott *et al.* (1994b).

10–200 msec before a startle-evoking stimulus (S2), and the response to the startle probe is inhibited. The ratio of startle amplitudes for paired S1–S2 trials divided by S2-only trials defines prepulse inhibition. The S1 activates inhibitory circuits that reduce the response to the probe, so the degree of prepulse inhibition is a measure of the salience of the S1 prepulse. Obviously, if an S1 tone cannot be heard, it will not produce prepulse inhibition; thus measurement of prepulse inhibition while varying the SPL of an S1 can be used to identify animals with hearing impairments (Willott *et al.*, 1994b). Figure 42.2 shows how prepulse inhibition can be used to demonstrate high-frequency hearing loss in DBA mice, an inbred strain that exhibits very rapid, early-onset progressive hearing loss (see below).

3. The Use of Aversive Events

Mild shock is used in several behavioral paradigms that can be used to indicate the salience or potency of auditory stimuli. They have not been widely applied to presbycusis research, but have promise in this regard. Fear-potentiated startle is widely used to investigate the neurobiological basis of anxiety (Davis *et al.*, 1993) and because fear-potentiated startle utilizes Pavlovian conditioning procedures, it is also used to investigate the neurobiological basis of learning and memory. If an auditory stimulus is used as a conditioned stimulus (CS), fear-potentiated startle can be used as a test for auditory behavior. In fear-potentiated startle, a mild shock is paired with the auditory CS for several trials, so that the CS elicits fear. When an animal is then startled in the presence of the CS, startle is potentiated. Thus, the effectiveness of an auditory CS can be measured as a function of age and/or presbycusis. In our experience, old mice (Willott, 1999) and mice with early presbycusis (Falls *et al.*, 1997) continue to exhibit fear-potentiated startle, providing a test of the behavioral salience of sounds in a learning context.

Avoidance conditioning utilizes the shock as a negative reinforcer—an animal will learn to make a behavioral response when an auditory stimulus is presented; this response prevents it from being shocked. Avoidance conditioning is rapid and can be used to test auditory discrimination and other abilities. In the lick suppression paradigm, the subject learns to associate

a mild shock to a drinking spout with the acoustic condition, and presentation of the stimulus causes the subject to stop licking. For example, Heffner and Donnal (1993) used lick suppression to compare spatial localization in young and middle-aged C57 mice with presbycusis; they learned that the shock depended on whether sounds came from one versus two locations. When young, the mice had a mean left/right localization threshold of 33° for broadband noise, but this dropped to 46° 5 months later. Both the loss of high-frequency sensitivity and altered central neural responses (cf. McFadden and Willott, 1994a,b) probably contributed to the localization deficit.

4. Appetitive Conditioning

A potential problem with aversive stimuli in aging research is that old animals that cannot perform well on the task might be subject to shocks that they cannot prevent. The use of positive reinforcers (food or water rewards) precludes such problems. Whereas appetitive tasks often take longer to learn, this is usually not a problem with aging research. Several studies can serve as examples for the use of positive reinforcement to evaluate age-related auditory changes. Harrison (1981) employed a cross-sectional design to evaluate the ability of aging Sprague–Dawley rats to localize sounds. Stimuli were 300-msec noise pulses at 70 dB SPL emitted from one of two loudspeakers. The rats, ages 12 and 30 months, were trained to press a lever (for food reward) close to the speaker emitting sound during a trial. Both old and young rats acquired the learned response quickly, but the percentage of correct responses reached an asymptotic level which was lower (i.e., fewer correct responses) for the older rats. In another study, Brown (1984) used a longitudinal design with Sprague–Dawley rats, training them to press one bar (on the left side of the testing apparatus) if a pink noise was presented from the left side and to press a bar on the right when the sound was presented on that side. By 21 months of age, accuracy of localization had declined from greater than 90% to less than 69% correct. Both studies indicate that aging is accompanied by decreased directional hearing ability in Sprague–Dawley rats. In another example, Sinnott *et al*. (1997) used food reinforcement in a go/nogo detection paradigm to demonstrate age-related changes in responses to of gerbils to human vowel sounds.

In summary, it is clear that a number of behavioral methodologies can be applied to the study of presbycusis in animals. Those mentioned here are applicable to a range of animals from rodents to humans and other primates. Additional techniques are available that may be less broadly applicable, such as more difficult or complex tasks that can be used with chimpanzees.

IV. The Animal Models

A. Mice

Mice have been used increasingly in the past two decades as subjects for auditory research (Willott, 1991; Henry and McGinn, 1992). For gerontological research, mice are unparalleled as experimental subjects with regard to the range of time courses and severity of age-related hearing loss, the various types of cochlear pathology that occur, availability of mutants for immunological and other disorders affecting hearing in adults, genetically engineered mice, and the advanced state of mouse genetics (Willott, 1996b). These factors, combined with the general practical and economic advantages of mice as gerontological research subjects, point to an exciting future in presbycusis research.

The auditory system of *Mus musculus* is functionally mature by age 1 to 1½ months (Shnerson and Pujol, 1983). Thus, in strains or mutants that hear well at this age it can be assumed that the auditory system has developed ''normally.'' That is, early-onset cochlear hearing loss and associated dramatic effects on auditory development have not occurred. Thus, age effects are not confounded by developmental ones. Subsequent changes related to gradual adult-onset hearing loss and/or biological aging can then be interpreted within the gerontological context. Strains vary from those which maintain rather good hearing throughout most of their life (e.g., CBA, C3H, many F1 strains) to those which exhibit very early progressive loss of hearing (e.g., the DBA/2J strain). In the latter regard, there are few if any members of other species that reliably exhibit progressive hearing loss before old age. Table 42.1 summarizes the literature showing properties of age-related hearing loss for a number of mouse strains and mutants. Especially noteworthy are the following.

TABLE 42.1. Age-Related Hearing Loss in Various Mouse Strains and Mutants

Mouse	Reference[a]	Description of hearing loss
Inbred strains		
CBA	1	Little age-related loss through 18 months to 2 years
CBA/J,	2,3,4	
CBA/H-T6J	5	
CBA/CaJ	6,7	
C3H/HeJ	8,9	Maintains good hearing for 18 months or more
C3H/HeSnJ	10	
SJL/J	11,12	Little loss of hearing at 200 days
BALB/c	5,13	Hearing loss appears during first year of life
	14,15	
WB	5,13	Hearing loss appears during first year
A/J	11,12	Relatively high AP thresholds at 50 days but little further loss at 200 days
AU/SsJ	11,12	AP threshold elevations from 4 to 64 kHz by 200 days
AKR/J	11,12	AP threshold elevations (especially high frequencies) by 200 days

TABLE 42.1. (Continued)

Mouse	Reference[a]	Description of hearing loss
C57BL/6	3,12,3	Progressive high frequency loss after 2 months, severe by 1 year, profound by 2 years
C57BL/6J	4,6	
C57b/16	16	
C57BR/cdJ	11,12	Similar to C57BL/6 but at an accelerated rate, losses already evident at 50 days
C3H/*lpr*	9	Elevation of thresholds by 10 months in conjunction with autoimmune disease
SAM-P/1	17	High and low frequency losses by 6 months, profound by 12 months
SAM-R/1	17	Similar to SAM-P/1 but slower progression, profound loss by 20 months
LP/J	18	Relatively high AP thresholds at 50 days, profound loss by 200 days
DBA/2J	5,14	Rapid age-related loss, beginning by 1 month, profound by 3–5 months
	19	
CD/1	20	Hearing loss probably too early, rapid for aging research
BXD strains	21	All 25 BXD strains exhibit hearing loss ranging from worse than DBA to C57-like
129/J	22	These strains exhibited elevated ABR thresholds before the age of 3 months; some might be useful as models of early-onset presbycusis; most are presumably not good candidates for studies wishing to use relatively old mice that can hear
129/ReJ		
129/SvJ		
ALR/LtJ		
ALS/LtJ		
BUB/BnJ		
C57BLKS/J		
C57BR/cdJ		The same study also identified more than 60 other inbred strains that had good hearing at 3 months; some of these may have potential as later onset or normal-hearing aging models
C57L/J		
I/LnJ		
MA/MyJ		
NOD/LtJ		
NOR/LtJ		
SKH2/J		
Outbred Strain		
NMRI	23	Gradual behavioral threshold elevations through 18 months, 20–30 dB for 60–80 kHz, <20 dB for 15–40 kHz
F1 hybrids		
CBA/BALB	5	Normal hearing to 2+ years
CBA/WB		
CBA/C57		
CBA/DBA		
DBA/BALB	5	Severe hearing loss during first year of life
DBA/WB		
DBA/C57		
C57/WB	5	Moderate hearing loss by 2 years but better than parental inbred strains
C57/BALB		
BALB/WB		
CBA/CaJ/ AU/SsJ	24	Excellent hearing to at least 1 year of age
"BD"	25	Moderate hearing loss during first year of life, profound by 18 months
Mutants		
Dancer	26	Progressive hearing loss between 1 and 13 months (but much variability among individuals)
Shaker-1	27,28	Some hearing, but early, rapid degeneration; deaf by 3–7 weeks of age
Waltzer		
Pirouette		
New	8	Profound hearing loss from birth, but cochlear pathology progressed through 18 months
Light (Blt)	29	Reduced endocochlear potentials during the first year of life
Hypophosphatemia	30	Abnormal phosphate metabolism; late-onset hearing loss
Trembler	31	PMP-22 homozygote; progressive hearing loss
Mov-13 transgenic	31	COL1A1 heterozygote heterozygotes; progressive hearing loss

[a] 1, Wenngren and Anniko, 1988a; 2, Henry and Chole, 1980; 3, Hunter and Willott, 1987; 4, Willott, 1986; 5, Erway *et al*., 1993; 6, Li and Borg, 1991; 7, Henry, 1982; 8, Kitamura *et al*., 1991; 9, Trune *et al*., 1989; 10, Trune *et al*., 1996; 11, Shone *et al*., 1991a; 12, Henry, 1983; 13, Jimenez *et al*., 2000; 15, Willott *et al*., 1998; 16, Mikaelian, 1979; 17, Saitoh *et al*., 1994, 1995; 18, Chole and Henry 1983a,b; 19, Willott *et al*., 1984; 20, Shone *et al*., 1991b; 21, Willott and Erway, 1998; 22, Zheng *et al*., 1999; 23, Ehret, 1974; 24, Henry *et al*., 1992; 25, Church and Shucard, 1986; 26, Wenngren and Anniko, 1988b; 27, Deol, 1956; 28, Steel *et al*., 1983; 29, Cable *et al*., 1993; 30, Steel *et al*., 1989; 31, Steel, 1995.

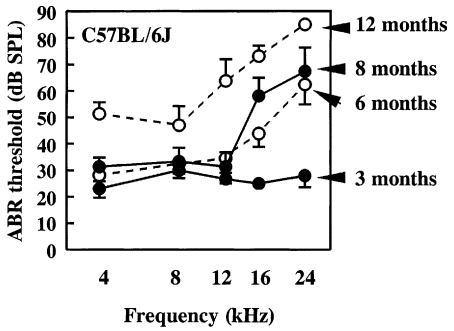

FIG. 42.3. ABR thresholds from C57 mice as they age. These thresholds were obtained longitudinally from the same set of mice. Earliest and most severe losses are at high frequencies. Data are from routine testing in our laboratory.

C57BL/6J mice (C57; also called B6) have been the most extensively studied animal model of age-related hearing loss. Peripheral function develops in an apparently normal fashion, being optimum between 1 and 2 months of age. Sometime during the following few months, however, high frequency sensitivity begins to decline. By 6 months of age loss of high frequency sensitivity (e.g., >20 kHz) is significant; by 1 year, losses are severe and have begun to encompass lower frequencies; after about 15 months of age, thresholds for all frequencies are typically in excess of 80 dB SPL (Mikaelian, 1979; Henry, 1983; Willott, 1986; Li and Borg, 1991). The advantage here is that the onset of hearing loss in most humans suffering from presbycusis likewise occurs after maturation has progressed beyond sensitive developmental periods; like C57 mice, most people begin their adult life with good hearing. Figure 42.3 shows ABR thresholds revealing age-related hearing loss in C57 mice.

It is now known that a recessive gene is responsible for the progressive hearing loss in C57 mice (Erway *et al*., 1993; Willott *et al*., 1995; Johnson *et al.*, 1997). The *Ahl* gene (for *a*ge-related *h*earing *l*oss) is found on chromosome 10 and results in damage to the cochlea, characterized by a consistent pattern of histopathology (Mikaelian, 1979; Henry and Chole, 1980; Cohen and Grasso, 1987; Willott and Bross, 1990; Li and Borg, 1991; Mizuta *et al*., 1993; Spongr *et al*., 1997). Prior to 2–3 months of age, little cochlear pathology is evident in C57 mice. By 6 months, degenerative changes of the organ of Corti (e.g., distortion, clumping, and loss of outer hair cells) have begun and are most pronounced in the basal turn. The loss of outer hair cells is more severe than, and precedes the loss of inner hair cells, and this is reflected by changes in otoacoustic emissions (Parham, 1997; Jimenez *et al*., 2000). Supporting cells also show degenerative changes. By 2 years of age the basal region of the C57 organ of Corti is virtually devoid of recognizable structures. A pronounced loss of spiral ganglion cells occurs in aging C57 mice, with nearly complete loss in the basal cochlea during the second year of life. Some atrophy of the stria vascularis is observed in aging C57 mice, but the most striking strial changes is an increase in pigment. Histopathological changes are shown in Fig. 42.4.

Vascular dysfunction occurs as well. Brown *et al*. (1995) evaluated vascular changes in the aging inner ear of C57 mice. Microvascular reactivity was monitored by laser Doppler flowmetry and assessed by the change in cochlear vascular conductance in response to the application of sodium nitrorusside (a vasodilating agent) to the round window. In C57 mice vascular conductance increased less than that of young mice and age-matched CBA mice, which continued to hear well (see Table 42.1). There were also differences in the duration of the vascular conductance change. The findings suggest that cochlear blood flow may be altered in hearing-impaired C57 mice.

DBA/2J mice (DBA; also called D2) exhibit optimal hearing before 25 days of age, but sensitivity rapidly deteriorates thereafter (Ralls, 1967; Willott, 1981; Willott *et al*., 1984). By 5 months of age, hearing loss is severe, and anatomical signs of degeneration seen in the outer hair cells, inner hair cells, and spiral garglion cells. DBA mice also possess the *ahl* gene, but also appear to have one or two additional genes with deleterious effects on hearing.

BALB/c mice have a time course and pattern of hearing loss somewhat like that of C57 mice, although different substrains may progress faster (Willott *et al*., 1998) or slower (Jimenez *et al*., 2000) than C57. BALB/c mice also possess a recessive gene but it is probably not *Ahl* (Erway *et al*., 1993). Like the other strains, BALB/c exhibits a progressive loss of hair cells and spiral ganglion cells that is most severe in the cochlear base and least severe in the middle turns; however, BALB/c mice have relatively more spiral ganglion cells loss in the apex.

BXD recombinant inbred strains of mice have been widely used in genetic studies using quantitative trait loci analysis and other methods. Numerous genetic markers have been identified for the BXD strains, thus providing a powerful method of examining correlations between phenotypes (e.g., hearing loss) and genotype. The BXD strains were derived by successive brother–sister matings from original F1 hybrids of C57 and DBA strains. As indicated above, both of the parental strains are homozygous for the *Ahl* gene, so all BXD strains possess the gene (and some are also homozygous for the other one or two hearing-loss genes of DBA). Whereas all 25 BXD strains exhibit progressive hearing loss (Willott and Erway, 1998), the severity ranges from worse-than-DBA to C57-like in time course and severity. This suggests that the number of hearing-loss genes (one, two, or three) and/or genetic background contribute to the severity of hearing loss. Ultimately, selected BXD strains can be used to ''vary'' the time course of hearing loss and elucidate the genetics of age-related hearing loss.

129-Related inbred strains are the source of most embryonic stem cells used to create targeted mutations. 129 Strains exhibit moderate hearing impairment at 7 months (Zheng *et al*., 1999). Because 129 and C57 mice are commonly used in genetic engineering, caution is warranted in the use of genetically altered mice in presbycusis research.

Mice with genetic mutations often have severe congenital or early-onset ear pathology (Steel *et al*., 1983; Steel, 1995). However, some of these mutants may be useful in aging research. For instance, the Dancer mutant succumbs to progressive hearing loss during the first year of life (Wenngren and Anniko, 1988b), making it a potential model for studying the genetics of presbycusis.

Mice that retain good hearing until relatively late in life include outbred NMRI mice, CBA strains, C3H strains, and

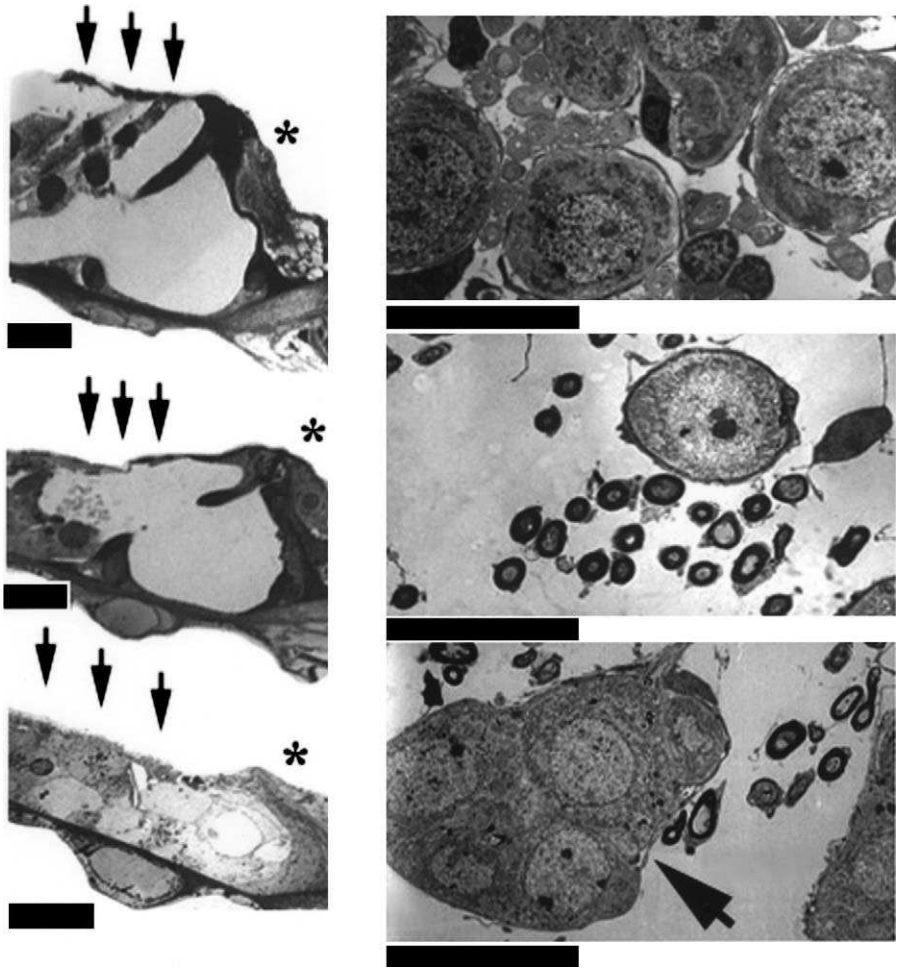

FIG. 42.4. Some age-related histopathological changes in the C57 mouse cochlea. Scale bars, 10 μm. The left column shows changes seen in the basal region of the organ of Corti. The arrows point to the location of the three rows of outer hair cells; the asterisks are above the inner hair cells. The top micrograph (a semithin section) shows the earliest histopathological changes, degeneration of outer hair cells in the outer row; such changes can be seen as early as 2–3 months of age in the extreme cochlear base. Progressive degeneration gradually occurs, as all outer hair cells in the basal cochlea disappear (middle micrograph); most inner hair cells remain intact. At the bottom is an electron micrograph showing complete loss of organ of Corti structures that occur during the second year of life. The organ of Corti, including the IHCs, is replaced by a nonfunctional sheet of cells. The right column shows electron micrographs of spiral ganglion cells. In young, normal-hearing mice, spiral ganglion cells are packed tightly within the cochlea (Rosenthal's canal). Compare this with the middle micrograph obtained from an older mouse, containing only a few somata and cross sections of myelinated axons. The bottom micrograph shows clumping of spiral ganglion cells (large arrow) that occurs in the spical region of the cochlea—another type of pathology that is typical of aging C57 mice.

a number of F1 hybrids. CBA mice in particular have been used extensively in research on aging and the auditory system, when a model of mild presbycusis is desired. They do not exhibit age-related hearing loss until relatively late in life (Henry, 1983; Willott, 1986; Wenngren and Anniko, 1988a; Willott *et al*., 1988a). CBA mice show virtually no loss of hair cells at 16 months of age; at 2 years of age a few inner hair cells have been lost, and there is a small loss of outer hair cells with a total loss only at the extreme cochlear base (Henry and Chole, 1980). Also, virtually no loss of spiral ganglion cells is observed during the first year of life of CBA mice, and only a minimal decrease throughout the cochlea is seen by 2 years. There are age-related changes in CBA mice, however. Suzuki *et al*. (1998) obtained evidence that ears of aging of CBA mice (21 months) had reduced capacity to maintain stable blood flow. They measured cochlear blood flow with laser Doppler flowmetry during intermittent occlusion of the anterior inferior cerebellar artery, which alters cochlear perfusion pressure and causes a decrease in blood flow. The compensatory dilatory response was reduced in the old mice. In another study, Ding *et al*. (1999) measured dehydrogenase and glycogen levels in the CBA cochlea and found evidence for altered energy metabolism in aging mice.

Another model for minimal presbycusis is the B6.CAST–$^{+ahl}$/+ congenic strain. These mice were produced from back crosses of C57 with CAST/Ei with marker assisted selection to produce a congenic line of B6 mice that lacks the *ahl* gene and, therefore, has normal hearing (Johnson *et al*., 1997). Because they are genetically very similar to C57 but hear normally, comparison of 6-month-olds of the two strains

can help to isolate the effect of hearing loss from other genetic influences on the central auditory system.

Mice with genetically determined age-related cochlear pathology prior to old age provide excellent models for central effects for peripheral pathology, while those maintaining good hearing can be used to evaluate central effects of biological aging. The most extensive and detailed life span studies on the central auditory system have been done with C57 and CBA mice, and they exemplify, respectively, aging with and without significant cochlear pathology, allowing elucidation of central effects of peripheral pathology and central effects of biological aging, respectively.

Anatomical studies of C57 mice have found that age-related sensorineural cochlear pathology is associated with changes in the neuropil volume of cochlear nucleus subdivisions and the size and number of neurons (Browner and Baruch, 1982; Willott *et al*., 1987, 1992, 1997; Briner and Willott, 1989; Willott and Bross, 1990). However, the occurrence of these effects depends upon the subdivision examined—the anterior ventral cochlear nucleus (AVCN), posterior ventral cochlear nucleus (PVCN), and dorsal cochlear nucleus (DCN). Those regions heavily innervated by the terminals of primary fibers from spiral ganglion cells (AVCN, PVCN, layer III of DCN) are relieved of much of their normal input from the impaired cochlea, and it is here that anatomical signs are most pronounced. In contrast, DCN layers I and II, which receive little direct primary fiber input, and higher-order structures such as the inferior colliculus, which receive no cochlear input, show fewer anatomical changes through the median life span age of 2 years (Willott *et al*., 1994a). Nonetheless, higher-order structures do exhibit age effects such as changes in synaptic density (Kazee *et al*., 1995), calcium binding proteins (O'Neill *et al*., 1997; Zettel *et al*., 1997; Idrizbegovic *et al*., 1999), and other neurobiological variables.

Some morphological changes in the cochlear nucleus of DBA and BALB/c mice are similar to those in C57 mice. However, because the time course of progressive cochlear pathology differs, so do the central changes. Figure 42.5 shows the relative age-related change in AVCN volume for C57, DBA, BALB/c, and normal-hearing CBA strains. Note that even though cochlear pathology and central changes are rapid and severe in DBA mice, the ultimate loss of neurons and AVCN volume reaches a stable level similar to that in C57 and BALB/c mice, with no additional changes thereafter. AVCN volume in normal-hearing CBA mice grows during year 1 before starting to decline gradually.

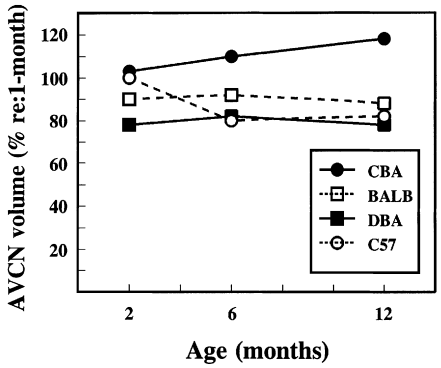

FIG. 42.5. Relative change in the volume of the anterior ventral cochlear nucleus of four inbred strains of mice. CBA mice retain good hearing as they age, and exhibit a maturational increase in AVCN volume. The other three strains exhibit early progressive hearing loss and lose AVCN volume. Data were obtained from Willott and Bross (1996) and Willott *et al*. (1987, 1998).

Neurophysiological studies have also evaluated central effects of peripheral pathology and central effects of biological aging in C57 and CBA mice. In either strain, neural coding activities that rely on populations of neurons must do so with diminished numbers due to threshold elevations and an increase in "sluggish neurons"—those that respond to sound, but do so unreliably (Willott *et al*., 1988a,b). Nonetheless, the responses of most ("nonsluggish") neurons in middle-aged C57 and very old CBA mice reveal a remarkable degree of "normalcy," at least when simple tones are used as stimuli. When more complex auditory processing is required, age effects become more prevalent, such as deficits in gap detection (Walton, *et al*., 1995, 1998; Ison *et al*., 1998).

As C57 mice lose high-frequency hearing, the way frequency is represented in the central auditory system is altered (Willott, 1986, Willott *et al*., 1988b). The inferior colliculus central nucleus is tonotopically organized: neurons in the superficial portions are sensitive to low-frequency sounds, whereas neurons in progressively deeper regions are sensitive to progressively higher frequencies (Stiebler and Ehret, 1985, Willott, 1986). In the ventral inferior colliculus, neurons respond very well to high-frequency sounds but do not respond to low or middle frequencies. As C57 mice age and lose functioning of the high frequency (basal) portion of the cochlea, neurons that were formerly (normally) sensitive only to high-frequency sounds become sensitive to lower frequencies. In this form of hearing-loss-induced plasticity there is a reorganization of frequency representation in older C57 mice [see Willott (1996a) for a detailed discussion]. The degree of reorganization of frequency representation differs considerably in different parts of the central auditory system. In the auditory cortex, hearing-loss-induced plasticity is even more pronounced than it is in the inferior colliculus as virtually the entire auditory cortex quickly becomes responsive to low and middle frequencies (Willott *et al*., 1993). By contrast in the AVCN and PVCN, the lowest-level central auditory system structures, neurons exhibit elevation of thresholds but little if any plasticity of the type seen at higher levels (Willott *et al*., 1991). Thus, the cochlear nucleus regions, which exhibited the greatest degenerative anatomical changes associated with central effects of peripheral pathology (see above), showed the least amount of physiological plasticity in the representation of frequency.

B. Gerbils

The Mongolian gerbil has an audibility curve similar to that of humans, despite its small size (small rodents typically have a higher frequency range of hearing, as demonstrated in mice). This makes gerbils an attractive animal model for auditory research. For gerontological studies, it shares the practical advantages of mice, being economical to maintain, with a life span of about 3 years. Mongolian gerbils are not explicitly inbred, which can be viewed as a plus because humans are also genetically diverse. Thus, consistent findings related to age are

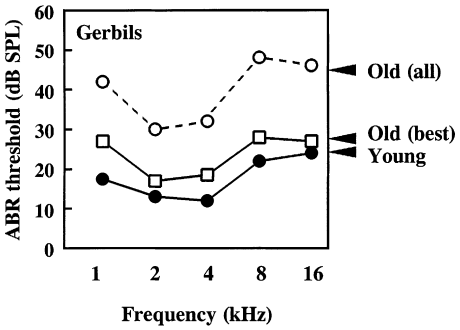

FIG. 42.6. Age-related hearing loss in gerbils. Because of the between-subject variability, ABR thresholds are shown from all old animals as well as the four best. The latter exhibit minimal loss compared to young gerbils, but overall a flat pattern of hearing loss is seen. Data were obtained from Boettcher *et al.* (1993).

presumably robust across genotypes. On the minus side, gerbils exhibit a great deal of variability in the severity and time course of presbycusis, one of the disadvantages shared by studies of humans.

As shown in Fig. 42.6, gerbils usually maintain good hearing through 16–18 months of age, then exhibit losses (Mills *et al.*, 1990). However, threshold elevations are typically not severe even by age 3 years (life expectancy), and the high-frequency losses are not substantially greater than the low-frequenciy losses (Henry *et al.*, 1980; Boettcher *et al.*, 1993a).

Scanning electron microscope material obtained by McGinn and Chole (cited in McGinn and Faddis, 1987) revealed little loss of hair cells or other cochlear pathology in gerbils as old as 30 months. Tarnowski *et al.* (1991) also found that inner hair cell loss was minimal in old gerbils, and whereas some outer hair cell losses occurred, they were quite variable between animals and correlated poorly with threshold shifts. Keithley *et al.* (1988) found a loss of spiral ganglion cells appearing first at 24–30 months of age. In the oldest animals (36–42 months) a 15–25% loss occurred. Recently, Zheng *et al.* (1998) presented evidence suggesting that the sensorineural damage that does occur involves apoptosis. The development of vacuoles was seen in the auditory nerve, but only central to the Schwann–glial border (Keithley *et al.*, 1988). The age-related development of vacuolar lesions in the gerbil cochlear nucleus appears to involve degeneration of dendrites (Faddis and McGinn, 1997), although their functional significance is not clear.

The somewhat flat pattern of hearing loss and moderate sensorineural pathology in old gerbils suggest strial pathology. This has been supported by a series of studies from the Medical University of South Carolina, which included quiet-aged gerbils. They found considerable evidence for the occurrence of progressive changes in the vasculature and integrity of the stria vascularis and associated events such as Na^+,K^+-ATPase activity and its effects on the endocochlear potential (Schulte and Schmiedt, 1992; Gratton *et al.*, 1995, 1996, 1997; Schmiedt, 1996; Adams and Schulte, 1997) and deposition of the glycoprotein laminin in strial capillaries (Sakaguchi *et al.*, 1997).

The South Carolina team also demonstrated a variety of electrophysiological changes in quiet-aged gerbils. ABRs had reduced amplitudes, altered latencies, stable responses to stimulus repetition, and abnormal response to noises separated by gaps in ABR wave IV but not wave II (Boettcher *et al.*, 1993a,b, 1995, 1996). Compound action potentials and auditory nerve fibers also showed age-related changes including altered input–output intensity functions, reduced frequency selectivity, and a change in spontaneous activity of nerve fibers (Hellström and Schmiedt, 1990, 1991, 1996, Schmiedt *et al.*, 1990, 1996).

C. Rats

Rats have some compelling advantages as an animal model for aging research. Most notably, a large gerontological database is accruing for neurogerontological research in rats, as is evident in other chapters (see also Willott, 1999). Thus, a great deal is known about the aging rat nervous system, and many neurobiological techniques are available. Moreover, rats have been the favorite subject in behavioral research, which should facilitate behavioral evaluations of auditory function in aging animals. Most of the presbycusis research on rats has used the Sprague–Dawley and Fischer 344 strains.

In Sprague–Dawley rats, Crowley and colleagues (1972a,b) found that the cochlear sensitivity for click stimuli was optimal at 12 months and then declined by about 12 dB at 24 months. Cooper *et al.* (1990) observed ABR threshold increases of about 18 dB at 3 kHz, 14 dB at 8 kHz, and 32 dB at 40 kHz in 24 to 29 month-olds. Longitudinally measured behavioral thresholds for narrow noise bands remained unchanged through 14 months but exhibited progressive elevations through 30–39 months (Turnock and Harrison, 1975; Harrison, 1981). Rats over 30 months old typically showed a hearing loss of 10–15 dB at frequencies above 32 kHz and below 1 kHz, a less pronounced age effect than that of Cooper *et al.* (1990). However, their ABR study used tone bursts whereas the behavioral work used filtered noise bands.

Keithley and Feldman (1982) observed only small degrees of hair cell degeneration beginning by 6 months of age, with greater vulnerability of outer hair cells as opposed to inner hair cells. The greatest losses of hair cells occurred at the cochlear base and apex. Keithley and Feldman (1979) found a significant reduction in the number of spiral ganglion cells by 23 months with little apparent further loss through 34 months. The losses occurred throughout the length of the cochlea, but were greatest near the base and apex. Type I ganglion cells (which synapse on inner hair cells) were lost to a greater degree than inner hair cells, suggesting that at least some type I spiral ganglion cells were degenerating for reasons other than the loss of the hair cells that they innervated. Conversely, they saw the disappearance of outer hair cells at the cochlear base, but not type II spiral ganglion cells presumed to innervate them, suggesting that outer hair cells can atrophy without involving neural cells. Hoeffding and Feldman (1988) showed that the median number of normal auditory nerve fibers was reduced by 21% at age 26.5 months and by 24% at 36 months. Old Sprague–Dawley rats also show evidence of altered basilar membrane composition with respect to fibronectin and the number of mesothelial cells (Keithley *et al.*, 1993).

Fischer 344 rats show a loss of sensitivity with aging that is probably more severe than that of Sprague–Dawley rats. By 25 months of age, there is an elevation in click thresholds of 20

dB (Simpson *et al*., 1985), whereas thresholds to tone pips (3, 8, and 40 kHz) were on the order of 30 dB or more (Cooper *et al*., 1986). Recent work indicates that older Fischer 344 rats exhibit decreased cochlear vascular function (Seidman *et al*., 1996). There is also evidence that deletions of mitochondrial DNA may contribute to presbycusis in these rats (Seidman *et al*., 1997).

A good deal of gerontological research has focused on the rat central auditory system. A series of studies on the medial nucleus of the trapezoid body of Sprague–Dawley rats revealed a number of age effects (Casey and Feldman, 1982, 1985a,b, 1988). These included a loss of neurons, increased pigment deposits, neuronal degeneration, and vascular abnormalities. Other studies have demonstrated age-related decrease in inhibition in the lateral superior olive (Finlayson, 1995), age-related increases in glial fibrillary acid protein (a marker for gliosis) in the cochlear nucleus (Jalenques *et al*., 1997), and hearing-loss-induced plasticity in the inferior colliculus (Keithley *et al*., 1994).

The inferior colliculus makes great use of gamma-aminobutyric acid (GABA) in its neural circuits. Caspary, Milbrandt, and colleagues have provided strong evidence that inhibitory processes involving the neurotransmitter GABA become diminished in the inferior colliculus of aging Fischer 344 rats (Caspary *et al*., 1990, 1995; Milbrandt *et al*., 1994, 1996, 1997; Raza *et al*., 1994; Milbrandt and Caspary, 1995). Older rats are characterized by fewer inferior colliculus neurons containing GABA (as shown by immunocytochemistry), decreased basal concentrations of GABA, decreased release of GABA by inferior colliculus neurons, decreased activity of glutamic acid decarboxylase (an enzyme involved in making GABA), changes in GABA receptors at synapses, and a decrease in the number of presynaptic terminals using GABA. Neural responses of inferior colliculus neurons are altered as well, some of which suggest decreased inhibition (Palombi and Caspary, 1996a,b).

D. Chinchillas

Chinchillas are a popular animal model for auditory research, having a number of technical advantages, a good ability to learn discrimination tasks, and an audibility curve which is similar to that of humans (Miller, 1970). With respect to gerontological auditory research, chinchillas appear to have low susceptibility to middle ear infections and a life span of about 20 years.

Bohne *et al*., (1990) provided a detailed, quantitative description of the histopathology of the inner ear of 80 chinchillas, some as old as 19.2 years. All aging animals exhibited some degeneration of the organ of Corti, but it was generally mild. Only 5% demonstrated primary neural degeneration. Outer hair cells degenerated by about 1% per year compared to 0.35% per year for inner hair cells. Many animals had circumscribed regions of hair cell loss, and these became more prevalent with age. Total degeneration of regions of the organ of Corti was rare. Some strial degeneration and lipofuscin accumulation were also typical of old chinchillas.

McFadden *et al*. (1997a) obtained auditory evoked potential thresholds and also assessed outer hair cell function with distortion product otoacoustic emissions and histopathological evaluations. Like Bohne *et al*. (1990), they found a small but significant decline in auditory sensitivity and outer hair cell function. The losses were greatest at higher frequencies, as shown in Fig. 42.7. This, along with the slow time course of hearing loss, recommend chinchillas as a model for the study of aging when mild cochlear pathology occurs. Because the old animals continue to hear relatively well, gerontological experiments elucidating suprathreshold auditory responses can be performed. In this regard, McFadden *et al*. (1997b) obtained several measures of suprathreshold auditory function (e.g., frequency resolution, forward and remote masking) before and after exposure to noise. They observed abnormal responses, even for frequency ranges where hearing thresholds were near normal.

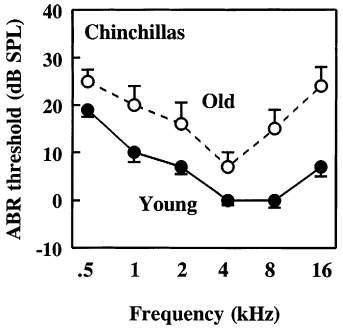

FIG. 42.7. Age-related hearing loss in chinchillas. High-frequency ABR threshold elevations are slightly greater. Data were obtained from McFadden *et al*. (1997a,b).

E. Guinea Pigs

Guinea pigs have been used widely in auditory research, and have been the subject of several aging studies. Dum *et al*. (1980) measured ABR thresholds and found them to be near normal at 15 months, but elevated by 30–40 dB at 24 months with little further change by 3 years. The threshold elevations were relatively "flat," being similar for frequencies of 500 to 15,000 Hz. Proctor *et al*. (1998) and Ingham *et al*. (1998) obtained similar found ABR threshold elevations in animals aged to 3 and 4 years, respectively. However, there appears to be considerable between-subject variability in ABR changes in aging guinea pigs (Nozawa *et al*., 1996). For example, Ingham *et al*. (1998) found no evidence for retrocochlear deficits, whereas Proctor *et al*. (1998) reported markedly reduced ABR amplitudes. In addition, Ingham *et al*. (1998) found evidence for conductive hearing loss in old animals (mean elevation for click thresholds was 32 dB), but 24% appeared to have severe sensorineural hearing loss in which ABRs could not be elicited. More than 40 years ago Pestalozza *et al*. (1957) also concluded that both middle ear conductive and cochlear sensorineural hearing loss occurred in older guinea pigs.

Covell and Rogers (1957) were the first to describe the histological features of the aging guinea pig inner ear. At 5 years of age, pathology of the stria vascularis was not consistent, but included regional atrophy and increased pigmentation. The most prominent age-related change was a decrease in the number of spiral ganglion cells near the cochlear apex and, to a

lesser extent, in the base (not the typical pattern observed in humans and other animals). Fiber degeneration proceeded in each of the bipolar directions (into the eighth nerve and toward the organ of Corti), suggesting primacy of neural degeneration. Degenerative changes in the organ of Corti were prominent only in the oldest guinea pigs and only in the regions of spiral ganglion cell loss. Recently, Ingham *et al*. (1999) reported about a 20% loss of outer hair cells restricted to the cochlear apex of 4.5-year-olds. Other studies were consistent with these in finding little hair cell degeneration, but failed to include particularly old animals (see Willott, 1991).

F. Cats

Historically, cats have been one of the most important animal models for auditory anatomical and physiological research, and much is known about the cat auditory system. However, their utility as subjects for aging research is limited by their relatively long life span and lack of availability of old research subjects, particularly ones that have been maintained under known environmental conditions.

Auditory thresholds become elevated in aging cats. Harrison and Buchwald (1982) reported that the mean thresholds for clicks presented in the free field were elevated by about 30–50 dB in cats ages 12 to 23 years. In older cats of unspecified ages, Schuknecht (1955) found atrophic changes in the organ of Corti and loss of efferent and afferent nerve fibers, especially nearer the cochlear base. However, the histopathological profiles were rather variable within the general framework of sensorineural atrophy. Behavioral audiograms differed substantially as well. Spoendlin (1970) presented electron micrographs from a 13-year-old cat "which apparently did not hear well." The supranuclear portion of the outer hair cells appeared normal but extremely large lysosomal bodies, filled with dense inclusions, were seen in the subapical area of outer hair cells. There was also a marked reduction in the efferent innervation of the organ of Corti. An age-related increase in stria vascularis pigment has been reported in old cats (Conlee *et al*., 1989).

G. Dogs

Whereas dogs are rarely employed in auditory research, they do exhibit age-related cochlear histopathology reminiscent of that observed in humans. Johnsson and Hawkins (1972, 1979) indicated that the inner ear of several dogs they examined histologically had changes that included strial atrophy, sensorineural degeneration, and vascular disturbances. Knowles *et al*. (1989) examined the middle and inner ears of dogs aged 1.5 to 17 years. The middle ear ossicles appeared normal, but the density of spiral ganglion cells was reduced, primarily in the basal turns of the cochlea. In dogs with elevated ABR thresholds, the density of spiral ganglion cells was reduced to 40% in the lower basal region. Some dogs had severe hearing loss and these had nearly complete loss of spiral ganglion cells in the basal turn. Recently, Shimada *et al*. (1998) supported these findings, showing similar cochlear changes in old dogs. All in all, dogs may be a good model for post mortem studies of cochlear histopathology and aging.

H. Nonhuman Primates

Monkeys and chimpanzees have the advantage of being relatively closely related to humans. Of course, this also results in their primary disadvantages—a rather long life span, great expense, and ethical/political issues that complicate their use in research. Consequently, only a few studies of presbycusis in nonhuman primates have been performed.

Bennett *et al*. (1983) used psychophysical techniques to show that old rhesus monkeys exhibited presbycusis, especially showing losses at high frequencies. Hawkins *et al*. (1985) presented histopathological data on 15 rhesus monkeys, ages 4 to 31 years. Missing hair cells were seen in aging monkeys, particularly in the extreme cochlear base and apex. Their three 31-year-olds had a complete loss of inner and outer hair cells and nerve fibers in the first 2–5 mm of the basal turn. Loss of nerve fibers was seen in the same regions as hair cell loss and was interpreted as being secondary. A partial loss of spiral ganglion cells in the basal turn was characteristic of older animals. There were few consistent changes in the stria vascularis, spiral ligament, and Reissner's membrane (except for an increased number of lipofuscin granules in the oldest group). Taken together, the authors saw the changes as similar, albeit of lesser magnitude, to those typical of sensorineural presbycusis in humans.

Johnsson and Hawkins (1972) examined the temporal bone of a 39-year-old chimpanzee. The animal exhibited relatively mild outer hair cell loss, mainly in the basal turn, and diffuse strial atrophy throughout the cochlea. More recently, Cone-Wesson *et al*. (1995) obtained distortion product otoacoustic emissions and ABR thresholds from three chimpanzees ages 5, 14, and 38. Compared to the younger chimps, the 38-year-old, Susie, had elevated ABR thresholds and reduced distortion product otoacoustic emission amplitudes, indicative of hearing loss similar to that observed in humans. Distortion product otoacoustic emission amplitudes are shown in Fig. 42.8.

I. Other Species

Little work on presbycusis has been done on other species of research animals. Bhattacharyya and Dayal (1985) evaluated surface preparations of cochleas from 4-year-old rabbits. While this is not an extremely old age for rabbits, which

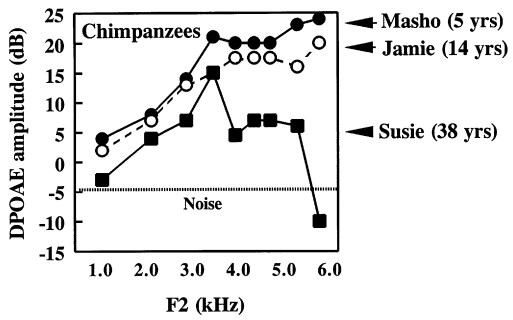

FIG. 42.8. Distortion product otoacoustic emissions from three chimpanzees. The responsiveness of the outer hair cells to low frequencies (indicated by the amplitude of the distortion product otoacoustic emission) is relatively normal in the old chimpanzee, Susie, but high frequency amplitudes are clearly reduced. Data were obtained from Cone-Wesson *et al.* (1995).

may live 6–10 years, mild damage to the organ of Corti was observed in both base and apex. In quail, Ryals and Westbrook (1988) found that age-related hair cell loss was minimal, amounting to less than 10% in the oldest birds. By contrast, 20–60% of spiral ganglion cells were lost in the old group, with the greatest losses occurring in the middle section of the papilla. Accumulation of lipofuscin was also seen in aged spiral ganglion cells. Despite the paucity of research, there is evidence of some age-related hearing loss, even in these species.

V. Some Topics Best Studied with Animal Models

As indicated earlier, ethical and practical issues often dictate the use of animal models. Three types of research requiring animal models are presented here as relevant examples.

A. Effects of Dietary Restriction

It is well established that restriction of caloric intake can have beneficial effects on the health and longevity of rodents (e.g., Ausman and Russell, 1990; Bronson and Lipman, 1996). Of course, controlled dietary restriction studies require the use of animal models. Several studies have evaluated age-related hearing loss in this respect. Feldman (1984) examined the cochleas of Sprague–Dawley rats whose lives were extended to 45–48 months by dietary restriction. The cochleas of the extremely old rats exhibited degenerative changes more severe than those of normally aged rats. While the extended life (restricted diet) animals showed substantially more severe pathology, there was no difference in severity between normal reared and restricted diet rats at age 26 months. Therefore, the time spectrum of age-related change was not reshaped. Rather, the additional passage of time resulted in a continuation of degenerative change that is normally terminated by the end of life. The story is not this simple, however. Willott *et al.* (1995) evaluated the effects of a calorically restricted diet on age-related hearing loss in five inbred strains (C57, CBA, DBA, BALB/c, WB), and their 10 possible F1 hybrid strains. Both ABR thresholds and postmortem cochlear histology indicated that genotype interacted with diet with regard to age-related hearing loss and cochlear pathology. The low calorie diet was associated with amelioration, exacerbation, or no effect depending on strain (see also Henry, 1986; Sweet *et al.*, 1988; Park *et al.*, 1990). Only in four strains was evidence obtained to indicate that the low calorie diet ameliorated age-related hearing loss.

B. Vulnerability to Noise-Induced Hearing Loss

An important question is whether the older ear is more or less susceptible to hearing loss from exposure to very intense noise. This is another area of research in which ethical and practical issues favor the use of animal models, especially given the variability in noise-induced hearing loss among humans (e.g., Mills *et al.*, 1996, 1997). Animal studies have begun to address the question, but it is a complex one. It appears that when the noise exposure is moderately intense and the threshold shift is temporary, there may be little or no age differences (Sun *et al.*, 1994; McFadden *et al.*, 1998). However, when very high levels of noise are involved, age may be associated with greater vulnerability (McFadden *et al.*, 1998). Studies on mice indicate that genetic influences may play a role as well. Specifically, mice possessing the *ahl* gene, such as C57, are more vulnerable to noise-induced hearing loss (Shone *et al.*, 1991a; Li and Borg *et al.*, 1993; Erway and Willott *et al.* 1996).

C. The Effects of the Acoustic Environment

At this time, little if anything can be done to effectively slow or ameliorate the progression of sensorineural presbycusis. We have been investigating a simple yet intriguing phenomenon that holds promise as a means of altering the severity and time course of progressive sensorineural hearing loss: exposure to augmented levels of controlled acoustic stimulation, what we have termed an augmented acoustic environment (Turner and Willott, 1998). With an appropriate augmented acoustic environment, the auditory system of hearing-impaired individuals can continue to be stimulated despite threshold elevations. In C57, DBA, BALB/c, and other strains of mice with progressive sensorineural hearing loss, exposure to a 70 dB SPL broad band noise augmented acoustic environment has been shown to alter age-related hearing loss. As seen in Fig. 42.9 ABR thresholds of BALB/c mice become elevated between ages 3 and 12 months (filled circles). However, in mice maintained with the augmented acoustic environment from age 25 days (open circles), hearing loss is minimal. Effects have thus far been demonstrated with ABR thresholds, spiral ganglion cells and hair cell counts, and behaviorally by increased amplitude of the acoustic startle response and improved prepulse inhibition (Turner and Willott, 1998; Willott and Turner, 1999).

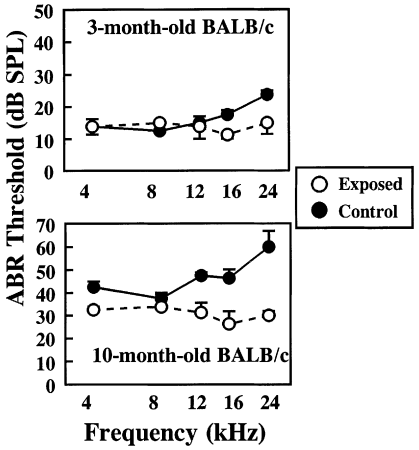

FIG. 42.9. The effect of exposure to an augmented acoustic environment on ABR thresholds. Exposed mice received a 70-dB SPL broad band augmented acoustic environment nightly from age 25 days. At age 3 months, control BALB/c mice have good thresholds, with only slight elevations at higher frequencies; exposed mice have better high frequency thresholds. By 10 months of age, BALB/c mice had significant hearing loss, but this was ameliorated in the exposed mice, particularly at high frequencies. In other words, the augmented acoustic environment slowed age-related hearing loss. Data are from Turner *et al.* (1999).

The augmented acoustic environment work is another example of auditory gerontological research requiring the use of animal models. Because the augmented acoustic environment has no effect on normal hearing mice, one must have subjects where the occurrence and time course of sensorineural hearing loss can be reliably predicted. Only then can augmented acoustic environment-exposed and nonexposed animals be compared to determine the specific effects of the augmented acoustic environment. This can be done most readily using inbred mice, such as C57 and DBA, as we have done.

VI. Evaluation of the Animal Models and Relationship to Humans

The animal model data demonstrate several important points (see also Willott *et al*., 1991).

- The various categories of presbycusis-related cochlear pathology seen in humans (Schuknecht, *et al*. 1974; Willott, *et al*. 1991) can all be seen in nonhuman animals. These include sensory, sensorineural, neural, vascular, cochlear–mechanical, and strial (metabolic) varieties.
- Because animals reared in institutional or commercial animal facilities are not usually exposed to excessive noise or ototoxic drugs, presbycusis appears to occur in mammals despite minimal ''contamination'' by industrial or urban environmental conditions.
- The differences in age-related pathology among different strains of mice demonstrate the role of genotype in certain forms of presbycusis. Genetic effects are also seen in the central auditory system. For instance, a loss of AVCN neurons was observed during the second year of life in CBA mice, but not in C57 mice (Willott *et al*., 1987), and the degree of cell loss in the modens of the trapezoid body differed for Sprague–Dawley and Fischer 344 rats (Casey and Feldman, 1982; Casey, 1990).
- At least some degree of sensorineural pathology appears to be a ubiquitous concomitant of aging across species.
- The cochlear base and, to a lesser extent, the apex are particularly vulnerable to age-related change in many species.
- Outer hair cells are more vulnerable than inner hair cells.
- The work on animal models demonstrates the complexity of age-related histopathological change in the central auditory system. Age-related changes differ among neuron types (e.g., bushy, multipolar, and octopus cells of the mouse cochlear nucleus). Within an individual, considerable variance in age-related histopathology occurs among neurons. The condition of octopus cells in aging mice ranged from normal to severely degenerated. In another example, Feldman and Vaughan (1979) noted that intermingled with neurons exhibiting signs of pathology or degeneration were many auditory cortical neurons of old rats which appeared to be free of pathology.
- The presence of high frequency hearing loss is likely to cause marked disruption of central frequency representation and other aspects of auditory processing (central effects of peripheral pathology). However, even in the absence of serious hearing loss, age-related changes can occur (central effects of biological aging).
- Very old individuals are likely to have a reduced number of central auditory system neurons. However, the losses are typically modest, even in the cochlear nucleus. The volume of central auditory system structures tends to be smaller in old individuals, with loss of neuropil contributing significantly to the reduced volume.
- There is considerable variability among individuals in the severity of presbycusis, the number of neurons surviving to old age, and many other age effects. Material from mice suggests that genetics plays a strong role in this regard. However, even in inbred mice, variance is substantial.
- While many similarities exist among humans and other species, the magnitude of age-related pathology is typically not severe in the animal models (with exceptions such as certain mouse strains). Whereas many humans are fortunate to retain relatively good hearing as they age, many others experience hearing loss that is more severe than most of the animal models. This may be due to a number of factors including the length of the life span and effects of environmental insults, diet, drugs, and genotype. While the involvement of these variables is far from being fully understood, research on animal models, with its ability to systematically control genetic and environmental variables, provides our best hope of future progress in elucidating their relevance to presbycusis.

References

Adams, J. C., and Schulte, B. A. (1997). Histopathologic observations of the aging gerbil cochlea. *Hear. Res.* **104**, 101–111.

Ausman, L. M., and Russell, R. M. (1990). Nutrition and aging. *In* ''Handbook of the Biology of Aging'' (E. L. Schneider and J. W. Rowe, eds.), 3rd ed., pp. 384–406. Academic Press, San Diego, CA.

Bennett, C. L., Davis, R. T., and Miller, J. M. (1983). Demonstration of presbycusis across repeated measures in a nonhuman primate species. *Behav. Neurosci.* **97**, 602–607.

Bhattacharya, T. P., and Dayal, V. S. (1985). Age-related cochlear hair cell loss in the chinchilla. *Ann. Otol. Rhinol. Laryngol.* **94**, 75–80.

Boettcher, F. A., Mills, J. H., and Norton, B. L. (1993a). Age-related changes in auditory evoked potentials of gerbils. I. Response amplitudes. *Hear. Res.* **71**, 137–145.

Boettcher, F. A., Mills, J. H., Norton, B. L., and Schmiedt, R. A. (1993b). Age-related changes in auditory evoked potentials of gerbils. II. Response latencies. *Hear. Res.* **71**, 146–156.

Boettcher, F. A., White, D. R., Mills, J. H., and Schmiedt, B. N. (1993c). Age-related changes in auditory evoked potentials of gerbils. III. Low-frequency responses and repetition rate effects. *Hear. Res.* **87**, 208–219.

Boettcher, F. A., Mills, J. H., Dubno, J. R., and Schmiedt, R. A. (1995). Masking of auditory brainstem response in young and aged Mongolian gerbils. *Hear. Res.* **89**, 1–13.

Boettcher, F. A., Mills, J. H., Swerdloff, J. L., and Holley, B. L. (1996). Auditory evoked potentials in aged gerbils: Responses elicited by noises separated by a silent gap. *Hear. Res.* **102**, 167–178.

Bohne, B. A., Gruner, M. M., and Harding, G. W. (1990). Morphological correlates of aging in the chinchilla cochlea. *Hear. Res.* **48**, 79–92.

Briner, W. B., and Willott, J. F. (1989). Ultrastructural features of neurons in the C57BL/6J mouse anteroventral cochlear nucleus: Young mice versus old mice with chronic presbycusis. *Neurobiol. Aging* **10**, 295–303.

Bronson, R. T., and Lipman, R. D. (1996). Reduction in rate of occurrence of age related lesions in dietary restricted laboratory mice. *Growth Dev. Aging* **55**, 169–184.

Brown, C. H. (1984). Directional hearing in aging rats. *Exp. Aging Res.* **10**, 35–38.

Brown, J. N., Miller, J. M., and Nuttall, A. L. (1995). Age-related changes in cochlear vascular conductance in mice. *Hear. Res.* **86**, 189–194.

Browner, R. H., and Baruch, A. (1982). The cytoarchitecture of the dorsal cochlear nucleus in the 3-month- and 26-month-old C57BL/6 mouse: A golgi impregnation study. *J. Comp. Neurol.* **211**, 115–138.

Cable, J., Jackson, I. J., and Steel, K. P. (1993). Light (Blt), a mutation that causes melanocyte death, affects stria vascularis function in the mouse inner ear. *Pigm. Cell Res.* **6**, 215–225.

Casey, M. A. (1990). The effects of aging on neuron number in the rat superior olivary complex. *Neurobiol. Aging* **11**, 391–394.

Casey, M. A., and Feldman, M. L. (1982). Aging in the rat medial nucleus of the trapezoid body. I. Light microscopy. *Neurobiol. Aging* **3**, 187–195.

Casey, M. A., and Feldman, M. L. (1985a). Aging in the rat medial nucleus of the trapezoid body. II. Electron microscopy. *J. Comp. Neurol.* **232**, 401–413.

Casey, M. A., and Feldman, M. L. (1985b). Aging in the rat medial nucleus of the trapezoid body. III. Alterations in capillaries. *Neurobiol. Aging* **6**, 39–46.

Casey, M. A., and Feldman, M. L. (1988). Age-related loss of synaptic terminals in the rat medial nucleus of the trapezoid body. *Neuroscience* **24**, 189–194.

Caspary, D. M., Raza, A., Lawhorn-Armour, B. A., Pippin, J., and Arneric, S. P. (1990). Immunocytochemical and neurochemical evidence for age-related loss of GABA in the inferior colliculus: Implications for neural presbycusis. *J. Neurosci.* **10**, 2363–2372.

Caspary, D. M., Milbrandt, J. C., and Helfert, R. H. (1995). Central auditory aging: GABA changes in the inferior colliculus. *Exp. Gerontol.* **30**, 349–360.

Chole, R. A., and Henry, K. R. (1983a). Otosclerotic lesions in the inbred LP/J mouse. *Science* **221**, 881–882.

Chole, R. A., and Henry, K. R. (1983b). Disparity in the cytocochleogram and the electrocochleogram in aging LP/J and A/J inbred mice. *Audiology* **22**, 384–392.

Church, M. W., and Shucard, D. W. (1986). Age-related hearing loss in BDF1 mice as evidenced by the brainstem auditory evoked potential. *Audiology* **25**, 363–372.

Cohen, G. M., and Grasso, J. S. (1987). Further observations on the degeneration of spiral ganglia in aging C57BL/6 mice. *Assoc. Res. Otolaryngol. Abstr.* **10**, 120.

Cone-Wesson, B. K., Fowler, C. G., and Sininger, Y. S. (1995). ABR and DPOAE in young, middle-aged and old chimpanzees. *Am. Speech Hear. Assoc.* **36**, A.

Conlee, J. W., Parks, T. N., Schwartz, I. R., and Creel, D. J. (1989). Comparative anatomy of melanin pigment in the stria vascularis. Evidence for a distinction between melanocytes and intermediate cells in the cat. *Acta Oto-Laryngol.* **107**, 48–58.

Cooper, W. A., Coleman, J. R., Newton, E. H., and Youatt, A. E. (1986). ABR changes to tonal stimuli in the senescent Fischer 344 rat. *Soc. Neurosci. Abstr.* **12**, 1280.

Cooper, W. A., Coleman, J. R., and Newton, E. H. (1990). Auditory brainstem responses to tonal stimuli in young and aging rats. *Hear. Res.* **43**, 171–180.

Covell, W. P., and Rogers, J. B. (1957). Pathological changes in the inner ears of senile guinea pigs. *Laryngoscope* **67**, 118–129.

Crowley, D. E., Schramm, V. L., Swain, R. E., and Swanson, S. N. (1972a). Analysis of age-related changes in electric responses from the inner ear of rats. *Ann. Otol.* **81**, 739–746.

Crowley, D. E., Schramm, V. L., Swain, R. E., Maisel, R. H., Rauchback, E., and Swanson, S. N. (1972b). An animal model for presbycusis. *Laryngoscope* **82**, 2079–2091.

Davis, M., Falls, W. A., Campeau, S., and Kim, M. (1993). Fear-potentiated startle: A neural and pharmacological analysis. *Behav. Brain. Res.* **58**, 175–198.

Deol, M. S. (1956). The anatomy and development of mutants pirouette, shaker-1 and waltzer in the mouse. *Proc. R. Soc. London, Ser. B* **145**, 206–213.

Ding, D., McFadden, S. L., Wang, J., Hu, B. H., and Salvi, R. J. (1999). Age- and strain-related differences in dehydrogenase activity and glycogen levels in CBA and C57 mouse cochleas. *Audiol. Neuro-Otol.* **4**, 55–63.

Dum, N. Schmidt, U., and von Wedel, H. (1980). Age-related changes in the auditory evoked brainstem potentials of albino and pigmented guinea pigs. *Arch. Oto-Rhino-Laryngol.* **228**, 249–254.

Ehret, G. (1974). Age-dependent hearing loss in normal hearing mice. *Naturwissenschaften* **61**, 506–507.

Erway, L. C., and Willott, J. F. (1996). Genetic susceptibility to noise-induced hearing loss in mice. *In* ''Scientific Basis of Noise-induced Hearing Loss'' (A. Axelsson *et al*., eds.), pp. 56–64. Thieme Medical Publishers, New York.

Erway, L. C., Willott, J. F., Archer, J. R., and Harrison, D. (1993). Genetics of age-related hearing loss in mice: I. Inbred and F1 hybrid strains. *Hear. Res.* **65**, 125–132.

Faddis, B. T., and McGinn, M. D. (1997). Spongiform degeneration of the gerbil cochlear nucleus: An ultrastructural and immunochemical evaluation. *J. Neurocytol.* **26**, 625–635.

Falls, W. A., Carlson, S., Turner, J. G., and Willott, J. F. (1997). Fear-potentiated startle in two strains of inbred mice. *Behav. Neurosci.* **111**, 855–861.

Feldman, M. L. (1984). Morphological observations on the cochleas of very old rats. *Assoc. Res. Otolaryngol. Abstr.* **7**, 14.

Feldman, M. L., and Vaughan, D. W. (1979). Changes in the auditory pathway with age. *In* ''Special Senses in Aging'' (S. S. Han and D. H. Coons, eds.), pp. 143–162. Institute of Gerontology, Ann Arbor, MI.

Finlayson, P. G. (1995). Decreased inhibition to lateral superior olive neurons in young and aged Sprague-Dawley rats. *Hear. Res.* **87**, 84–95.

Gratton, M. A., Smyth, B. J., Schulte, B. A., and Vincent, D. A., Jr. (1995). Na, K-ATPase activity declines in the cochlear lateral wall of quiet aged gerbils. *Hear. Res.* **83**, 33–40.

Gratton, M. A., Schmiedt, R. A., and Schulte, B. A. (1996). Age-related decreases in endocochlear potential are associated with vascular abnormalities in the stria vascularis. *Hear. Res.* **102**, 181–190.

Gratton, M. A., Smyth, B. J., Lam. C. F., Boettcher, F. A., and Schmiedt, R. A. (1997). Decline in the endocochlear potential corresponds to decreased Na,K-ATPase activity in the lateral wall of quiet-aged gerbils. *Hear. Res.* **108**, 9–16.

Harrison, J., and Buchwald, J. (1982). Auditory brainstem responses in the aged cat. *Neurobiol. Aging* **3**, 163–171.

Harrison, J. M. (1981). Effects of age on acquisition and maintenance of a location discrimination in rats. *Exp. Aging Res.* **7**, 467–476.

Hawkins, J. E., Miller, J. M., Rouse, R. C., Davis, J. A., and Rarey, K. (1985). Inner ear histopathology in aging rhesus monkeys (Macaca mulatta). In ''Behavior and Pathology of Aging Rhesus Monkeys,'' pp. 137–154. Liss, New York.

Heffner, R. S., and Donnal, T. (1993). Effect of high-frequency hearing loss on localization in mice (C57BL/6J). *Asso. Res. Otolaryngol. Abstr.* **16**, 49.

Hellström, L. I., and Schmiedt, R. A. (1990). Compound action potential input-ouput functions in young and quiet-aged gerbils. *Hear. Res.* **53**, 217–222.

Hellström, L. I., and Schmiedt, R. A. (1991). Rate/level functions of auditory-nerve fibers in young and quiet-aged gerbils. *Hear. Res.* **50**, 163–174.

Hellström, L. I., and Schmiedt, R. A. (1996). Measures of tuning and suppression in single-fiber and whole-nerve responses in young and quiet-aged gerbils. *J. Acoust. Soc. Am.* **100**, 3275–3285.

Henry, K. R. (1982). Age-related auditory loss and genetics: An electrocochleographic comparison of six inbred strains of mice. *J. Gerontol.* **37**, 275–282.

Henry, K. R. (1983). Ageing and audition. *In* ''Auditory Psychobiology of the Mouse'' (J. F. Willott, ed.), pp. 470–493. Thomas, Springfield, IL.

Henry, K. R. (1986). Effects of dietary restriction on presbyacusis in the mouse. *Audiology* **25**, 329–337.

Henry, K. R., and Chole, R. A. (1980). Genotypic differences in behavioral, physiological, and anatomical expressions of age-related hearing loss on the laboratory mouse. *Audiology* **19**, 369–383.

Henry, K. R., and McGinn, M. D. (1992). The mouse as a model for human audition: A review of the literature. *Audiology* **31**, 181–190.

Henry, K. R., McGinn, M. D. and Chole, R. A. (1980). Age-related auditory loss in the Mongolian gerbil. *Arch. Oto-Rhino-Laryngol.* **228**, 233–238.

Henry, K. R., McGinn, M. D., Carter, L. A., and Savoska, E. A. (1992). Auditory brainstem function of the F1 offspring of the cross of CBA/CaJ and AU/SsJ inbred mice. *Audiology* **31**, 190–197.

Hoeffding, V., and Feldman, M. L. (1988). Changes with age in the morphology of the cochlear nerve in rats: Light microscopy. *J. Comp. Neurol.* **276**, 537–546.

Hoffman, H. S., and Ison, J. R. (1980). Reflex modification in the domain of startle. I. Some empirical findings and their implications for how the nervous system processes sensory input. *Psychol. Bull.* **87**, 175–189.

Hunter, K. P., and Willott, J. F. (1987). Aging and the auditory brainstem response in mice with severe or minimal presbycusis. *Hear. Res.* **30**, 207–218.

Idrizbegovic, E., Willott, J. F., Bogdanovic, N., and Canlon, B. (1999). Aging and the total number of calbindin D-28k and parvalbumin immunopositive neurons in the dorsal cochlear nucleus of CBA/CaJ mice. *Assoc. Res. Otolaryngol. Abstr.* **22**, 65–66.

Ingham, N. J., Thornton, S. K., Comis, S. D., and Withington, D. J. (1998). The auditory braintem reponse of aged guinea pigs. *Acta Oto-Laryngol.* **118**, 673–680.

Ingham, N. J., Comis, S. D., and Withington, D. J. (1999). Hair cell loss in the aged guinea pig cochlea. *Acta Oto-Laryngol.* **119**, 42–47.

Ison, J. R., Agrawal, P., Pak, J., and Vaughn, W. J. (1998). Changes in temporal acuity with age and with hearing impairment in the mouse: A study of the acoustic startle reflex and its inhibition by brief decrements in noise level. *J. Acoust Soc. Am.* **104**, 1696–1704.

Jalenques, I., Burette, A., Albuisson, E., and Romand, R. (1997). Age-related changes in GFAP-immunoreactive astrocytes in the rat ventral cochlar nucleus. *Hear. Res.* **107**, 113–124.

Jimenez, A. M., Stagner B. B., Martin, G. K., and Lonsbury-Martin, B. L. (2000). Age-related loss of distortion product otoacoustic emissions in four mouse strains. *Hear. Res.* (in press).

Johnson, K. R., Erway, L. C., Cook, S. A., Willott, J. F., and Zheng, Q. Y. (1997). A major gene affecting age-related hearing loss in C57BL/6J mice. *Hear. Res.* **114**, 83–92.

Johnsson, L.-G., and Hawkins, J. E., Jr. (1972). Strial atrophy in clinical and experimental deafness. *Laryngoscope* **82**, 1105–1125.

Johnsson, L.-G., and Hawkins, J. E., Jr. (1979). Age-related degeneration of the inner ear. *In* "Special Senses in Aging" (S. S. Han and D. H. Coons, eds.), pp. 119–135. Institute of Gerontology, Ann Arbor, MI

Kazee, A. M., Han, L. Y., Spongr, V. P., Walton, J. P., Salvi, R. J., and Flood, D. G. (1995). Synaptic loss in the central nucleus of the inferior colliculus correlates with sensorineural hearing loss in the C57BL/6 mouse model of presbycusis. *Hear. Res.* **89**, 109–120.

Keithley, E. M., and Feldman, M. L. (1979). Spiral ganglion cell counts in an age-graded series of rat cochleas. *J. Comp. Neurol.* **188**, 429–442.

Keithley, E. M., and Feldman, M. L. (1982). Hair cell counts in an age-graded series of rat cochleas. *Hear. Res.* **8**, 249–262.

Keithley, E. M., Ryan, A. F., and Woolf, N. K. (1988). Spiral ganglion cell density in young and old gerbils. *Hear. Res.* **38**, 125–134.

Keithley, E. M., Ryan, A. F., and Woolf, N. K. (1993). Fibronectin-like immunoreactivity of the basilar membrane of young and aged rats. *J. Comp. Neurol.* **327**, 612–617.

Keithley, E. M., Lo, J., and Ryan, A. F. (1994). 2-deoxyglucose uptake patterns in response to pure tone stimuli in the aged rat inferior colliculus. *Hear. Res.* **80**, 79–85.

Kitamura, K., Nomura, Y., Yagi, M., Yoshikawa, Y., and Ochikubo, F. (1991). Morphological changes of cochlea in a strain of new-mutant mice. *Acta Oto-Laryngol.* **111**, 61–69.

Knowles, K., Blauch, B., Leipold, H., Cash, W., and Hewett, J. (1989). Reduction of spiral ganglion neurons in the aging canine with hearing loss. *Zentralbl. Veterinärmed.* **36**, 188–199.

Lambert, P. R., and Schwartz, I. R. (1982). A longitudinal study of changes in the cochlear nucleus in the CBA mouse. *Arch. Otolaryngol. Head Neck Surg.* **90**, 787–794.

Li, H. S., and Borg, E. (1991). Age-related loss of auditory sensitivity in two mouse genotypes. *Acta Oto-Laryngol.* **111**, 827–834.

Li, H. S., and borg, E. (1993). Auditory degeneration after acoustic trauma in two genotypes of mice. *Hear. Res.* **68**, 19–27.

McFadden, S. L., and Campo, P. (2000). Cubic distortion product otoacoustic emissions in young and aged chinchillas exposed to low-frequency noise. *J. Acoust. Soc. Am.* (in press).

McFadden, S. L., and Willott, J. F. (1994a). Responses of inferior colliculus neurons in C57BL/6J mice with and without sensorineural hearing loss: Effects of changing the azimuthal location of an unmasked pure-tone stimulus. *Hear. Res.* **78**, 115–131.

McFadden, S. L., and Willott, J. F. (1994b). Responses of inferior colliculus neurons in C57BL/6J mice with and without sensorineural hearing loss: Effects of changing the azimuthal location of a continuous noise masker on responses to contralateral tones. *Hear. Res.* **78**, 132–148.

McFadden, S. L., Campo, P., Quaranta, N., and Henderson, D. (1997a). Age-related decline of auditory function in the chinchilla *(Chinchilla laniger). Hear. Res.* **111**, 114–126.

McFadden, S. L., Quaranta, N., and Henderson, D. (1997b). Suprathreshold measures of auditory function in the aging chinchilla. *Hear. Res.* **111**, 127–135.

McGinn. M. D., and Faddis, B. T. (1987). Auditory experience affects degeneration of the ventral cochlear nucleus in mongolian gerbils. *Hear. Res.* **31**, 235–244.

Mikaelian, D. O. (1979). Development and degeneration of hearing in the C57/b16 mouse: Relation of electrophysiologic responses from the round window and cochlear nucleus to cochlear anatomy and behavioral responses. *Laryngoscope* **34**, 1–15.

Mikaelian, D. O., and Ruben, R. J. (1964). Hearing degeneration in shaker-1 mouse. *Arch. Oto-Laryngol.*, **80**, 418–430.

Milbrandt, J. C., and Caspary, D. M. (1995). Age-related reduction of [^{3}H] strychnine binding sites in the cochlear nucleus of the Fischer 344 rat. *Neuroscience* **67**, 713–719.

Milbrandt, J. C., Albin, R. L. and Caspary, D. M. (1994). Age-related decrease in $GABA_B$ receptor binding in the Fischer 344 rat inferior colliculus. *Neurobiol. Aging* **15**, 669–703.

Milbrandt, J. C., Albin, R. L. Turgeon, S. M., and Caspary, D. M. (1996). $GABA_A$ receptor binding in the aging rat inferior colliculus. *Neuroscience* **73**, 449–458.

Milbrandt, J. C., Hunter, C., and Caspary, D. M. (1997). Alterations of $GABA_A$ receptor subunit mRNAs levels in the aged Fischer 344 rat inferior colliculus. *J. Comp. Neurol.* **379**, 455–465.

Miller, J. D. (1970). Audibility curve of the chinchilla. *J. Acoust. Soc. Am.* **48**, 513–523.

Mills, J. H., Schmiedt, R. A., and Kulish, L. F. (1990). Age-related changes in auditory potentials of Mongolian gerbil. *Hear. Res.* **46**, 201–210.

Mills, J. H., Lee, F. -S., Dubno, J. R., and Boettcher, F. A. (1996). Interactions between age-related and noise-induced hearing loss. *In* "Scientific Basis of Noise-induced Hearing Loss" (A. A. Axelson, H. M. Borchgrevink, R. P. Hamernik, P. A. Hellstrom, D. Henderson, and R. J. Salvi, eds.), pp. 193–212. Thieme, New York.

Mills, J. H., Boettcher, F. A., and Dubno, J. R. (1997). Interaction of noise-induced permanent threshold shift and age-related threshold shift. *J. Acoust. Soc. Am.* **101**, 1681–1686.

Mizuta, K., Nozawa, O., Morita, H., and Hoshino, T. (1993). Scanning electron microscopy of age-related changes in the C57BL/6J mouse cochlea. *Scanning Microsc.* **7**, 889–896.

Nozawa, I., Imamura, S., Fujimori, I., Hashimoto, K., Shimomura, S., Hisamatsu, K., and Murakami, Y. (1996). Age-related alterations in the

auditory brainstem responses and the compound action potentials in guinea pigs. *Laryngoscope.* **106**, 1034–1039.

O'Neill, W. E., Zettel, M. L., Whittemore, K. R., and Frisina, R. D. (1997). Calbindin D-28k immunoreactivity in the medial nucleus of the trapeoid body declines with age in C57BL/6, but not CBA/CaJ mice. *Hear. Res.* **112**, 158–166.

Palombi, P. S., and Caspary, D. M. (1996a). Physiology of the Fischer 344 rat inferior colliculus: Responses to contralateral monaural stimuli. *J. Neurophysiol.* **76**, 3114–3125.

Palombi, P. S., and Caspary, D. M. (1996b). Responses of young and aged Fischer 344 rat inferior colliculus neurons to binaural tonal stimuli. *Hear. Res.* **100**, 59–67.

Parham, K. (1997). Distortion product otoacoustic emissions in the C57BL/6J mouse model of age-related hearing loss. *Hear. Res.* **112**, 216–234.

Parham, K., and Willott, J. F. (1988). The acoustic startle response in young and aging C57BL/6J and CBA/J mice. *Behav. Neurosci.* **102,** 881–886.

Park, J. C., Cook, K. C., and Verde, E. A. (1990). Dietary restriction slows the abnormally rapid loss of spiral ganglion neurons in C57BL/6 mice. *Hear. Res.* **48**, 275–280.

Pestalozza, G., Davis, H., Eldredge, D. H., Covell, W. P., and Rogers, J. B. (1957). Decreased bio-electric potentials in the ears of senile guinea pigs. *Laryngoscope* **67**, 1113–1122.

Proctor, T. B., Velde, T. M., Dayal, V. S., Bhattacharyya, T. K., Artwohl, J., and Towle, V. L. (1998). Auditory brain stem response in young and old guinea pigs. *Am. J. Otol.* **19**, 226–229.

Ralls, K. (1967). Auditory sensitivity in mice: *Peromyscus* and *Mus musculus. Anim. Behav.* **15**, 123–128.

Raza, A., Milbrandt, J. C. Arneric, S. P., and Caspary D. M. (1994). Age-related changes in brainstem auditory neurotransmitters: Measures of GABA and acetylcholine function. *Hear. Res.* **77**, 221–230.

Ryals, B. M., and Westbrook, E. W. (1988). Ganglion cell and hair cell loss in Coturnix quail associated with aging. *Hear. Res.* **36**, 1–8.

Saitoh, Y., Hosokawa, M., Shimada, A., Watanabe, Y., Yasuda, N., Takeda, T., and Murakami, Y. (1994). Age-related hearing impairment in sensecence-accelerated mouse (SAM). *Hear. Res.* **75**, 27–37.

Saitoh, Y., Hosokawa, M., Shimada, A., Watanabe, Y., Yasuda, N., Murakami, Y., and Takeda, T. (1995). Age-related cochlear degeneration in senescence-accelerated mouse. *Neurobiol. Aging* **16**, 129–136.

Sakaguchi, N., Spicer, S. S., Thomopoulos, G. N., and Schulte, B. A. (1997). Increased laminin deposition in capillaries of the stria vascularis of quiet-aged gerbils. *Hear. Res.* **105**, 44–56.

Schmiedt, R. A. (1996). Effects of aging on potassium homeostasis and the endocochlear potential in the gerbil cochlea. *Hear. Res.* **102**, 125–132.

Schmiedt, R. A., Mills, J. H., and Adams, J. C. (1990). Tuning and suppression in auditory nerve fibers of aged gerbils raised in quiet or noise. *Hear. Res.* **45**, 221–236.

Schmiedt, R. A., Mills, J. H., and Boettcher, F. A. (1996). Age-related loss of activity of auditory nerve fibers. *J. Neurophysiol.* **76**, 2799–2803.

Schuknecht, H. F. (1955). Presbycusis. *Laryngoscope* **65**, 407–419.

Schuknecht, H. F. (1974). "Pathology of the Ear." Harvard University Press, Cambridge, MA.

Schulte, B. A., and Schmiedt, R. A. (1992). Lateral wall Na, K-ATPase and endocochlear potential decline with age in quiet-reared gerbils. *Hear. Res.* **62**, 46–56.

Seidman, M. D., Khan, M. J., Dolan, D. F., and Quirk, W. S. (1996). Age-related differences in cochlear microcirculation and auditory brain stem response. *Arch. Otolaryngol. Head Neck Surg.* **122**, 1221–1226.

Seidman, M. D., Bai, U., Khan, M. J., and Quirk, W. S. (1997). Mitochondrial DNA deletions associated with aging and presbyacusis. *Arch. Oto-Laryngol. Head Neck Surg.* **123**, 1039–1045.

Shimada, A., Ebisu, M., Morita, T., Takeuchi, T., and Umemura, T. (1998). Age-related changes in the cochlea and cochlear nuclei of dogs. *J. Vet. Med. Sci.* **60**, 41–48.

Shnerson, A., and Pujol, R. (1983). Development: electrophysiology and behavior. *In* "Auditory Psychobiology of the Mouse" (J. F. Willott, ed.), pp. 395–425. Thomas, Springfield, IL.

Shone, G., Altschuler, R. A., Miller, J. M., and Nuttall, A. L. (1991a). The effect of noise exposure on the aging ear. *Hear. Res.* **56**, 173–178.

Shone, G., Raphael, Y., and Miller, J. M. (1991b). Hereditary deafness occurring in cd/1 mice. *Hear. Res.* **57**, 153–156.

Simpson, G. V., Knight, R. T., Brailowsky, S., Prospero-Garcia, O., and Scabini, D. (1985). Altered peripheral and brainstem auditory function in aged rats. *Brain Res.* **348**, 28–35.

Sinnott, J. M., Street, S. L., Mosteller, K. W., and Williamson, T. L. (1997). Behavioral measures of vowel sensitivity in Mongolian gerbils *(Meriones unguiculatus):* Effects of age and genetic origin. *Hear. Res.* **112**, 235–246.

Spoendlin, H. (1970). Changes due to aging. *In* "Ultrastructure of the Peripheral Nervous System and Sense Organs" (A. Bischoff, ed.), pp. 260–261. Thieme, Stuttgart.

Spongr, V., Flood, D. G., Frisina, R. D., and Salvi, R. J. (1997). Quantitative measures of hair cell loss in CBA and C57BL/6 mice through their life spans. *J. Acoust. Soc. Am.* **101**, 3546–3553.

Steel, K. P. (1995). Inherited hearing defects in mice. *Annu. Rev. Genet.* **29**, 675–701.

Steel, K. P., Niaussat, M. M., and Bock, G. R. (1983). The genetics of hearing. *In* "Auditory Psychobiology of the Mouse" (J. F. Willott, ed.), pp. 341–394. Thomas, Springfield, IL.

Steel, K. P., Barkway, C., Glenn, N., and Brown, A. (1989). Hearing impairment in two mouse mutants with hypophosphataemia. *Hered. Deafness Newsl.* **3**, 20–21.

Stiebler, I., and Ehret, G. (1985). Inferior colliculus of the house mouse. I. Quantitative study of tonotopic organization, frequency representation, and tone-threshold distribution. *J. Comp. Neurol.* **238**, 65–76.

Sun, J. C., Bohne, B. A., and Harding, G. W. (1994). Is the older ear more susceptible to noise damage? *Laryngoscope* **104**, 1251–1258.

Suzuki, T., Ren, T., Nuttall, A. L, and Miller, J. M. (1998). Age-related changes in cochlear blood flow response to occlusion of anterior inferior cerebellar artery in mice. *Ann. Otol. Rhinol. Laryngol.* **107**, 648–653.

Sweet, R. J., Price, J. M., and Henry, K. R. (1988). Dietary restriction and presbycusis: Periods of restriction and auditory threshold losses in the CBA/J mouse. *Audiology* **27**, 305–312.

Tarnowski, B. I., Schmiedt, R. A., Hellström, L. I., Lee, F. S., and Adams, J. C. (1991). Age-related changes in cochleas of Mongolian gerbils. *Hear. Res.* **54**, 123–134.

Trune, D. R., Craven, J. P., Morton, J. I., and Mitchell, C. (1989). Autoimmune disease and cochlear pathology in the C3H/lpr strain. *Hear. Res.* **38**, 57–66.

Trune, D. R., Kempton, J. B., and Mitchell, C. (1996). Auditory function in the C3H/HeJ and C3H/HeSnJ mouse strains. *Hear. Res.* **96**, 41–51.

Turner, J. G., and Willott, J. F. (1998). Exposure to an augmented acoustic environment alters progressive hearing loss in DBA/2J mice. *Hear. Res.* **118**, 101–113.

Turnoer, J. G., Bross, L. S., Sundin, V. S., and Willott, J. F. (1999). The effects of an augmented acoustic environment on progressive sensorineural hearing loss in BALB/cJ and BXD recombinant inbred mice. *Assoc. Res. Otolaryngol. Abstr.* **22**, 154.

Turnock, M. T., and Harrison, J. M. (1975). Effects of age on hearing in rats. *J. Acoust. Soc. Am.* **58** (Suppl. 1), S90.

Walton, J. P., Frisina, R. D., and Meierhans, L. R. (1995). Sensorineural hearing loss alters recovery from short-term adaptation in the C57BL/6J mouse. *Hear. Res.* **88**, 19–26.

Walton, J. P., Frisina, R. D., and O'Neill, W. E. (1998). Age-related alteration in procesing of temporal sound features in the auditory midbrain of the CBA mouse. *J. Neurosci.* **18**, 2764–2776.

Wenngren, B.-I., and Anniko, M. (1988a). A frequency-specific auditory brainstem response technique exemplified in the determination of age-related auditory thresholds. *Acta Oto-Laryngol.* **106**, 238–243.

Wenngren, B.-I, and Anniko, M. (1988b). Age-related auditory brainstem response (ABR) threshold changes in the dancer mouse mutant. *Acta Oto-Laryngol.* **106**, 386–392.

Willott, J. F. (1981). Comparison of response properties of inferior colliculus neurons of two inbred mouse strains differing in susceptibility to audiogenic seizures. *J. Neurophysiol.* **45**, 35–47.

Willott, J. F. (1986). Effects of aging, hearing loss, and anatomical location on thresholds of inferior colliculus neurons in C57BL/6 and CBA mice. *J. Neurophysiol.* **56**, 391–408.

Willott, J. F. (1991). "Aging and Audition: Anatomy. Physiology, and Psychophysics." Singular Press, San Diego, CA.

Willott, J. F. (1996a). Auditory system plasticity in the adult C57BL/6J mouse. *In* "Auditory Plasticity and Regeneration" (R. J. Salvi, D. H. Henderson, V. Colletti, and F. Fiorino, eds.), pp. 297–316. Thieme, New York.

Willott, J. F. (1996b). Aging and the auditory system. In "Pathobiology of the Aging Mouse, ILSI Monographs on the Pathobiology of Aging Animals" (U. Mohr, D. L. Dungworth, C. C. Capen, W. W. Carlton, J. P. Sundberg, and J. M. Ward Eds.) pp. 179-204. ILSI Press, Washington, DC.

Willott, J. F. (1999). "Neurogerontology: Aging and the Nervous System." Springer, New York.

Willott, J. F., and Bross, L. S. (1990). Morphology of the octopus cell area of the cochlear nucleus in young and aging C57BL/6J and CBA/J mice. *J. Comp. Neurol.* **300**, 61–81.

Willott, J. F., and Bross, L. S. (1996). Morphological changes in the anteroventral cochlear nucleus that accompany sensorineural hearing loss in DBA/2J and C57BL/6J mice. *Dev. Brain Res.* **91**, 218–226.

Willott, J. F., and Carlson, S. (1995). Modification of the acoustic startle response in hearing-impaired C57BL/6J mice: Prepulse augmentation and prolongation of prepulse inhibition. *Behav. Neurosci.* **109**, 396–403.

Willott, J. F., and Erway, L. C. (1998). Genetics of age-related hearing loss in mice. IV. Cochlear pathology and hearing loss in 25 BXD recombinant inbred mouse strains. *Hear. Res.* **119**, 27–36.

Willott, J. F., and Turner, J. G. (1999). Prolonged exposure to an augmented acoustic environment ameliorates age-related auditory changes in C57BL/6J and DBA/2J mice. *Hear. Res.* **135**, 78–88.

Willott, J. F. Kulig, J., and Satterfield, T. (1984). The acoustic startle response in DBA/2 and C57BL/6 mice: Relationship to auditory neuronal response properties and hearing impairment. *Hear. Res.* **16**, 161–167.

Willott, J. F., Jackson, L. M., and Hunter, K. P. (1987). Morphometric study of the anteroventral cochlear nucleus of two mouse models of presbycusis. *J. Comp. Neurol.* **260**, 472–480.

Willott, J. F., Parham, K., and Hunter, K. P. (1988a). Response properties of inferior colliculus neurons in young and very old CBA/J mice. *Hear. Res.* **37**, 1–14.

Willott, J. F., Parham, K., and Hunter, K. P. (1988b). Response properties of inferior colliculus neurons in middle-aged C57BL/6J mice with presbycusis. *Hear. Res.* **37**, 15–28.

Willott, J. F., Parham, K., and Hunter, K. P. (1991). Comparison of the auditory sensitivity of neurons in the cochlear nucleus and inferior colliculus of young and aging C57BL/6J and CBA/J mice. *Hear. Res.* **53**, 78–94.

Willott, J. F., Bross, L. S., and McFadden, S. L. (1992). Morphology of the dorsal cochlear nucleus in young and aging C57BL/6J and CBA/J mice. *J. Comp. Neurol.* **321**, 666–678.

Willott, J. F., Aitkin, L. M., and McFadden, S. M. (1993). Plasticity of auditory cortex associated with sensorineural hearing loss in adult C57BL/6J mice. *J. Comp. Neurol.* **329**, 402–411.

Willott, J. F., Bross, L. S., and McFadden, S. L. (1994a). Morphology of the inferior colliculus in C57BL/6J and CBA/J mice across the life span. *Neurobiol. Aging* **15**, 75–183.

Willott, J. F., Carlson, S., and Chen, H. (1994b). Prepulse inhibition of the startle response in mice: Relationship to hearing loss and auditory system plasticity. *Behav. Neurosci.* **108**, 703–713.

Willott, J. F., Erway, L. C., Archer, J. R., and Harrison, D. (1995). Genetics of age-related hearing loss in mice: II. Strain differences and effects of caloric restriction on cochlear pathology and evoked response thresholds. *Hear. Res.* **88**, 143–155.

Willott, F. F., Milbrandt, J. C., Bross, L. S., and Caspary, D. M. (1997). Glycine immunoreactivity and receptor binding in the cochlear nucleus of C57BL/6J and CBA/CaJ mice: Effects of cochlear impairment and aging. *J. Comp. Neurol.* **385**, 405–414.

Willott, J. F., Turner, J. G., Carlson, S., Ding, D., Bross, L. S., and Falls, W. M. (1998). The BALB/c mouse as an animal model for progressive sensorineural hearing loss. *Hear. Res.* **115**, 162–174.

Zettel, M. A., Frisina, R. D., Haider, S., and O'Neill, W. E. (1997). Age-related changes in calbindin D-28k and calretinin immunoreactivity in the inferior colliculus of CBA/CaJ and C57BL/6 mice. *J. Comp. Neurol.* **386**, 92–110.

Zheng, Q. Y., Johnson, K. R., and Erway, L. C. (1999). Assessment of hearing in 80 inbred strains mof mice by ABR threshold analyses. *Hear. Res.* **130**, 94–107.

Zheng, Y., Ikeda, K., Nakamura, M., and Takasaka, T. (1998). Endonuclease cleavage of DNA in the aged cochlea of Mongolian gerbil. *Hear. Res.* **126**, 11–18.

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James R. Ison

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The Development of Animal Models for the Study of Presbycusis: Building a Behavioral Link between Perception and Physiology

Elderly listeners often complain that they cannot understand what people are saying to them, a problem that may result in part from their inability to hear critical frequencies in the speech signal and in part by a failure in auditory processing that results in speech that is heard only in a distorted and near incomprehensible form. Behaviorally based psychophysical studies in laboratory animals have shown that the sensory attenuation that accompanies aging occurs in species other than humans and have served to validate other types of animal models, most often using evoked potentials, that have provided considerable insight into the physiological bases of attenuation. These animal models now have great potential as testing platforms for potential therapies intended to maintain or recover physiological function and, thus, sensory ability. In comparison, the nature of the processing deficits that may yield perceptual distortion is less well understood even in aged humans, let alone in laboratory animals. This chapter briefly reviews the development of animal models that demonstrate age-related sensory attenuation of simple auditory signals and then describes our behavioral study of a potential source of perceptual distortion in age-related changes in temporal acuity in mice. Our findings are similar to those of our colleagues who study neural temporal acuity in the aged central auditory nervous system of mice, and similar also to the data of our colleagues who study sensory temporal acuity in aged humans. This behavioral link between neurophysiology and sensation and perception supports the hypothesis that a diminished reactivity to threshold and suprathreshold gaps in noise in particular types of cells in the human auditory midbrain is responsible for distorting the perceptual representation of speech.

I. The Need for Animal Models of the Presbycusic Listener

The intent of the present work is to describe briefly the possible sensory and perceptual bases of age-related hearing loss, and the continuing search for ways of capturing these psychological attributes in animal models that may aid in the better understanding of their physiological bases and, potentially, in the development of strategies for prevention and remediation. Many aged human listeners have two complaints about their diminished hearing abilities. One is that they can no longer hear certain familiar, pleasant, and important sounds, such as the telephone or the door bell, or birds singing and children playing. This is a sensory phenomenon of diminished sensitivity. The other is that while they may certainly hear people talking and saying something, they may then find it impossible to make out what it being said. This aspect of their hearing loss that affects speech perception is a very serious matter because it undermines human communication in a great variety of contexts, and besides presenting obvious practical problems, it may lead also to social isolation from colleagues, family, and friends (Working Group, CHABA, 1988).

A question of fundamental interest is the degree to which these two have the same cause or, instead, result from separable malfunctions in basically different types of sensory/perceptual and physiological mechanisms that will require different therapeutic strategies for their remediation. The search for animal models that capture the critical elements of these two complaints and would support an analysis of their underlying causes is a challenging task: particularly challenging is the development of models that focus on the perception of complex acoustic signals, and yet it is this pursuit that seems especially important because it is here that age-related changes in brain structure and brain function are likely to play a decisive role in producing the perceptual deficits of the aging human listener.

II. Evidence for Attenuation and Distortion as Sensory Bases of Presbycusis

Plomp (1978) has described two very general categories of hearing loss in human listeners, one that he called "class A" (for attenuation) and the other "class D" (for distortion). He argued that a class A loss may be remedied by amplification, while class D is not. Drawn in broad strokes, the recent studies of these concepts of attenuation and distortion have been allied with different experimental and theoretical approaches to the understanding of presbycusis in aged humans, each suggesting a different type of underlying sensory or perceptual deficit and having a different physiological basis and a different anatomical location.

For the first of these approaches, by far the more familiar, the fact that once normally heard signals are no longer audible is understood as being a simple reflection of the clinical observation that the sensory threshold for significant spectral frequencies of acoustic stimulation is elevated in the elderly listener. Age-related changes in the audiogram are readily detected in the clinic, and there is considerable epidemiological evidence showing that a majority of aged humans exhibit a progressive loss of sensitivity for simple tonal stimuli, an attenuation effect that begins with the high frequencies and then becomes increasingly apparent at lower and lower frequencies with advancing age (for example, Pearson *et al*., 1995; and Fig. 43.1). The change in absolute thresholds that is seen in the clinic must certainly correlate with the loss of audibility of certain sounds in the real world. As this loss of audibility must ultimately encroach on critical speech frequencies, it is plausible to hypothesize that it is responsible for the changes in speech understanding of "more-or-less audible" speech. For example, the identifying hallmarks of speech presence might be readily heard, such as its overall amplitude envelope or its relatively low-frequency vowel structure, while the especially informative high-frequency spectral cues for consonants fall below the detection threshold; thus "speech" would be heard, but not comprehended.

The evidence for the class D loss in hearing that causes specific sorts of difficulties for complex acoustic stimuli over and above those due to the class A deficit seems persuasive to many investigators, but the supporting data are by no means as pervasive as those for the class A deficit, and there are no large-scale epidemiological studies that identify and then describe the incidence of a class D hearing loss. Then further, it is possible that the complete independence of these forms of hearing loss may be quite rare, that is, the presence of distortion may largely occur following attenuation of the auditory signal, but attenuation may not inevitably yield distortion.

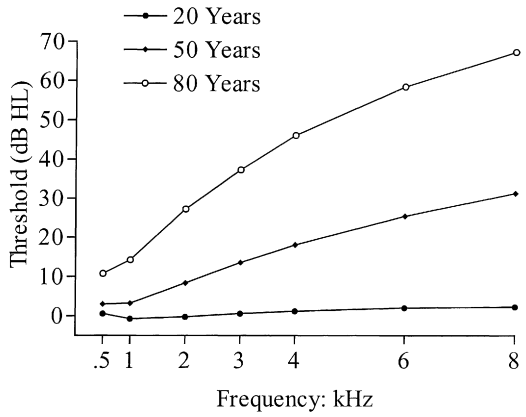

FIG. 43.1. Changes in hearing thresholds as a function of signal frequency in men of different ages. Adapted with permission from Pearson *et al*. (1995, Table III, p. 1199).

Independence is perhaps best encountered as a characteristic of a neurological disorder called "word deafness," which is seen in some patients who, in contrast to patients with classic aphasia, have normal reading and writing abilities and even normal speech, but share a total inability to understand the spoken word (e.g., Buchtel and Stewart, 1989). Word deafness appears to result as a consequence of bilateral damage to the auditory cortex and thus does not provide an apt model for most presbycusic listeners. However, these case histories are important because they provide evidence of great theoretical significance in their demonstration of the complexity of the processing mechanisms that are necessary for maintaining the clarity of the speech signal. They show also, first, that speech may be uninterpretable even though its entire spectral content is perfectly audible (the audiograms of these patients are typically normal), and second, at least some of these case histories provide evidence that the deficit in speech understanding is associated with a deficit in a very simple sensory dimension, namely, temporal acuity. For this reason the tragic neurological accident that leads to word deafness has had important implications for the search for animal models of presbycusis. It is relatively easy to measure temporal acuity in laboratory animals, and though it should not yet be argued that the aged presbycusic listener has subtle neurological problems at the level of the auditory cortex, it certainly seems reasonable to hypothesize that changes in temporal acuity may be in part responsible for a class D hearing loss.

In further support of the idea that distortion and attenuation may be at least in part independent, there is a larger class of apparently neurologically intact human listeners who also have normal or near-normal audiograms, but share a very similar and isolated problem in understanding speech. They differ in one respect from the neurological patients, which is, namely, that their problem is manifest only in the presence of masking noise and not in quiet, as the word-deaf patient cannot understand speech presented in either noise or quiet. Middelweerd *et al*. (1990) studied a relatively large group of patients of this sort, who, with their mean age of just 36 years, would not be classified as being "aged listeners," but nonetheless express this particular presbycusic-like complaint. Middelweerd *et al*. (1990) found a small and inconsequential difference between this patient group and a control group of listeners both in the audiogram and in speech perception measures obtained in quiet, but reported that there were large and clinically significant differences between the two groups when speech was presented in noise. It is not known if this group of patients had a deficit in their temporal acuity or even if they would have problems in processing simple signals in noise, which is a deficit in sensory processing that could be readily investigated in the psychoacoustics laboratory: but clearly, they have problems in understanding speech in noise in the absence of any apparent deficit in their absolute thresholds.

The third source of data pointing to the importance of distortion as a distinct species of hearing loss that is set apart from changes in audibility is presented often incidentally in the reports of experiments that have attempted to characterize aged and hearing-impaired listeners as a group. Thus, for example, Glasberg and Moore (1989) report a correlation matrix for hearing impaired and mostly aged listeners (mean age 61 years) which provided measures of the relationships between speech reception thresholds in quiet and in noise with a variety of psychoacoustic measures. They showed that the correlation between an absolute threshold measure and speech reception threshold in quiet was extremely strong, essentially accounting for all of the variance in the speech thresholds ($r=0.96$). However, this correlation between the speech reception threshold and absolute thresholds was reduced in size when the speech reception threshold was obtained in noise, and in this noise condition the speech reception threshold was instead correlated with measures of acuity and resolution in the temporal and spectral domains (gap detection thresholds and frequency discrimination). Similarly, Ison *et al.* (1998) compared speech reception threshold in quiet and masking noise in a group of aged listeners with but modest hearing loss ($n=21$; mean age, 68 years) with a second group of normal young listeners ($n=19$; mean age, 22) (Fig. 43.2). The speech reception threshold measured in quiet was correlated with absolute threshold measures to the same extent in both young and old subjects, and, indeed, this difference in the class A deficit accounted for all of the apparent age difference between the groups in their speech thresholds. This consequence of the deficit in audibility occurred only in the quiet condition, and, in striking contrast, the correlations between speech reception thresholds obtained in noise and their absolute detection thresholds were close to zero. These data indicated that the age difference in speech perception in noise was not determined by their small class A deficits apparent in the audiogram and provides additional evidence for the independence of the class D hearing loss. It is also of interest that in psychoacoustic work using a largely overlapping group of subjects, these elderly listeners also showed a deficit in temporal acuity.

There are other scattered observations indicating that the hearing abilities of aged listeners may be quite heterogeneous in their composition, and that although decrements in audibility may fully account for speech perception losses in many listeners, in other listeners decrements in temporal or spectral resolution may prove to be more important. For example, Lutman and Clark (1986) measured speech identification thresholds in noise for 23 mostly aged listeners (mean, 60 years old; range, 44–72), all having substantial hearing loss and all wearing hearing aids. Their speech measures were related to audiometric thresholds obtained at 2 kHz, but also they were related independently to gap detection and frequency resolution thresholds: these data are certainly consistent with the suggestion that changes in audibility (class A deficits) and changes in frequency and temporal acuity (which are presumably two important bases of class D deficits) make independent contributions to speech perception. However, the authors also noted that their correlation could be attributed in large part to the presence of a small group of subjects with very high gap thresholds who also required very high signal/noise ratios to correctly identify the speech signals: this observation agrees with the outcome of most aging research in its showing that the aged form a heterogeneous group of persons having diverse capabilities and, perhaps, diverse causative factors lying behind the differences of their performance from that of the young.

III. The Development of Animal Models to Study Attenuation

The reports above, and others like them, suggest that indeed changes in both the audibility and in the perceived clarity of

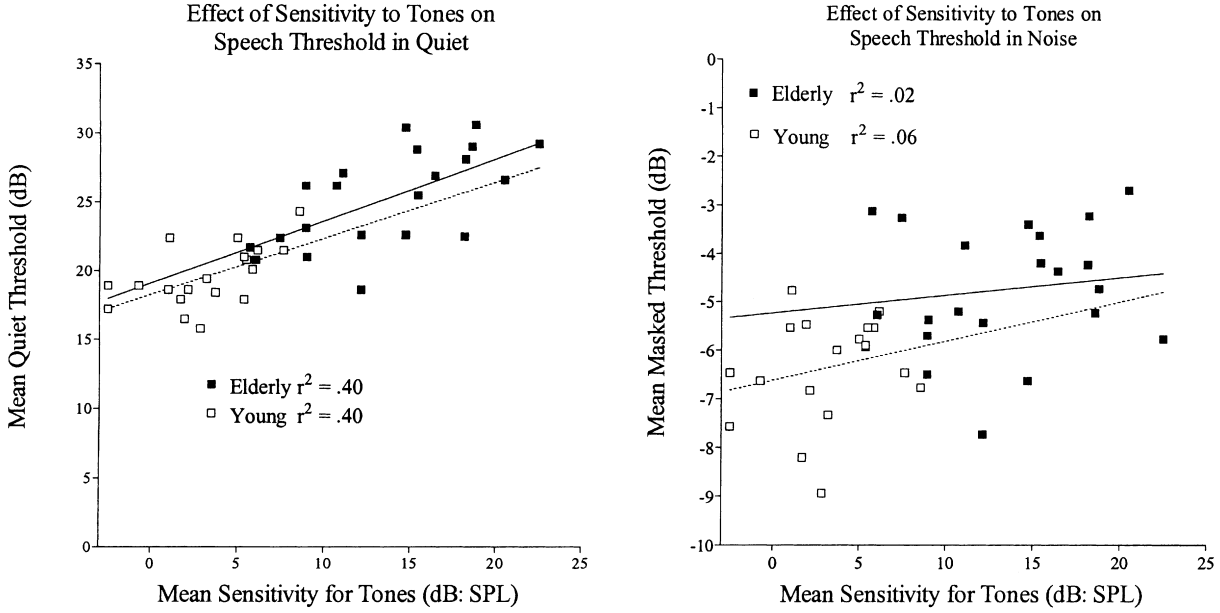

FIG. 43.2. Regression of speech reception thresholds in quiet (left) and noise (right) on the group mean audiogram, in groups differing in age (solid lines and symbols are the aged group, dotted lines and open symbols the young group). From Ison *et al.* (1998).

acoustic signals may provide two partially dissociable consequences of age that make it difficult for many aged listeners to understand speech, especially in noisy and reverberant conditions. The question then is whether it is possible to design animal models that might capture these two sensory effects in the laboratory, models that will help to understand the physiological bases of age-related hearing loss, and afford an animal testing platform for proposed therapeutic strategies prior to their clinical application.

In humans, changes in audibility are readily obtained in clinical audiograms, using a simple procedure in which the critical question asked of the listener is whether or not he or she heard the test tone. Long (1994) has persuasively argued that similar psychophysical tests based in the behavior of animals are critical to illuminating the problem of how neurophysiological events are expressed in sensation and perception. She presented the basic principles common to these tests, their difficulties in implementation, and many of their findings for the standard psychoacoustic dimensions of threshold determination, frequency processing, temporal acuity, and so forth. Comparative hearing researchers have been concerned with the limits of animal perception at least since the time of Yerkes (1905), and behavioral audiograms are available for many species of animals (see, for example, Fay, 1988). Unfortunately, there are some serious obstacles in the design and in the application of most standard psychophysical tests in animals, some of which may especially detract from their use with aged populations. This is not to say that laboratory procedures to measure sensory events in animals have not been exceedingly powerful and useful, for at best they have both of these attributes. However, many of these procedures require the use of sophisticated training procedures that are extraordinarily time intensive, and this alone severely limits the numbers of subjects that can be run in any one experiment. As a result, it is not unusual for the published and paradigmatic classical audiograms characteristic of different species to have been obtained for just two or three young animals of that species.

The results of psychophysical tests in animals are typically reliable and readily replicated in young normal animals. Thus, comparing the audiograms obtained in the hooded rat by Heffner *et al*. (1994), with those obtained in just 3 albino rats by Kelly and Masterton (1977), reveals the stability of outcomes that can be obtained in the best of these experiments. However, the restrictive use of only small numbers is a very serious impediment to aging research, especially if the degree of variability among old animals should approach that of aging human listeners. In addition, most psychophysical behavioral tests require animals who are well motivated and cooperative and are able to perform learned responses under stimulus control, and this sometimes under conditions of time pressure: aged animals in these tests may be affected by age-related deficiencies in a great variety of necessary perceptual–motor and integrative talents, and thus their performance must always be interpreted with great care.

Stimulus-controlled performance of learned behavior is undoubtedly the ''gold standard'' of behavioral psychophysics. Other tests have been developed that do not depend on the animal's having learned a discriminative reaction to the presence of a stimulus, often using the elicitation or the modification of simple reflex behavior. These tests are more readily applied to large numbers of subjects and do not require the use of highly motivated and well trained cooperative subjects, but to say that they represent sensory and perceptual events in the same way that human detection experiments can be thought to represent sensory events demands some strong assumptions and, often, arguments from analogy. Nevertheless, there are tradeoffs and compromises in animal testing that must be considered carefully. At best, a study of presbycusis in an animal model should not depend alone on one sort of experimental procedure, but has been embedded into an empirical and theoretical context that borrows from different research domains an achieve a coherent picture of hearing in the aging animal.

There are very few published behavioral audiograms in any aged animal that approach the level of completeness typical of the human audiogram, but one most impressive set of behavioral audiograms in the animal literature was reported by Bennett *et al*. (1983). They studied three groups of rhesus monkeys, an old group ($n=3$, 31 years of age), a middle-aged group ($n=2$, 24 years of age), and a young group (9 years of age). Thresholds for frequencies between 125 Hz and 32 kHz were assessed in an operant conditioning procedure in which the monkey learned to move its head toward the drinking tube on hearing or failing to hear a tone that normally would be presented at a particular time. The experiment required 3 years for its completion. The authors used an adaptive tracking procedure to follow detection thresholds, each examination period consisting of three 2-month-long tests given 1 year apart. The data provided by Bennett *et al*. are presented in Fig. 43.3. Like older human listeners, the middle-aged monkeys showed a loss of high frequency hearing but little change in the low frequencies compared to that seen in the youngest group. The oldest group of monkeys showed a general loss of sensitivity across the spectrum that was exaggerated for the highest frequencies, this too being consistent with the audiograms of very old human listeners. Bennett *et al*. reported also that the three oldest subjects were quite different from each other despite their long common environmental history. This is interesting: old human listeners can be very different from each other in their hearing abilities and so, apparently, are monkeys.

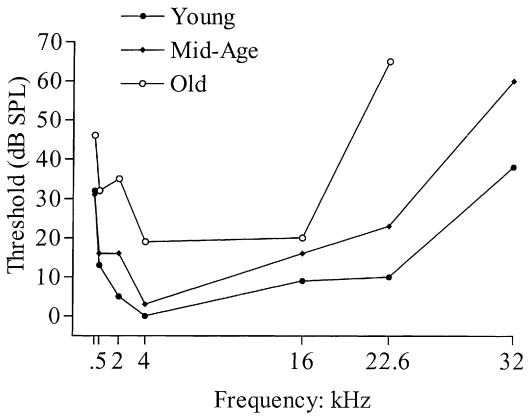

FIG. 43.3. Changes in hearing thresholds with age in monkeys, *Macaca mulatta,* as a function of age, from 9 to 31 years. Data adapted with permission from Bennett *et al*. (1983), copyright © 1983 American Psychological Association.

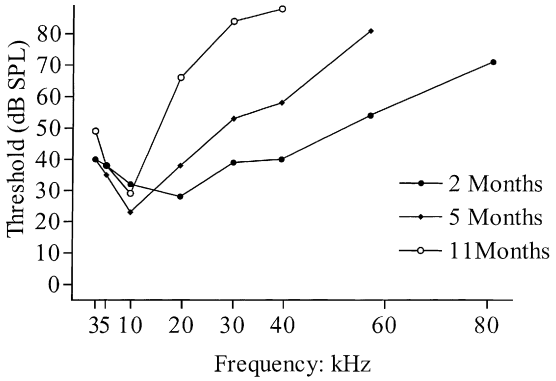

FIG. 43.4. Age related changes in threshold across frequency in three groups of aging mice. The data were adapted from Mikaelian *et al*. (1974), with permission.

Another impressive behavioral analysis of age-related changes in hearing was published for the C57/B16 mouse by Mikaelian *et al*. (1974). This was a seminal paper in its introduction of a mouse model of human presbycusis that has subsequently led to an active exploration of the genetic bases of hearing loss, its peripheral basis in the organ of Corti, and its central consequences for both structure and function. These authors trained groups of water-deprived mice to press a lever for a water reinforcement on the presentation of a tone, and then followed their thresholds for various periods of time as the mice (17 in total) aged variously from about 2 to 21 months. Figure 43.4 is a graph of data selected from their more extensive set of groups, showing thresholds at 2, 5, and 11 months. The pattern of loss is very similar to that exhibited in both humans and monkeys, in its becoming evident first at high frequencies and then progressing in its severity with age. Mikaelian *et al*. also examined the organ of Corti under the light microscope in some of these mice. They observed a loss of hair cells and supporting cells in the basal high-frequency region beginning as early as 3 months of age in this mouse, even as it entered early adulthood. This structural deterioration then progressed toward the apex and was accompanied by losses in the spiral ganglion cells, these anatomical changes roughly in concert with the loss of sensitivity.

At about the same time, Ehret (1974) published an extensive series of behavioral audiograms in a different strain of mouse that did not suffer from a relatively early onset and genetically based hearing impairment. He had developed a Pavlovian conditioning procedure in mice in which tone presentation was followed by a brief shock, so that as the mouse came to anticipate the shock it was possible to observe a facial grimace or eye blink on the presentation of the tone. Following this initial training a series of different frequencies and levels of tonal stimulation were presented in order to determine the threshold levels of stimulation that yielded responses. Ehret reported thresholds for 32 house mice ranging between 2 months and 18 months of age. The youngest mice were the most sensitive, and while subtle age differences were present variously across the tested frequency spectrum, the most systematic age-related changes were at the higher frequencies: here the performance of older mice diverged from that of younger mice at progressively lower frequencies with advancing age, an outcome very similar to that described above for humans, monkeys, and the younger mouse with the early onset age-related hearing loss.

These procedures were then replicated and extended in an interesting and provocative report by Henry and Chole (1980) who used the same behavioral procedure in two strains of mice, one, the C57BL/6 related to the strain studied by Mikaelian *et al*., which suffers from a rapid onset age-related hearing loss, and the other the CBA/J strain that, like the house mouse studied by Ehret, maintains its hearing for 18 months or more. These authors also measured auditory evoked potentials in many of the same mice, and in some of these mice they dissected out the organ of Corti in order to count the numbers of inner and outer hair cells present across the basilar membrane under the light microscope. The stimuli ranged across the spectrum between 5 and 80 kHz, and the mice ranged from 45 to 760 days in age, with sample sizes of seven or eight at the younger ages and just two or three at the oldest ages. This study thus provided a very useful comparative analysis of the correspondence between behavioral and electrophysiological functional measures and an important component of its physiological substrate, between different strains and age groups of mice.

The behavioral and the electrophysiological audiograms obtained in the youngest CBA mice were quite similar to each other, though neither showed the same sharpness of the V-shaped tuning curve that is apparent in the data of Mikaelian *et al*. The behavioral thresholds also were more variable than the electrophysiological measures, but the group means suggested a high frequency loss occurring between 200 and 470 days of age and then a large spectrumwide loss in the oldest animals. The authors noted that this audiometric pattern in the very old CBA mice resembled that of a conductive loss, and indeed, they found middle ear blockage on postmortem examination. Behavioral thresholds for the C57BL mouse were more in line with those of Mikaelian at least qualitatively, for they showed a substantial specific high frequency loss between 65 and 100 days of age, and then a complete flattening of the audiogram near the limits of the sound production equipment at 200 days and beyond. However, while electrophysiological and behavioral measures were roughly equivalent in the CBA mouse, for the C57BL mice the young electrophysiological thresholds were very much lower than their behavioral estimates, by 40 to 50 dB. Henry and Chole ascribe the poor behavioral results in the C57BL mouse to its hyperactivity and greater emotionality in the testing situation. The contrast between these behavioral results and those described by Mikaelian *et al*. reveals the importance of fitting the behavioral testing paradigm to the needs of the particular laboratory animal, with a suggestion that the Pavlovian conditioning used by Ehret and by Henry and Chole was not appropriate for the C57 strain. The appearance of discrepant observations such as this points to the need for a unifying conceptual approach to understand the apparently different outcomes of multidisciplinary paradigms.

Henry and Chole found that there were no changes in either inner or outer hair cell counts in the CBA mouse up through 470 days of age. In the C57BL mouse there were no losses in hair cell counts at 100 days of age, but a substantial loss

in thresholds of 20 to 30 dB for the higher frequencies compared to the thresholds obtained at 45 days of age. Although this might be thought of as indicating a serious disparity between structure and function in this mouse, it is now apparent that the counts of hair cells available to the methods of light microscopy are not the most sensitive measures of receptor structure, compared to the examination of stereocilia under electron microscopy. At 200 days and beyond changes in hair cell counts were more in line with changes in the audiogram.

This small list of behavioral audiograms in aged animals can be considered to be the primary justification for the belief that the sensory impact of simple sounds presented at near threshold values is diminished by age in laboratory animals just as it is in human listeners; they are also the primary justification for asserting that class A hearing losses can be modeled in the behavior of the laboratory animal. However, this short list is very far from the sum total of experimental studies of age-related changes in sensitivity in animals, but most of the studies of hearing loss in animals use not behavioral techniques but study instead some type of auditory evoked potential, often the brain-stem response.

A considerable body of research with both human listeners and animals makes it quite reasonable to argue that a physiologically based study of neural sensitivity to acoustic stimulation can serve as an able proxy for a behavioral measure of its sensory consequence. Indeed, most direct comparisons of behavioral thresholds and thresholds of auditory nerve fibers show a very strong and convincing relationship (see, for example, Pickles, 1988, p. 84). Moreover, measures of frequency-specific brain stem auditory evoked responses are routinely used in the clinic for estimating sensory thresholds in infants and difficult-to-test populations (Schwartz and Schwartz, 1991). It happens that these evoked potential estimates of sensory thresholds can be obtained as quickly and as conveniently in animals as they can in humans, which is often a welcome contrast with behavioral procedures. Further, their ready acceptance as a surrogate for psychophysically obtained audibility measures in the clinical examination of difficult-to-test humans and their overall correlation with behaviorally obtained audiograms in humans argue that they already serve audiology very well at a practical level: thus, it has seems eminently reasonable to use evoked potential measures rather than behavioral measures to study class A deficits in laboratory animals. Figure 43.5 describes brain stem auditory-evoked responses measures of threshold (the ''ABR'') obtained in our laboratory for C57BL/6J mice ranging from 6 weeks to 5 months of age. The qualitative appearance of these data is exactly that described by Mikaelian in his behavioral assessment of audibility thresholds in a closely related strain of mice, though in our observations the onset of severe hearing loss was much earlier than in his. Our 6-week-old and 3-month-old mice gave approximately the same thresholds as obtained by Mikaelian *et al*. at 2 months of age, but our 5-month-old mice had a much greater loss at the high frequencies. Given the similar values obtained in the younger mice this difference in the older mice seems unlikely to result from the difference in methodologies, but rather from real individual differences that might be anticipated in small groups of old mice (Li and Borg, 1991). More to the point, the overall similarity of these two sets of data attests to the validity of the use of an electrophysiological measure of detection thresholds as a reasonable surrogate for behavioral measures of audibility.

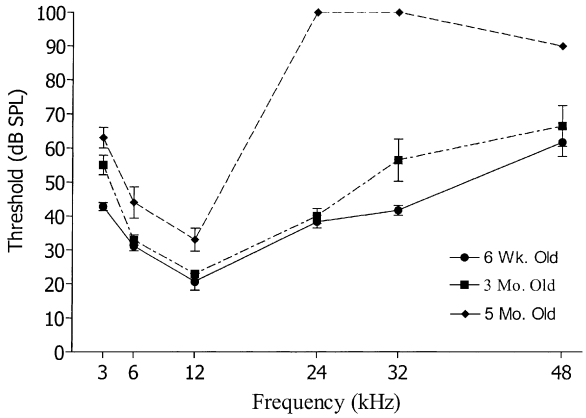

FIG. 43.5. Absolute thresholds in C57BL/6J mice obtained at three different ages, 6 weeks ($n=9$), 3 months ($n=7$), and 5 months ($n=5$). Group means $\pm$ SEM.

From a practical standpoint, the electrophysiological methods have much to recommend them, for the ABR thresholds can be obtained in less than 1 h, while the behavioral thresholds may take many days, even weeks, of training and testing. As a result of this, there are many more examples of the use of evoked potentials for assessing age-related changes in detection thresholds in animals, compared to the use of behavioral thresholds. The list of aged animals that have been tested includes almost all of the laboratory animals common to auditory research, such as mice, rats, guinea pigs, chinchillas, cats, marmosets, and so forth. As a result of this large body of work, there can be little doubt that age-related loss in electrophysiologically defined threshold measures of stimulus detection is a universal phenomenon across mammalian species.

One may object that these measures of neural thresholds, most of them obtained under anesthesia, do not really assess sensory or perceptual experience. However, the general overall correspondence of the data so obtained with the data characteristic of more direct measures of sensation seems sufficient justification for their use. Besides, a philosopher with an even stricter interpretation of the nature of scientific constructs might point out that neither learned behaviors nor psychophysical judgments allow direct inspection of sensory experience in either laboratory animals or human listeners. Of course, all of the conclusions we draw from all of these experiments, regardless of their indicator response, are simply inferences about the sensory and perceptual experiences of our subjects, and their validity lies very simply in their ability to generate coherent and interesting ideas and data. Most certainly, the use of various sorts of evoked potentials in animal models of age-related hearing loss has generated an enormous understanding of the changes in the organ of Corti that underlie the decrements in sensitivity that accompany aging. Most recently these methods, together with some simple behavioral methods of reflex modification, are being used in research that is attempting to prevent early-onset age-related changes in hearing (Sundin and Willott, 1999; Willott *et al*., 2000).

IV. An Animal Model for Studying Distortion

As described earlier, some human listeners who have normal or near normal audiograms have trouble perceiving speech, sometimes when they are in relatively quiet surroundings but particularly when people are speaking in an unfavorable acoustic environment in which competing noises and reverberation mask the speech signal: it is these data that suggest the importance of distortion in limiting the listening abilities of the aged. If it were true that the phenomenon of distortion is specific to language processing and human speech then it would of course be impossible to develop an animal model that would allow us to study its neural basis. However, the working hypothesis that the concept of ''distortion'' describes a set of related phenomena resulting from deficits in processing complex acoustic stimuli along perceptual dimensions other than audibility seems quite reasonable and is likely to be productive. For example, it may be that distortion results because of changes in mechanisms that aid in the processing of signals presented in noise; or spatial location, frequency analysis, or the analysis of signals that rapidly change in time; or mechanisms that maintain the integrity of a complex stimulus on the basis of the common fate of its components.

The loss of audibility of simple acoustic stimuli presented at near-threshold levels almost certainly results in large part or perhaps entirely from age-related degenerative changes in the cochlea. However, while the loss of clarity in a complex acoustic signal might result from changes in the ear, in principle it might well accompany all sorts of functional changes at any level of the nervous system where age-related degenerative processes result in the loss of neurons, perturb neuronal connectivity, or reduce transmitter release or receptor sensitivity. Not surprisingly, our understanding of the perceptual processing deficits that result in the distorted representation of acoustic events in animal models of aging and their physiological bases is much behind our understanding of attenuation. This state of affairs results in part because of a degree of uncertainty in our knowledge of the important dimensions of perceptual processing that are necessary for a faithful representation of an acoustic event even in young human listeners, as well as the difficulties in measuring such subtle effects in laboratory animals. Behavioral studies of auditory thresholds in aging animals have provided the crucial information leading to the subsequent development of the physiological bases of class A hearing loss, and it seems reasonable to suppose that comparable studies of perceptual processing deficits in aging animals will serve the same important purpose for understanding class D hearing loss.

There are several potential perceptual bases of distortion, but the following discussion focuses on temporal acuity for gaps in noise as this is the area in which our laboratories have made the most progress in collecting interdisciplinary data showing parallels between mouse behavior and both human sensory performance and animal physiology. Then further, we think that deficits in temporal acuity are likely to be present in the aged listener and are likely to have a disturbing effect of the clarity of a complex acoustic signal. There are both *a priori* and data-driven reasons for supposing that temporal acuity must be important for speech perception in the human listener. The speech signal can be thought of as a complex array of sinusoidal acoustical signals that vary over time in composition and amplitude. Kewley-Port (1983) reported that certain phonemes differ only in temporal profiles of their frequency spectra on a 5-msec time scale. Our ability to identify these phonemes must depend not only on our detecting their spectral components but also on our encoding their rapidly changing temporal pattern.

Shannon *et al*. (1995) used the temporal envelope of a speech signal to modulate a wideband noise signal and then added onto this envelope an increasing number of narrow spectral bands filtered out from the speech signal. These authors reported that speech understanding was excellent even for a severely degraded signal in the spectral domain, as long as its overall dynamic pattern was maintained. Rosen (1992) has reviewed a set of these experiments that all point to the importance of the temporal cues of the speech envelope for speech understanding. These examples indicate that the ability to track rapidly changing signals must be critically important for accurate speech perception. A further interesting footnote to these ideas was provided by Turner *et al*. (1995), who showed that the ability to recognize the speech signal in amplitude modulated noise was not diminished in hearing-impaired listeners when allowances were made for their class A deficit: they argued that this supported the hypothesis that deficits in temporal acuity seen in some listeners are not limited by their peripheral hearing loss, but results from independent changes in central auditory processing mechanisms.

One of the most dramatic examples of the importance of central processing for the temporal analysis of acoustic signals is presented in the description by Buchtel and Stewart (1989) of the diminished psychoacoustic abilities of their patient with word deafness who was introduced briefly above. In addition to his near total inability to understand the spoken language, this patient suffered a major deficit in his ability to distinguish between the presentation of one versus two tone pips. He required not the usual interval between the pips of about 15 msec to make the correct discrimination, but, remarkably, he required the pips to be separated by about 300 msec. That this was an auditory rather than a cognitive deficit was shown by his normal ability to discriminate between one and two light flashes at various separations.

Some further suggestive evidence for the hypothesis that deficits in temporal acuity are the basis of the problems in speech perception seen in some aged listeners is provided in a classic report by Tyler *et al*. (1982). These authors showed that in a group of listeners differing in age and in degree of hearing loss, temporal processing as assessed by gap detection was strongly correlated with a measure of speech perception in noise. This correlation was primarily due to differences in gap detection in an older subgroup of subjects, older than the median of 58 years of age: in this subgroup the percentage of the variance accounted for by differences in gap detection approached 70%.

There are many experimental reports showing that temporal acuity as measured by gap detection thresholds does decline with age and also with high frequency hearing (for example, Buus and Florentine, 1985). Other data have shown that high frequency components of a wideband carrier make significant contributions to gap detection in human listeners (for example, Buus and Florentine, 1985; Snell *et al*., 1994) and in mice

(Allen *et al*., 2000): thus it might be anticipated that age-related attenuation of the high-frequency components of a gap carrier would result in a decrease in gap sensitivity. The question is, then, whether age by itself might have an independent effect on temporal acuity. Here a report by Snell (1997) is important in its showing longer gap thresholds in elderly listeners compared to those of younger listeners, even though the aged listeners were a select group with near normal hearing. The mean differences between the groups were not large, on the order of 1 to 2 msec across the various conditions, but about one-third of the elderly subjects were outside of the range of the younger subjects, and several elderly listeners had particularly poor temporal acuity. In interpreting these results, it is important to note that the correlations between temporal acuity and measures of high frequency hearing were negligible and accounted essentially for none of the variance in temporal acuity. Of course, this does not mean that in general high frequency hearing ability would not correlate with temporal acuity among a group of listeners who differed considerably in their degree of hearing impairment, but it does mean that in these groups in which aged differences were considerable (26 versus 70 years of age) and differences in thresholds were minimal, the effect of age on temporal acuity was indeed independent of changes in audibility.

Our behavioral data on gap detection obtained in mice are reasonably comparable to those obtained by Snell in the values obtained for thresholds in the mouse compared to the human listener, and in the mean differences in threshold between young and old mice. We are studying gap detection in the CBA mouse, the same mouse strain used by Henry and Chole (1980) as a model of hearing loss that occurs very late in its life span, beyond about 18 months of age. The behavioral method that we use is called ''reflex modification audiometry'' (Young and Fechter, 1983). The method has been used to measure sensory processing in a variety of species (see Ison and Hoffman, 1983; Hoffman and Ison, 1991), including humans. In its present application, the mouse sits in a small cage mounted on top of an accelerometer, and occasionally hears a brief noise burst that produces a flinch-like startle reaction, which is detected by the accelerometer. It is possible to use this startle reflex itself as a measure of suprathreshold hearing (Parham and Willott, 1988, for example), but for reflex modification the basic observations of interest are provided by presenting small threshold and near-threshold stimuli just prior to the startle stimulus. When presented at lead times of about 100 msec, the weak prestimulus inhibits the reflex response to the louder stimulus, and the presence of inhibition is used as objective evidence for prestimulus detection. Similar work with humans (Ison and Pinckney, 1983) has shown that gap thresholds obtained with this procedure approximate those obtained by conventional psychophysical methods. Besides its sensitivity to near-threshold stimulus levels, the great advantage of this methodology is that it does not require that the animal be trained at the task. It has also been shown that reflex inhibition for suprathreshold noise pulses presented in quiet does not vary as a function of age in mice, indicating that the inhibitory mechanisms themselves do not appear to change with age, even though reflex expression to the startle eliciting stimulus is considerably reduced in old mice (Ison *et al*., 1997).

Over 200 mice were run in our experiment on gap thresholds in mice of different ages (reported in Barsz *et al*., 2000), 109 below 1 year of age (range of 2 to 8 months); 42 over 1 year but less than 2 years (range 13 to 20 months); and 67 over the age of 2 years (age 24 to 29 months). The fact that many animals can be tested quite efficiently in little time is one of the great advantages of the method, compared to those methods that require the use of training, but the thresholds we obtain are about the same as those obtained with these other methods (Wagner *et al*., 2000). It is also important that the method can be used to indicate the salience of suprathreshold stimuli, which we now believe may be more important than the simple assessment of threshold values for aging research.

The prestimulus was a gap that ranged in duration from 0 to 15 msec, that was presented 60 msec before the startle eliciting stimulus. Fig. 43.6 shows the type of data characteristic of experiments of this sort. The size of the response in each gap condition was ''normalized'' with respect to the baseline size of the uninhibited control condition which had no gap presented. It can be seen in all age groups that as the gap duration increased the relative size of the startle reflex diminished, rapidly when the gap increased over the range of 2 to 6 msec and more slowly afterward. The majority of mice showed an abrupt inhibitory effect for gaps between 2 and 4 msec in duration, with stragglers accounting for most of the drop in the response at the longer intervals.

It is very clear in these data that the asymptotic inhibitory effect of the gaps declined with age, this indicating that the longer gaps were less salient in the older mice. The individual data of each mouse were further analyzed in order to determine a gap threshold, this defined as the smallest gap duration that provided at least one-half of the asymptotic level of inhibition, thus providing threshold values that were independent of the asymptotic peak of inhibition. Fig. 43.7 shows the mean gap thresholds for these mice when again ranked in 1-year categories, the youngest group showing the smallest thresholds and the older group showing the largest thresholds.

These age effects for both asymptotic relative response values and for the mean gap thresholds were highly significant in the analysis of variance. These mean differences between

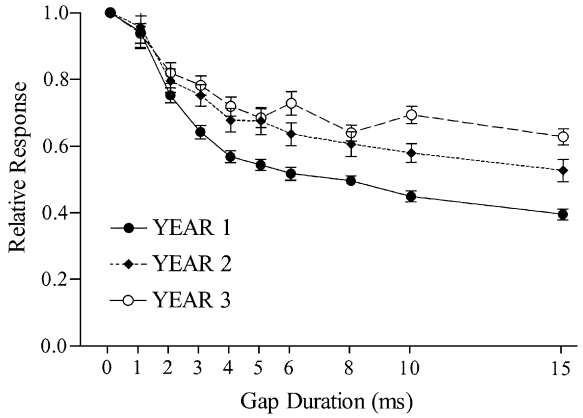

FIG. 43.6. Amplitude of the startle response (relative to the baseline control value) as a function of gap duration, in mice less than 1 year old ($n = 109$), between 1 and 2 years of age ($n = 67$), and more than 2 years old ($n = 67$).

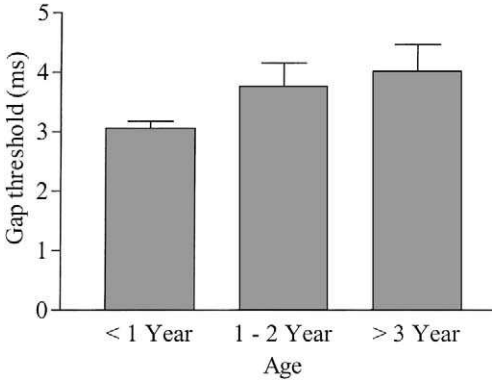

FIG. 43.7. Mean gap thresholds in mice of different ages.

the three groups in their threshold values are not extraordinarily large, but in fact they are about equivalent to the mean differences obtained in young versus old human listeners, as was shown in the data of Karen Snell. Then further, the variance in the scores within the oldest group of mice was significantly larger than that obtained in the youngest mice, and although many of the oldest mice had normal thresholds of 2 and 3 msec, about 10% had thresholds beyond the range of the younger mice.

How might these effects be explained? Our mouse experiment was different in design from that of Snell's human experiment because the mice were not preselected for their having particularly good absolute thresholds. Thus, like an unselected group of human listeners, it might be expected that, for the aged groups as a whole, measures of absolute sensitivity would be reduced in comparison to young mice. In fact, this was the case. We were able to measure thresholds in about one-third of these mice using the ABR, 37 of the youngest group, 17 of the middle-aged group, and 14 of the oldest group. These threshold data are given in Fig. 43.8. There were significant differences in sensitivity with age, with the groups being somewhat different throughout the spectrum, but different especially beyond about 20 to 24 kHz. At these higher levels, and depending on the frequency, the youngest mice had thresholds about 5 to 10 dB below those of the middle-aged group and 10 to 20 dB below those of the oldest group of mice.

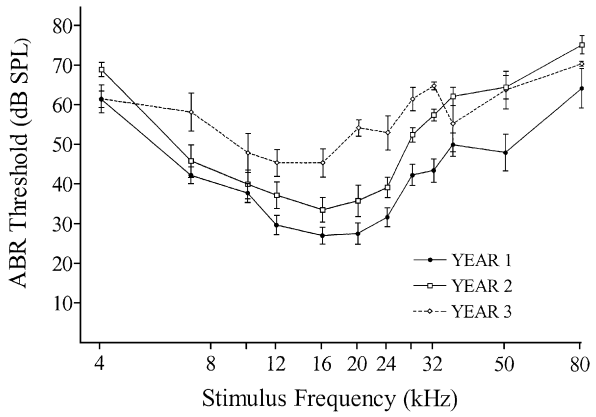

FIG. 43.8. Mean ABR thresholds (± SEM) in CBA mice of difference ages, within the first year (mean, 5 months), second year (mean, 16 months), or third year (mean, 25 months) of life.

Although there was no correlation between age in months and ABR thresholds within the first year of life (the range was just 3 to 8 months) there were substantial individual differences in threshold sensitivity, with the interquartile range within this group being about 20 dB. The presence of these individual differences in sensitivity to simple tone pips as given in the ABR data made it possible to determine whether differences in gap threshold would be affected by a class A hearing loss in mice. A correlation analysis of these data showed that sensitivity to moderately high frequency stimuli (the average of the thresholds taken from 28 to 50 kHz) was significantly correlated with gap detection thresholds, with the differences in threshold accounting for about 20% of variance in gap levels ($r = +0.46$). This correlation could perhaps have been anticipated because in other work we have found that in mice, as in humans, gap detection is better if the carrier for the gap has these high frequency components (Allen *et al.*, 2000), but it is an interesting confirmation of human data. It might then also be anticipated that the older mice would be less sensitive to the gaps simply because of their relatively poor sensitivity to high frequency stimuli. Indeed this may have contributed to an overall effect of age on gap detection, but in fact, we were not able to find a significant correlation between gap detection thresholds and ABR thresholds. However, we did find that in the two older groups of mice small gap thresholds could be found both in mice with poor threshold sensitivity for high frequency stimulation and in mice with good sensitivity to high frequency stimuli, but very large gap thresholds were found only in older mice that showed poor sensitivity to high frequencies in the ABR measures. The data suggest that a class A deficit may have been necessary but not sufficient to produce a deficit in gap detection thresholds.

In contrast to the small effect of age on gap detection thresholds, shown in Fig. 43.7, there was a relatively large effect of age on the asymptotic levels of inhibition, as can be seen in Fig. 43.6. A correlation analysis showed that differences in sensitivity to relatively high frequency stimuli was also correlated with the relative size of the response at the longest gap durations, the least sensitive mice showing also the least inhibitory effect of gaps at these suprathreshold gap durations ($r = 0.37$, $P < 0.01$). However, in addition, there was an equally strong effect of age alone on the level of asymptotic inhibition, there being a significant correlation between age at the time of the behavioral test and the residual inhibition values after regressing inhibition on sensitivity ($r = 0.38$, $P < 0.01$).

How might increased age contribute to a decline in the salience of suprathreshold gaps, beyond its effect on measures of sensitivity to high frequency stimuli? We believe the answer to this lies in the central nervous system, because this behavioral effect has a parallel in the neurophysiological data for suprathreshold gap durations obtained in Joe Walton's laboratory. In the young mouse both the behavioral gap threshold and the overall time course of the growth of reflex inhibition with an increase in gap duration are very similar to the neural threshold and neural responsivity in phasic neurons in the inferior colliculus (Walton *et al.*, 1997). A later study of these types of cells in both young and old CBA mice (reported in Walton *et al.*, 1998) found two neural effects that resemble the behavioral effects shown here in Figs. 43.6 and 43.7. The first finding

was that the mean thresholds for the phasic cells were slightly elevated in the older mice, overall by about 1 to 3 msec depending on the type of cell. Many neurons in the old mouse had the same very sharp sensitivity as those of the young mouse, but there were proportionately fewer such cells in old compared to young mice. The second finding was that the recovery function to noise onset at the end of the gap was substantially prolonged in the older mice, with most young cells having fully recovered for gap durations between 5 and 10 msec in duration, whereas most cells in the older mice were not fully recovered even at gap durations of 20 to 30 msec. These age-related differences in neural responsivity to the end of a suprathreshold gap were obviously substantial, and, in contrast to the effects of age on gap thresholds, there was very little overlap between the young and the old neurons on this measure.

Thus in both sets of data, behavioral and neurophysiological, the major effects of age were seen in the diminished response to suprathreshold gaps, rather than in the gap detection threshold. This parallel outcome supports the thesis that the neural substrate for behavioral gap detection is in these phasic cells of the inferior colliculus and that the most apparent effect of age is to diminish the salience of suprathreshold events.

Our behavioral data point to two discrete and separable effects of age that diminish the perceptual significance of brief gaps in noise in the mouse, just as others have shown that two effects of age may be responsible for the changes in gap detection in human listeners. One effect is attenuation, which reduces the audibility of the high-frequency components of the carrier for the gap. In the conceptual scheme provided by Plomp, this is clearly a class A hearing loss. The other is manifest in the mouse both at the level of the neural processing in the central auditory neural system and in its behavior, and may be thought of most easily as a diminished neural responsivity to gaps which at a sensory level is represented by diminished salience, and in behavior as reduced inhibition. The ability to detect gaps has been shown to be strongly correlated to differences in speech perception among aged listeners, and the temporal attributes of the spoken language have been shown to contain an abundance of discriminative cues essential to its understanding. Thus we suspect that a diminished sensitivity to the dynamic acoustic structure of speech must result in a type of presbycusic hearing loss that may reasonably be thought of as distortion.

V. Conclusions and Thoughts for the Future

We think that in these data we have shown the importance of studying both threshold and suprathreshold effects in aged animals, and have found evidence for both class A and class D hearing loss in the acoustically guided behavior of old animals. Old mice, like old human listeners, show diminished threshold sensitivity to gaps, and, perhaps more important, show a diminished responsiveness to gaps presented at suprathreshold values. Evidence for the partial independence of class A and class D deficits in our data seems particularly interesting from the standpoint of trying to understand where class D deficits might arise. It has seemed reasonable to attribute class A deficits to deficits at the level of the organ of Corti and class D deficits to deficits in brain function. This distinction is not completely well founded, for it has been shown that changes in sensitivity for particular frequencies might have effects other than simple attenuation at those frequencies. Thus, at the level of the auditory nerve Woolf *et al*. (1981) found that the loss of outer hair cells reduced the threshold sensitivity of high frequency fibers to high frequency stimulation but also reduced their ability to phase lock to lower frequency stimuli presented at above-threshold values. Given the importance of phase locking to certain forms of temporal processing (in particular, sound localization) it may be expected that in this case the class A hearing loss would be accompanied by a suprathreshold class D loss. In line with this reasoning, in the present experiment the class A loss was clearly correlated with a diminished suprathreshold inhibitory effect of gaps and so may be presumed to contribute to the class D hearing loss: but the separate correlation of age with inhibition is evidence that there is an additional class D loss independent of changes at the periphery.

The strong correspondence between the behavioral data and the findings from electrophysiology provided in Walton's laboratory provides a persuasive argument for the basis of this class D loss in the reduced neural response of the aged inferior colliculus at the end of the suprathreshold gap. Together these data suggest that the amelioration of the two problems of presbycusis must have two goals, one to solve the problems of the periphery, the other to solve the problems of the aged central auditory nervous system. We can imagine that this might involve neuropharmacological treatment that would boost the function of the aged neuron, perhaps by enhancing its reactivity to important acoustic events, such as gaps in noise. Then further, given recent demonstrations of neuroplasticity within the auditory system that results from exposure to certain stimulus conditions, such as the augmented acoustic environment studied by Willott *et al*. (2000), it may be possible to find other means for enriching the auditory environment in a way that will maintain auditory function with age.

The focus in the present chapter is on the contributions that an animal model of age-related changes in temporal processing might make to our understanding of presbycusis in human listeners. Obviously the distortion of complex acoustic signals may result from other sorts of deficits, such as changes in the abilities to extract signals from noise, locate sound objects in space, attend to particular signals and properly ignore others, or maintain the coherence of a complex sound object by synchronizing the processing of its component elements, for example. It seems likely that these problems too will have their bases in a combination of both peripheral and central neural mechanisms. The search for their neural bases must certainly profit from the continued development of animal models that provide the link between the physiological and the sensory–perceptual bases of behavior and offer a laboratory method for studying therapeutic effectiveness.

References

Allen, P. D., Barsz, K., Ison, J. R., Walton, J. P., and O'Neill, W. E. (2000). Gap-detection in behaving mice for octave band noise compared to inferior colliculus gap-detection for neurons of different best frequency. *Soc. Neurosci. Abstr.* **26** (in press).

Barsz, K., Walton, J., Ison, J., and Snell, K. (2000). Changes in gap detection in the aging mouse: Behavioral measures and their correlates in the inferior colliculus. *Assoc. Res. Otolaryngol. Abstr.* **23**, 154.

Bennett, C. L., Davis, R. T., and Miller, J. M. (1983). Demonstration of presbycusis across repeated measures in a nonhuman primate species. *Behav. Neurosci.* **97**, 602–607.

Buchtel, H. A., and Stewart, J. D. (1989). Auditory agnosia: Apperceptive or associative disorder? *Brain Lang.* **37**, 12–25.

Buus, S., and Florentine, M. (1985). Gap detection in normal and impaired listeners: The effect of level and frequency. *In* "Time Resolution in Auditory Systems" (A. Michelsen, ed.), pp. 159–179. Springer-Verlag, New York.

Ehret, G. (1974). Age-dependent hearing loss in normal hearing mice. *Naturwissenschaften* **11**, 506–507.

Fay, R. R. (1988). "Hearing in Vertebrates: A Psychophysical Data Book" Hill-Fay Associates, Winnetka, IL.

Glasberg, B. R., and Moore, B. C. (1989). Psychoacoustic abilities of subjects with unilateral and bilateral cochlear hearing impairments and their relationship to the ability to understand speech. *Scand. Audiol. Suppl.* **32**, 1–25.

Heffner, H. E., Heffner, R. S., Contos, C., and Ott, T. (1994). Audiogram of the hooded Norway rat. *Hear. Res.* **73**, 244–247.

Henry, K. R., and Chole, R. A. (1980). Genotypic differences in behavioral, physiological and anatomical expressions of age-related hearing loss in the laboratory mouse. *Audiology* **19**, 369–383.

Hoffman, H. S., and Ison, J. R. (1991). The application of reflex modification to the analysis of sensory processing in developmental and comparative research. *In* (B. A. Campbell, H. Hayne, and R. Richardson, eds.), "Attention and Information Processing in Infants and Adults: Perspectives from Human and Animal Research" pp. 83–111. Erlbaum, Hillsdale, N. J.

Ison, J. R., and Pinckney, L. A. (1983). Reflex inhibition in humans: Sensitivity to brief silent periods in white noise. *Percep. Psychophys.* **34**, 84–88.

Ison, J. R., and Hoffman, H. S. (1983). Reflex modification in the domain of startle: II. The anomalous history of a robust and ubiquitous phenomenon. *Psychol. Bull.* **94**, 3–17.

Ison, J. R., Bowen, G. P., Pak, J., and Gutierrez, E. (1997). Changes in the strength of prepulse inhibition with variation in the startle baseline associated with individual differences and with old age in rats and mice. *Psychobiology* **25**, 266–274.

Ison, J. R., Agrawal, P., and Frisina, D. R. (1998). An initial study of the beneficial effect of spatial separation of signal and noise on masking in the free field in the aged mouse, in comparison to that in the aged human listener. *Soc. Neurosci. Abstr.* **24**, 904.

Kelly, J. B., and Masterton, B. (1977). The auditory sensitivity of the albino rat. *J. Comp. Physiol. Psychol.* **91**, 930–936.

Kewley-Port, D. (1983). Time-varying features as correlates of place of articulation in stop consonants. *J. Acoust. Soc. Am.* **73**, 322–335.

Li, H. S., and Borg, E. (1991). Age-related loss of auditory sensitivity in two mouse genotypes. *Acta Oto-Laryngol.* **111**, 827–834

Long, G. R. (1994). Psychoacoustics. *In* "Comparative Hearing: Mammals" (R. R. Fay and A. R. Popper, eds.), pp. 18–56. Springer-Verlag, New York.

Lutman, M. E., and Clark, J. (1986). Speech identification under simulated hearing-aid frequency response characteristics in relation to sensitivity, frequency resolution, and temporal resolution. *J. Acoust. Soc. Am.* **4**, 1030–1040.

Middelweerd, M. J., Festen, J. M., and Plomp, R. (1990). Difficulties with speech intelligibility in noise in spite of a normal pure-tone audiogram. *Audiology* **29**, 1–7.

Mikaelian, D. O., Warfield, D., and Norris, B. A. (1974). Genetic progressive hearing loss in the C57/b16 mouse: Relation of behavioral responses to cochlear pathology. *Acta Oto-Laryngol.* **77**, 327–334.

Parham, K., and Willott, J. F. (1988). Acoustic startle response in young and aging C57BL/6J and CBA/J mice. *Behav. Neurosci.* **102**, 881–886.

Pearson, J. D., Morrell, C. H., Gordon-Salant, S., Brant, L. J., Metter, E. J., Klein, L. L., and Fozard, J. L. (1995). Gender differences in a longitudinal study of age-associated hearing loss. *J. Acoust. Soc. Am.* **97**, 1196–1205.

Pickles, J. O. (1988). "An Introduction to the Physiology of Hearing," 2nd ed. Academic Press, New York.

Plomp, R. (1978). Auditory handicap of hearing impairment and the limited benefit of hearing aids. *J. Acoust. Soc. Am.* **63**, 533–549.

Rosen, S. (1992). Temporal information in speech: Acoustic, auditory and linguistic aspects. *Philos. Trans. R. Soc. London Ser. B Biol. Sci.* **336**, 367–373.

Schwartz, D. M., and Schwartz, J. A. (1991). Auditory evoked potentials in clinical pediatrics. Chapter 8 *In* "Hearing Assessment" (W. F. Rintelmann, ed.). 2nd ed., pp. 429–476. Allyn & Bacon, Needham Heights, MA.

Shannon, R. V., Zeng, F. G., Kamath, V., Wygonski, J., and Ekelid, M. (1995). Speech recognition with primarily temporal cues. *Science* **270**, 303–304.

Snell, K. B. (1997). Age-related changes in temporal gap detection. *J. Acoust. Soc. Am.* **101**, 2214–2220.

Snell, K. B., Ison, J. R., and Frisina, D. R. (1994). The effects of signal frequency and absolute bandwidth on gap detection in noise. *J. Acoust. Soc. Am.* **96**, 1458–1464.

Sundin, V. S., and Willott, J. F. (1999). Effects of exposure to an augmented acoustic environment on hearing loss in DBA/2J mice. *Soc. Neurosci. Abstr.* **25**, 133.

Turner, C. W., Souza, P. E., and Forget, L. N. (1995). Use of temporal envelope cues in speech recognition by normal and hearing-impaired listeners. *J. Acoust. Soc. Am.* **97**, 2568–2576.

Tyler, R. S., Summerfield, Q., Wood, E. J., and Fernandes, M. (1982). Psychoacoustic and phonetic temporal processing in normal and hearing-impaired listeners. *J. Acoust. Soc. Am.* **72**, 740–752.

Wagner, E., Klump, G. M., and Hamann, I. (2000). Gap detection in Mongolian gerbils. *Assoc. Res. Otolaryngol. Abstr.* **23**, 27.

Walton, J. P., Frisina, R. D., Ison, J. R., and O'Neill, W. E. (1997). Neural correlates of behavioral gap detection in the inferior colliculus of the young CBA mouse. *J. Comp. Physiol. A* **181**, 161–176.

Walton, J. P., Frisina, R. D., and O'Neill, W. E. (1998). Age-related alteration in processing of temporal sound features in the auditory midbrain of the CBA mouse. *J. Neurosci.* **18**, 2764–2776.

Willott, J., Bross, L., and Turner, J. (2000). Effects of exposure to an augmented acoustic environment on BABL/cj mice: Interaction of age and gender. *Assoc. Res. Otolaryngol. Abstr.* **23**, 782.

Woolf, N. K., Ryan, A. F., and Bone, R. C. (1981). Neural phase-locking properties in the absence of cochlear outer hair cells. *Hear. Res.* **4**, 335–346.

Working Group on Speech Understanding and Aging, Committee on Hearing, Bioacoustics and Biomechanics (CHABA). (1988). Speech understanding and aging. *J. Acoust. Soc. Am.* **83**, 859–895.

Yerkes, R. M. (1905). The sense of hearing in frogs. *J. Comp. Neurol. Psychol.* **15**, 279–304.

Young, L. S., and Fechter, L. D. (1983). Reflex inhibition procedures for animal audiometry: A technique for assessing oto-toxicity. *J. Acoust. Soc. Am.* **73**, 1686–1693.

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Donald G. Sims and Robert Burkard

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Rehabilitation for Presbycusis

Current and promising future treatments for presbycusis are reviewed. Proceeding from the evidence that compensatory functional reorganization of the brain takes place with aging, rehabilitation efforts should include appropriate treatment of the peripheral and central components of age-related hearing handicaps. Treatment may include addressing age-related declines in neurochemistry, but also may include behavioral training in compensatory communication strategies following the establishment of successful hearing aid use. This chapter has one section that summarizes animal experiments which may lead to human biochemical, bioelectric, or other interventions to reduce or reverse the effects of aging in the auditory system; the second section summarizes current and potentially fruitful future approaches to audiologic rehabilitation. In this chapter, we provide the reader with cross-discipline vantage points in order to facilitate collaborative, applied research. © 2001 Academic Press.

I. Introduction

One of the challenges of research based on the scientific method is that you must hold constant, manipulate, or make random all sources of variability in an experiment so causality can be clearly shown. Usually this sort of experimental control is best achieved in animal research. The price paid for this approach is that we can never be sure that these experimental results can be applied to humans. Scientists will, therefore, always be challenged to find an application of their work that is relevant to the human condition, while clinicians will always be challenged by scientists to be more scientific in their approach to human research. This chapter represents an attempt by an audiologist and a hearing scientist to ''mingle'' their views of hearing and hearing loss and thus enable the reader to see current and future aural rehabilitation strategies from both a clinical and a scientific perspective. In this way, the scientist can review current practice and research of the clinical audiologist's art in order to be alert to areas of application. In addition, the audiologist can view the scientific horizon for clinical treatments and participate in the evaluation of the efficacy of emerging medical interventions. As a reader of a book on neurophysiology of auditory aging, it would be easy to bypass the ''soft'' science of applied, aural rehabilitation. It would be similarly easy for the audiologist to dismiss the hard science of neurophysiology as not applied enough to be useful. We urge the reader to take a few minutes to complete this review of aural rehabilitation from both perspectives. If amelioration of hearing impairments in the aged is the ultimate goal of work in this area, then we have a responsibility to be appraised of the full spectrum of treatments currently available for the elderly patient with a hearing problem, as well as to consider what the future might hold in terms of aural rehabilitation for this population.

II. Aural Rehabilitation in the (Near?) Future

The hearing health care professional working with an elderly patient currently has a limited number of rehabilitative options open to them. They can provide a technical solution to hearing impairment in the form of assistive listening devices, hearing aids, or cochlear implants. They can educate their clients so that they can optimally control their communicative situation or use multimodality strategies to enhance their speech understanding. Despite the benefits of amplification and aural rehabilitation, the combination of hearing impairment and advanced age still, unfortunately, results in social withdrawal and a feeling of isolation in many elderly patients. This section of the chapter will offer a conceptual model of age-related hearing decrements and a discussion of possible treatments for presbycusis that might become reality during the reader's lifetime. Ponce de Leon failed in his quest to find the fountain of youth. While Florida may never reveal the secrets of immortality, it seems likely that recent scientific studies may provide the basis for the prevention or treatment of age-related hearing loss.

What is presbycusis? Many people live well into the eighth or ninth decade of life and show remarkably normal hearing, while others show a steady decrement in their audiometric thresholds starting in early adulthood. While it is comforting to know that hearing loss is not an inevitable consequence of aging, the reasons underlying this remarkable variability in age-related hearing loss are largely unknown. Schuknecht

(1974) identified four different types of age-related hearing loss, relating patterns of audiometric findings to underlying anatomical changes. Whereas this is helpful as a classification scheme of presbycusis, this still does not explain the underlying causes of presbycusis, or its variable expression. It seems likely that the development of effective prevention or treatment of age-related hearing loss will follow an improved understanding of the underlying causes of presbycusis.

A. Is Presbycusis Purely Peripheral?

In the following paragraphs, we will review evidence that the central auditory system undergoes a functional reorganization in response to damage to the peripheral auditory system. We will argue that the constellation of symptoms known to comprise presbycusis is not solely the result of peripheral damage, but also the consequence of the functional reorganization of the central auditory nervous system following peripheral damage.

1. Evidence of Functional Reorganization Following Hearing Loss

A large body of evidence demonstrates that the adult brain is capable of substantial functional changes, both as a result of the normal learning process, or in response to injury. Let us review evidence that the central auditory nervous system is capable of functional reorganization in adults. The most direct evidence is available from studies of nonhuman mammals, and we will briefly review some of this literature and assume that these findings can be generalized to humans.

There is evidence that learning can produce changes in the cortical representation of sound. Bakin and Weinberger (1990) used classical conditioning to investigate changes in cortical receptive fields in guinea pigs. They implanted an electrode array in the auditory cortex. Following recovery from surgery, responses from these electrodes were recorded in the awake guinea pig to determine the receptive field of the near-field responses from the various electrodes in the electrode array. Animals were exposed to a classical conditioning paradigm, where a tone stimulus was followed in some trials by a mild electrical shock. Other animals received the tonal stimulus or the shock, in random order, but unpaired. For the animals exposed to the conditioning procedure, there was an increase in the neural response magnitude to the conditioning acoustic stimulus, and a reduction in response magnitude at other frequencies. In some instances, this actually led to a change in response best frequency to the conditioning frequency. Sensitized animals showed an overall increase in response across the receptive field, but did not show a change in tuning. Bakin and Weinberger (1990) found that the effects of conditioning lasted up to 24 hr (the longest time interval they studied). Weinberger *et al*. (1993) found evidence that these receptive field modifications could last as long as 8 weeks following training. Moreover, they found these changes in animals that were anesthetized for the cortical recording session (but not the conditioning session). Recanzone *et al*. (1993) trained monkeys to perform a specific frequency discrimination task. The monkeys improved in this task as a result of training. These trained animals were then studied physiologically by recording multiple-unit responses from primary auditory cortex. When compared to control animals, the trained animals showed an expanded frequency representation, sharper tuning, and increased response latencies for the frequency region used for the frequency discrimination task. These differences were not observed for other frequency regions. These studies demonstrate clear changes in the processing of acoustic stimuli in the auditory cortex that appear to be the result of learning.

There is also evidence of functional reorganization in adult animals following cochlear damage. One line of evidence shows changes in the tonotopic map. Kaltenbach *et al*. (1992) exposed hamsters to a damaging high level, high frequency tone, and investigated changes in the tonotopic map of the dorsal cochlear nucleus. In the nonexposed animals, there was a systematic increase in best frequency as the electrode moved in the lateral to median direction through the dorsal cochlear nucleus. Responses of the tone-exposed animals showed a gap in best frequency near the exposure frequency, or an expanded representation of the frequencies bordering the exposure frequency. Thus, the tonotopic map can be modified in response to peripheral hearing loss. However, Kaltenbach *et al*. (1992) noted that thresholds were often elevated in the region of expanded representation and suggested that these changes were in all probability the result of peripheral changes (i.e., the best frequency was shifted to a different frequency because the low threshold tip had been substantially elevated due to tone exposure). Robertson and Irvine (1989) produced cochlear lesions of limited extent in guinea pigs, and studied changes in the tonotopic organization of the auditory cortex. At long survival times (more than 1 month), the tonotopic map changes revealed an expansion in representation of tonal frequencies adjacent to the region of cochlear damage. In addition, thresholds in this expanded frequency region were very similar to normal thresholds at these frequencies. In a second experiment, Robertson and Irvine (1989) studied cortical tonotopic organization within several hours of exposure to the damaging tone. It was found that responses were shifted in best frequency in frequency regions adjacent to the damaged region, but in this case thresholds were substantially elevated compared to control values. These data suggest that changes in the map immediately following cochlear damage are likely the direct result of peripheral damage, with units in the cortical region whose best frequencies were previously in the region of greatest cochlear damage showing responses to frequencies remote from best frequency. However, the emergence of normal thresholds in these ''reorganized'' regions appears to be the result of functional reorganization. Willott *et al*. (1993) found expanded frequency representation of the midfrequency region of primary auditory cortex in aging C57 mice, as their high frequency thresholds increased due to progressive, adult-onset hearing loss. Thresholds in the expanded midfrequency region were low (near normal). These studies suggest that following traumatic or progressive hearing loss that the auditory cortex is capable of a functional reorganization of frequency representation across the auditory cortex.

A second line of evidence that the auditory system is capable of reorganization is based on evoked potential amplitude changes following cochlear injury. Salvi *et al*. (1990) exposed chinchillas to a high intensity tone that produced a substantial threshold shift for mid and high frequency tone bursts. Despite

the threshold elevation, responses as measured from the inferior colliculus often showed larger than normal responses to high level tone-burst stimuli. Popelar *et al*. (1987) investigated responses from the auditory nerve, inferior colliculus, and auditory cortex in awake guinea pigs before and after they were exposed to white noise (120 dB SPL, 1 hr). Evoked potential thresholds at 1 hr following exposure were elevated, and threshold changes were similar at all three levels of the auditory system, and recovered to near baseline values by 1 week postexposure. At 1 hr postexposure, auditory nerve and inferior colliculus response amplitudes were reduced for all tone-burst levels. In contrast, auditory cortex response amplitude was increased at 1 hr postexposure. Response amplitudes progressively moved toward baseline values (i.e., auditory nerve and inferior colliculus responses increased, auditory cortex responses decreased) and approximated baseline values by 1–2 weeks postexposure. It is interesting to note that when guinea pigs were anesthetized with urethane, all three responses (auditory nerve, inferior colliculus, auditory cortex) showed amplitude reduction following noise exposure. Thus, not only can responses from the cortex show response enhancement following hearing loss, despite smaller response amplitudes from more caudal auditory regions, but this cortical response enhancement is sensitive to anesthesia. Robert Harrison and colleagues (Wake *et al*., 1993, 1994; Takeno *et al*., 1994a,b) found that carboplatin, an antineoplastic agent, can selectively damage inner hair cells in the chinchilla. Trautwein *et al*. (1996) investigated the effects of carboplatin on the compound action potential of the auditory nerve. They found a decrease in the amplitude of the compound action potential, with little or no compound action potential threshold change until a near total loss of inner hair cells was observed. Burkard *et al*. (1997) reported a substantial decrease in the amplitude of the inferior cortex potential following inner hair cell loss, but little change in threshold. Qiu *et al*. (2000) evaluated the effects of inner hair cell loss while recording the auditory nerve, inferior colliculus, and auditory cortex potential in unanesthetized chinchillas. Threshold changes were minimal at all electrode locations, even when inner hair cell loss was quite substantial. Auditory nerve response amplitude decreased in proportion to inner hair cell loss. Inferior colliculus response amplitude also tended to decrease with increasing inner hair cell loss, but this decrease was less than that seen for the auditory nerve response. Finally, and most importantly, auditory cortex response amplitudes in many cases increased following inner hair cell loss. In some chinchillas, amplitude enhancement was most prominent at 2 weeks after carboplatin, and then reduced to near baseline values at 5 weeks after carboplatin. Others showed an increase in auditory cortex response amplitude that was similar at 2 and 5 weeks after carboplatin. We believe that cortical amplitude enhancement following inner hair cell loss is the result of auditory plasticity in response to the reduced peripheral input. These observations are of fundamental importance in the present context, because they demonstrate that substantial inner hair cell loss produces little threshold shift, and hence a normal audiogram does not guarantee a normal complement of hair cells and that, following inner hair cell loss, and the subsequent reduction in neural input to the central auditory system, cortical activity can actually be enhanced. Perhaps some of the age-related changes in auditory abilities are not directly a consequence of age-related hearing loss itself, but rather the result of the subsequent functional reorganization.

The preceding paragraphs demonstrate that the auditory nervous system is capable of functional reorganization as not only part of the normal learning process, but also in response to cochlear damage. This suggests that the social and clinical manifestations of hearing loss are not only the result of the peripheral hearing loss per se, but also possibly the result of compensatory functional reorganization. It appears that normal hearing (i.e., normal hearing thresholds) does not guarantee that the cochlea is normal, as substantial inner hair cell loss often results in only small threshold shifts (Qiu *et al*., 2000). Furthermore, functional reorganization may appear as cortical response enhancement, suggesting that such reorganization is not strictly the result of threshold shift. In terms of our discussion of the aging auditory system, it appears that we cannot conclude that the auditory system of older human subjects is ''normal'' based on thresholds alone. Furthermore, functional reorganization is possible, despite ''normal'' thresholds. These considerations suggest that elderly patients could exhibit decrements in auditory performance, despite a normal audiogram, and that these decrements could be the result of peripheral damage or compensatory functional reorganization. Thus, aural rehabilitation in the future needs to focus on a reduction of peripheral hearing loss (cochlear damage in its broadest sense) and on developing the ability to modify functional reorganization in response to cochlear damage.

B. Preventing Cochlear Hearing Loss

Let us accept the notion that peripheral hearing loss in the elderly is responsible for many of their communicative problems, regardless of whether it is the hearing loss itself or the resulting functional reorganization. Thus, the problem of presbycusis might best be solved by an ounce of prevention rather than a pound of cure. Age-related hearing loss appears to have multiple causes. For example, Schuknecht (1974) lists four distinct forms of presbycusis based on clinical manifestations and anatomical changes: cochlear, strial, neural, and cochlear–conductive. Whether age-related hearing loss is the accumulation of ototoxicity over a lifetime, the result of an adult-onset genetically based hearing loss, or simply an inevitable consequence of getting old, prevention of cochlear hearing loss with aging would presumably improve an elderly patient's quality of life. There is currently an emerging body of evidence that sensorineural hearing loss can be reduced by appropriate treatment prior to exposure to ototoxic agents.

Hu *et al*. (1997) investigated the effects of *R*-phenylisopropyladenosine on noise-induced hearing loss. *R*-phenylisopropyladenosine has been found to upregulate antioxidant enzyme activity, and in this study was topically delivered through the round window of one ear of each chinchilla, while normal saline was delivered to the opposite ear. Within several hours of *R*-phenylisopropyladenosine or saline application, each chinchilla was exposed to an octave band of noise centered at 4 kHz (105 dB SPL, 4 hr). Distortion product otoacoustic emission input/output functions and inferior colliculus evoked potential thresholds were determined at various postexposure times (15 min to 20 days), and hair cell counts

were made (20 days postexposure). Distortion product otoacoustic emissions were somewhat larger in the *R*-phenylisopropyladenosine-treated ears than the saline-treated ear. In addition, average thresholds were lower (better) in the *R*-phenylisopropyladenosine-treated ears than in the saline-treated ears. Finally, outer hair cell loss was reduced in the *R*-phenylisopropyladenosine-treated ears as compared to that seen in the saline-treated ears, while inner hair cell loss was similar for both ears. It appears that *R*-phenylisopropyladenosine can be effective in reducing noise-induced hearing loss. Hight *et al*. (1999) investigated the effects of glutathione monoethyl ester on hearing loss to high level impulse noise. Glutathione monoethyl ester was applied to one round window approximately 40 min prior to exposure to 50 pairs of 150 dB peak SPL impulses. There was a nonsignificant trend for permanent threshold shift to be less in the glutathione monoethyl ester-treated ears. There was significantly less outer hair cell loss in the glutathione monoethyl ester-treated ears than in the control ears. Wang *et al*. (1999) infused leupeptin (a calpain inhibitor) into one cochlea in a group of chinchillas using an osmotic pump. Leupeptin reduced noise-induced hair cell loss. Leupeptin had little influence on inner hair cell loss and actually increased outer hair cell loss due to carboplatin.

Canlon *et al*. (1988) investigated the effects of exposure to a low level ''conditioning'' noise prior to exposure to a higher level exposure that is potentially damaging to the auditory system. They found a reduction in threshold shift in those animals receiving the ''conditioning'' exposure, as compared to those who received only the ''damaging'' exposure. It appears that prior exposure to a nondamaging sound can reduce the damage to a ''damaging'' sound. This phenomenon has been popularly referred to as toughening. Jacono *et al*. (1998) provides a possible explanation for toughening. They investigated antioxidant activity in the cochleae of animals exposed to a variety of sounds. They found increases in glutathione reductase, γ-glutamyl cysteine synthetase, and catalase activity that were more prominent in the stria than in the organ of Corti. These data suggest that toughening may reduce noise-induced hearing loss by upregulation of free radical scavengers. When the damaging exposure occurs, the upregulated ability to scavenge reactive oxygen species provides protection above and beyond that available in the non toughened animals.

One might ask what toughening and noise-induced hearing loss have to do with prevention of age-related hearing loss. This rhetorical question is answered by Willott and Turner (1999), who studied age-related hearing loss in two strains of mice (C57BL/6J and DBA/2J) who develop a progressive, adult-onset hearing loss. Beginning at 25 days of age, mice were exposed to a 70 dB SPL broadband noise for 12 hr each evening. When compared to control animals, the mice exposed to the conditioning noise showed improved auditory performance, including lower (better) auditory brain stem response thresholds. The noise conditioning did not eliminate threshold decrement with aging, but did appear to reduce its magnitude, for measurement ages of 3–14 months in C57BL/6J mice and 2–9 months in DBA/2J mice. Both of these strains of mice exhibit an adult-onset genetically caused sensorineural hearing loss, and thus this study cannot be viewed as evidence that toughening can reduce ''normal'' age-related hearing loss. It does, however, suggest that progressive (and thus age-dependent) hearing loss can be reduced by toughening. Furthermore, if we combine the results of Jacono *et al*. (1998) and Willott and Turner (1999), then it seems likely that at least some forms of age-related hearing loss can be influenced by upregulating the free-radical scavenging abilities of the cochlea.

C. Age-Related Changes in the Central Auditory System: Age-Related Changes in GABA

GABA is an inhibitory neurotransmitter in the central nervous system, GABAergic neurons are prevalent in the neocortex of all mammals, and their characteristics in primates has been well studied (Jones, 1993). We suggest that a downregulation of GABA-A receptors is one possible mechanism leading to the cortical enhancement observed following inner hair cell loss. Furthermore, an age-related decline in GABA-A receptors may represent a compensatory mechanism for overcoming a reduction in peripheral input from the aging auditory system.

Caspary *et al*. (1990) evaluated age-related changes in GABA in neurons from the central nucleus of the inferior colliculus. They counted the number of inferior colliculus neurons that were immunolabeled for GABA and found a 36% reduction in GABA-containing neurons in the ventrolateral inferior colliculus of old as compared to young adult rats. Caspary *et al*. (1995) reviewed the work of his own group and others, and reported that not only was there a decrease in the number of immunoreactive GABA neurons in the inferior colliculus with advancing age, but that there were reduced concentrations of GABA, and decreased GABA-B receptor binding, as well as other evidence of reduced GABA activity in the inferior colliculus of the aged animal. Taken together, this work provides evidence of reduced GABA activity in the central auditory system of aged animals.

D. Age-Related Changes in Antioxidant Enzymes

Mei *et al*. (1999) studied age-related changes in antioxidant enzymes in 3-, 18-, and 28-month-old rat inferior colliculus. They found age-dependent reductions in both total superoxide dismutase and catalase. This reduction in inferior colliculus antioxidant enzymes with advancing age suggest that age-related changes in the central auditory system might be the result of a decrease in free radical scavenging ability of the aging nervous system. Thus, upregulation of free radical scavengers in the central nervous system might represent one approach to a reduction in central manifestations of age-related hearing loss.

E. Can Responses of the Central Auditory System Be Modified?

Let us accept the idea that some manifestations of hearing loss are the result of functional reorganization of the auditory nervous system following hearing loss. Is there any evidence that these changes can be experimentally influenced? Halonen *et al*. (1991) found that intraperitoneal administration of vigabatrin increased the level of GABA in cortical and subcortical regions. In addition, at some doses, there was a decrease in the concentration of several excitatory amino acids (glutamate,

aspartate, and glutamine) in cortical and subcortical structures. Should it become desirable to reverse the age-dependent decline in GABA reported by Caspary and colleagues, then drugs like vigabatrin can be used to upregulate GABA. Kilgard and Merzenich (1998) have recently shown that pairing a specific auditory stimulus with electrical stimulation of the nucleus basalis of the basal forebrain produces dramatic cortical reorganization in the rat auditory cortex. This demonstrates that functional reorganization can be experimentally induced, should this be deemed desirable. Although preliminary in nature, it appears that electrical stimulation or systemic introduction of specific drugs offer therapeutic possibilities for either promoting functional reorganization or reversing such reorganization.

Fujiyoshi *et al*. (1994) investigated the shiverer mouse, which shows central nervous system abnormalities due to an autosomal-recessive deletion of a gene for myelin basic protein. These mice show abnormalities in central myelin, but normal peripheral myelin. Shiverer mice showed greatly reduced central myelin in the auditory nerve, as compared to control animals. In addition, there was prolongation of the interwave intervals in the auditory brain stem responses of shiverer mice, compared to the controls. Transgenic shiverer mice were created by inserting the myelin basic protein gene into the fertilized eggs of shiverer mice. These transgenic shiverer mice showed levels of myelin basic protein that were less than those seen in the control mice, but greater than those seen in the shiverer mice. Similarly, the central myelin on the auditory nerve of transgenic shiverer mice was less than that seen in control mice, but more than that seen in the shiverer mice. Finally, auditory brain stem response interwave intervals were greatest in the shiverer mice, intermediate for transgenic shiverer mice, and smallest for the control mice. It appears from this study that genetically based changes to the central auditory nervous system can be partially reversed by gene therapy.

F. Summary

Presbycusis can be viewed as a combination of peripheral hearing loss and the resulting functional reorganization. In response to cochlear damage, functional reorganization can take the form of a modification of the tonotopic map the central auditory nervous system, in particular the auditory cortex. In addition, functional reorganization can take the form of amplitude enhancement in the central auditory system. Enhancement of responses from the central nervous system following peripheral hearing loss may be the result of a downregulation of GABA. If we view presbycusis as a combination of peripheral changes and the resulting functional reorganization, then treatment of presbycusis can possibly take the form of either reducing the magnitude of peripheral hearing loss with aging, or modulating the functional reorganization that occurs in response to the peripheral hearing loss. Importantly, conditioning noise and several drugs (glutathione monoethyl-ester, *R*-phenylisopropyl-adenosine, calpain) are effective in reducing hair cell loss as well as threshold changes if administered prior to noise exposure. In addition, Willott and Turner (1999) have found that long-term conditioning with a moderate level broadband noise can reduce the magnitude of adult-onset hearing loss in several strains of mice. Thus, both noise-induced and genetic (age)-induced hearing loss can be reduced by exposure to a conditioning stimulus, and noise-induced hearing loss can be reduced by either systemic application or direct application to the cochlea of free-radical scavengers or calpain inhibitors. Centrally, there appears to be a reduction in GABA-mediated inhibition with aging, but Halonen *et al*. (1991) have demonstrated an upregulation of GABA by systemic administration of vigabatrin. In addition, recent experimental studies have demonstrated functional reorganization in auditory cortex by electrical stimulation of the nucleus basalis of Meynert (Kilgard and Merzenich, 1998). These studies suggest that one can promote or reverse functional reorganization of the auditory nervous system. As we improve our differentiation of age-related hearing loss into peripheral and central changes, we should also work to differentiate those central changes that are the consequence of aging itself from those that are the result of functional reorganization in response to peripheral changes. It seems likely than in some instances, functional reorganization actually improves auditory function, while in others it produces a decrement in function. Thus, the ability to promote or reverse functional reorganization could prove to be effective ways of reducing age-related decrements in auditory function. Finally, Fujiyoshi *et al*. (1994) has shown that severe abnormalities of auditory brain stem responses in shiverer mice can be improved by gene transfer. Thus, age-related hearing loss may not be inevitable, but rather in many is likely to be the result of adult-onset genetically based hearing loss. Once these hearing loss genes are identified, then approaches to prevention of hearing loss by gene transfer will be feasible.

We have discussed future approaches to aural rehabilitation in the elderly that focus on reversal/prevention of adult-onset hearing loss and the promotion/reversal of functional reorganization. These suggested approaches are based on very preliminary studies that simply offer hope that age-related hearing loss can be reduced.

III. Audiologic Rehabilitation: The Need

An elderly person put his hearing aid in his denture-cleaning solution overnight, another person removed his canal-type hearing aid while eating a bowl of pretzel nuggets and suddenly realized that he had just pulverized their hearing aid in one vigorous bite. An 80-year-old, ex-farmer purchased two expensive hearing aids, only to find that he could not manipulate the tiny volume controls because of his massive, callused hands. We all have probably had contact with an older person who bought the hearing aid reluctantly after many complaints by spouse and family, used it for a few weeks and then put it in the dresser drawer, never to be used again.

All of these incidents illustrate that fitting a hearing aid does not necessarily solve the problems of older adults with hearing loss. Specifically, elderly patients may have a constellation of problems associated with senescence, which need to be considered. It is, therefore, important for older persons with hearing loss to have access to a full scope of quality medical as well as other professional services such as aural rehabilitation. This section of the chapter will describe current approaches to the rehabilitative treatment of age-related hearing loss (presbycusis).

A. Why Audiological Interventions?

Audiologists are trained to assist not only in the quantification and diagnosis of the hearing loss, but in the rehabilitation of persons with hearing impairments. Their aural rehabilitation training provides advanced knowledge and skills regarding the fitting of hearing aids and the use of assistive listening devices. Further, they can provide auditory training, lip-reading training, and personal counseling that aids in their adjustment to the use of amplification and improved communication strategies. Each of these areas will be reviewed briefly so that the reader will understand the necessity and the scope of services that are available.

B. Aging and Hearing Loss (Presbycusis) Defined

1. Characteristic Hearing Thresholds

Initially, presbycusis results in a gradual change in hearing sensitivity, but can eventually end with a communication impairment of sufficient magnitude to result in an isolated, frustrated, and sometimes angry person with a severely reduced quality of life. Typically, hearing loss begins by the fourth or fifth decade of life in the higher frequencies, and has little consequence for communication or enjoyment of life in its early stages. The hearing loss gradually increases in the higher frequencies and spreads to the mid frequencies over the next 10–30 years (Gates *et al*., 1990). Initially, the aging person adapts to the gradually changing hearing thresholds without conscious effort or knowledge of the hearing loss. However, as the degree of hearing loss worsens the person may begin to experience communication difficulties that are severe enough for them to seek professional help. For this magnitude of hearing loss, patients probably obtain sufficient benefit from a hearing aid to continue its use. However, the level of communicative demands of individual lifestyles, occupations, and other factors often dictate whether they seek professional help. One person with a 40-dB HL hearing loss may not seek help or need to use a hearing aid if he/she is not socially or occupationally active and is not motivated to be so. However, another person with a 30-dB HL hearing loss, who is highly active, may be a full-time hearing aid user because missing a word or two could be critical to success on the job. Thus, disability (i.e., the communication dysfunction) and handicap (i.e., the psychosocial manifestations) cannot be predicted from hearing test results. Therefore, management of hearing-impaired clients is not based on the audiometric hearing loss alone (Erdman and Demorest, 1998).

C. Audiological and Otological Assessment

Gates *et al*. (1990) lists the causes of presbycusis as intrinsic, age-related degeneration, noise damage, biologic effects of diseases, ototoxic substances, and diet, superimposed on a genetic mechanism. This complex of etiologies warrants comprehensive medical and audiological examination so that the range of the patient's age-related hearing problems may be addressed. The otolaryngologist may need to address the age-related changes to the outer and middle ear (White and Regan, 1987), as well as age-related changes to the inner ear (Schuknecht, 1974). Older persons may also have the associated symptoms of tinnitus and/or vertigo. Some of these symptoms may respond to medical management. The audiologist's and otolaryngologist's collaboration provides the data necessary to document treatment efficacy.

Assessment of speech recognition performance in adverse listening situations is an important additional component of audiometry with the elderly because performance on standard phonetically balanced word tests (in quiet) is often normal, yet patients' complain of communication difficulties in noise. The source of these difficulties can be related to simple loss of audibility of some of the acoustic cues for speech (Souza and Turner, 1994). However, it is possible that more central structures of the auditory system may also be involved (see Gordon-Salant and Fitzgibbons, 1993). Also, Jerger (1992) presents evidence of cognitive dysfunction at cortical sites with aging. Humes *et al*. (1994) and Lesner and Kricos (1995) recommend that the audiologist sample phonemic-word, and sentence-level speech recognition in background noise in order to fully characterize speech perception deficits and thus determine the need for aural rehabilitation.

Otoacoustic emissions are basically a test of outer hair cell function. Normal otoacoustic emissions suggest normal outer hair cell function. In addition, otoacoustic emissions are influenced by stimulation of the contralateral ear. This contralateral suppression appears to be the result of activation of the cochlear efferents innervating outer hair cells. Thus, measurement of otoacoustic emissions can illuminate the condition of the efferent auditory system which may be important for sound localization and understanding speech in adverse background noise situations. Auditory evoked potentials, including the auditory brain-stem response, are powerful clinical tools for hearing screening, threshold estimation, site-of-lesion testing, and intraoperative monitoring. Future research into stimulation protocols for challenging the temporal processing capabilities of the brain stem may yield important diagnostic information (Walton *et al*., 1999; Sims and Burkard, 2000).

D. Assessment of Hearing Handicap

Garstecki and Erler (1996), Alberti *et al*. (1984), and Erdman and Demorest (1998) have described the necessity for careful determination of the broad scope of handicaps that result directly and indirectly from an individual's hearing loss. Use of hearing handicap scales enables the audiologist to detect and specify significant communication and adjustment difficulties, select from an array of rehabilitative interventions, and document treatment outcomes for consumers and third-party payers. Also, hearing handicap surveys can assist the hearing-aid fitting process by illuminating specific, troublesome listening situations. The audiologist can then program the aid with various signal processing algorithms and frequency responses to minimize the listener's difficulties. There are several standardized surveys, and each test instrument has different purposes and strengths. The few listed below are used in many clinics, but many more are available (see review by Lesner and Kricos, 1995).

1. The Hearing Handicap Inventory for the Elderly

The Hearing Handicap Inventory for the Elderly (HHIE) was designed by Ventry and Weinstein (1982) to determine

whether non-institutionalized individuals have sufficient difficulty with understanding spoken communication to warrant professional follow-up. A brief screening version (Ventry and Weinstein, 1983; Weinstein, 1986) consists of 10 questions, which can be quickly completed in the clinic waiting room. Research results have indicated that the brief version has adequate sensitivity and specificity to screen for significant hearing-related communication difficulty (American Academy of Audiology, 1991; Jupiter and DiStasio, 1998).

2. The Denver Scale

The Denver Scale of Communication Function for Senior Citizens Living in Retirement Centers, by Zarnoch and Alpiner (as cited and reproduced in Hull, 1997), has been designed specifically for the institutionalized elderly client to determine their need for aural rehabilitation.

3. Communication Profile for the Hearing Impaired

Once the hearing loss has been identified, by the HHIE or by audiometric evaluation, further assessment of the psychosocial effects of the hearing loss is required. The Communication Profile for the Hearing Impaired (CPHI) is a 145-item survey, which has been normed on large, national samples of hearing-impaired adults (Erdman and Demorest, 1998; Demorest and Erdman, 1987). This comprehensive assessment tool has several subscales: communication performance, communication importance, communication environment, communication strategies, and psychological adjustment. This instrument is useful in two ways. First, it can alert the practitioner to an individual's specific needs and monitor the outcomes of rehabilitation. Second, CPHI's normative research across diverse populations of hearing-impaired individuals may be helpful in documenting the adjustment variables that influence the success of aural rehabilitation.

E. Hearing Aids

Once the hearing loss has been documented by audiologic assessment, medical interventions have been exercised and the degree of communication handicap determined via interview and handicap surveys, the audiologist has the necessary information to determine whether a hearing aid is needed and if the client is motivated to proceed with purchase. Because a binaural hearing aid fitting costs between $1800 and $6000, hearing aids are a major commitment by the patient.

1. New Hearing Aid Techniques and Technologies

The most frequent hearing complaint of the older person is the understanding of speech in a background of noise. Unfortunately, listening in noise with a hearing aid can be extremely difficult, as background noises are amplified along with the speech. Furthermore, a person with sensorineural hearing loss frequently perceives high intensity sounds as loud, or even uncomfortably loud (i.e., recruitment or hyperacusis). Thus, when loud environmental sounds are made even louder by amplification, the hearing aid user may quickly decide that amplification is more harmful than helpful. Audiological research has focused on these problems with some success in prescription fittings for loudness control (Storey *et al*., 1998). Within the past 5 years, hearing aids have been developed that have low power, digital signal processing chips, CMOS memory, and directional, multiple microphone arrays. This technology enables even more fitting flexibility for making speech audible, while compressing this audible speech into a substantially reduced dynamic range (between threshold of audibility and threshold of discomfort). These hearing aids have client-controlled, multiple ''programs'' which are designed for various listening circumstances (e.g., crowded restaurant, one-on-one listening, music appreciation, telephone reception). Frequently, audiologists work with computer-assisted algorithms that are directly interfaced to the hearing aid so that fittings take place on the individual's ear, rather than by estimations of the required fitting prescription based on averaged data. Digital hearing aids' noise reduction algorithms have not shown significant improvements in speech recognition performance in noise, but most hearing-impaired persons subjectively ''prefer'' them when cost is not a factor (Newman and Sandridge, 1998). The use of directional microphones or multiple-microphone arrays has been shown to provide significant improvements in speech reception performance in background noise (Schuchman *et al*., 1999). Also, digital circuitry for management of acoustic feedback (hearing aid squeal) is a definite benefit for those using higher gain or open ear aid fittings. More research is needed to determine the ultimate potential of these circuits. In the near future, digital hearing aid circuits may allow reduction of periodic types of noise (e.g., engine noise) through automatic detection and adaptive filtering.

2. Hearing Aid Fitting

The audiologist can help the hearing-impaired person sort through the hearing aid options by negotiating an informed priority list and discussing hearing aid style and feature options from several instrument manufacturers. The audiologist will then fit the hearing aid to restore hearing sensitivity for the important speech frequency range, and control loudness such that comfortable use in all listening situations is assured. The ultimate purpose of any fitting, however, is improved speech recognition in the user's typical listening situations. Once a prescription is determined, it is frequently verified by the use of *in situ* acoustic measurements of the aid's performance in the user's ear and by assessment of speech recognition in quiet and background noise. The process is best accomplished over the course of several visits, and adjustments are made as the user acclimates to the hearing aid.

3. Hearing Aid Types

Choosing a hearing aid has become a complex task involving economic, health, lifestyle, and cosmetic issues. Second generation digital instruments are smaller, and can be used in completely-in-the-canal aids. However, hearing aid components such as a telecoil for use with the telephone, client-controlled volume control, and programming may need to be sacrificed with the completely-in-the-canal or in-the-ear style aids due to lack of space in an individual's ear canal. Handheld remote controls are available with some hearing aids to improve the older client's ability to manipulate controls with

these small instruments, but some patients find it inconvenient to use and easy to misplace.

Behind-the-ear hearing aids represent 15–20% of the market and are typically fitted to persons with moderate to profound hearing losses. Behind-the-ear hearing aids enable coupling with other assistive listening devices such as FM or infrared sound transmission systems. These systems are increasingly important if the level of hearing loss is moderate to profound, or if the user must hear in high background noise or in long-distance situations (see below). Generally, behind-the-ear systems are lower in cost than custom-assembled completely-in-the-canal systems, have fewer problems with acoustic feedback, and need to be repaired less often.

For those persons with severe to profound hearing loss who receive limited benefit from conventional amplification (e.g., aided speech recognition scores of less than 40% using the CID Everyday Sentences), the cochlear implant is often useful (Allum, 1996; Koch, 1996). The cochlear implant requires surgery and costs approximately of $30,000–$40,000. However, many of the implanted persons are able to use the telephone for the first time in many years. Most of the remainder of the users can use lipreading and their implant to reestablish functional receptive communication.

An intriguing alternative to the implant uses digital signal processing to transpose high frequency speech sound to lower, audible frequency ranges. Currently, this sort of frequency-transformation algorithm is being used with children with profound hearing loss, but it may also be an alternative to the cochlear implant with the elderly. With either the cochlear implant or the transposing hearing aid, extended periods of individual aural rehabilitation are required to fine-tune the fittings. The user needs intensive retraining for speech perception with these devices' representation of sound.

4. Assessment of Improvement via Hearing Aid Fitting

Survey scales have been shown to be effective for documenting the client benefit from hearing aid fitting. The APHAB (Abbreviated Profile of Hearing Aid Benefit) designed by Cox and Alexander (1995) uses 24 questions concerning self-ratings of communication performance and problems with and without a hearing aid. In addition, the client can document the results of two different hearing aids as part of the hearing aid evaluation process. The current version has a normative database for the elderly. The APHAB can be administered and scored on a personal computer in a few minutes.

The Client Orientated Scale of Improvement (COSI) (Dillon *et al*., 1997) is a quick and simple means of documenting hearing aid benefit. The specifics of the five ''most troublesome'' listening situations are documented. These are rank-ordered by the client from ''most troublesome'' to ''least troublesome'' and then scaled by the client as to frequency of occurrence or severity. When the hearing aid fitting has been completed, the client again ranks the severity and frequency of the five problem listening situations.

F. Assistive Devices

Assistive devices, a term referring to both assistive listening devices and alerting devices, are an integral component of comprehensive hearing-care services (Sandridge and Lesner, 1995). Persons with hearing impairment must listen in less than ideal situations that limit the performance of their hearing aid. With an assistive listening devices, effective listening distance can be increased, signal-to-noise ratio improved, and effects of adverse room acoustics minimized. Sandridge (1995) provides a complete review of systems that work with a hearing aid, or work independently of hearing aids. For example, there are simple hard-wired earphone extensions for the TV speaker, telephone equipment options, and sophisticated FM radio or infrared systems connected to the person's own hearing aid. Each system has its advantages and disadvantages in terms of fidelity, cost, susceptibility to interference from other sources, ease of use, maintenance, portability, and the specific communication situation for which it is designed (e.g., one-on-one conversation, in church, in a meeting, in a classroom, etc.).

Alerting devices are useful for anyone with any degree of hearing loss. Even while wearing amplification, many people will miss the telephone ring, the car blinker, the alarm clock or smoke alarm if they are some distance from the sound source. The audiologist can assist elderly persons in their selection from a wide array of alerting devices designed for these problems.

G. Psychological Adjustment

Trychin (1995), Clark and Martin (1994), and Luterman (1996) have documented the need and described counseling as a vital component in audiologic rehabilitation. Without patient counseling, daily hearing aid utilization declines for new hearing aid users. With counseling, patients report greater reductions in perceived handicap (Brooks, 1979). Even minimal amounts of counseling can significantly decrease the most commonly reported difficulties of the elderly, e.g., listening in background noise, dexterity concerns, and the belief that hearing loss is not significant enough to warrant a hearing aid (Brooks, 1989). Trychin (1995) states that a typical counseling program provides information regarding hearing loss, assistive listening devices, service providers, and resources. Participants also practice effective communication behavior that will reduce hearing problems and alter client's dysfunctional responses to communication problems when they do occur. Clients as well as their significant others (Tye-Murray and Schumm, 1994) need to develop realistic, nonjudgmental attitudes toward hearing loss.

H. Auditory and Lipreading Training

There are three traditional components within the context of aural rehabilitation: amplification, auditory training, and lipreading. Most clinicians, however, now focus on hearing aid selection and orientation, with an apparent loss of confidence in both auditory training and lipreading instruction as primary therapies (Erber, 1996). Some studies support that view (e.g., Lesner *et al*., 1987), but other studies show positive results (e.g., Walden *et al*., 1981). Recent successful auditory and lipreading training results have utilized computer-assisted instruction to enable the necessary, sustained drill and practice for auditory training and lipreading training (see Sims and Gottermeier, 2000). With a laptop computer (or Web-based dis-

tance learning), intensive auditory training and lipreading training will become more feasible. Clients will be able to obtain this training at home, with reduced treatment cost and with less need for individual therapy sessions for the entire course of auditory rehabilitation treatment.

1. Auditory Training

Auditory training is the process by which a person with impaired hearing develops perceptual skills through guided listening practice (Carhart, 1960; Cole and Gregory, 1986). During therapy sessions, an adaptive approach is used with a particular combination of stimulus and response (e.g., word identification). As therapy progresses, the clinician will present more challenging listening activities (e.g., word identification in noise). If the client experiences difficulties, remedial stimulus–response tasks that contain greater acoustic differences are presented. Recent techniques are using time-based variations in training stimuli to sequence the difficulty level. For example, Fast ForWord (Tellal *et al*., 1996) utilizes computer-processed lengthening of prerecorded phonetic stimuli. Lengthening enables the client to hear previously indistinct cues. Training progresses by gradual shortening of the acoustic speech elements to normal durations. Katz (personal communication) is using phonetic synthesis exercises with cochlear implant clients. In this technique, phonetic elements or words are presented in isolation for identification. These elements are then presented in a phoneme-by-phoneme sequence, and the listener is asked to assemble these individual sounds into words. This training challenges the auditory-memory synthesis abilities that are required to follow conversational speech.

2. Lipreading (Speechreading) Training

When amplification does not restore functional speech perception, hearing-impaired persons use vision to compensate for the missing auditory information, even when vision is impaired as well (Karp, 1988). In fact, research with normally hearing persons has repeatedly shown a robust influence of lipreading cues on speech perception when the auditory and visual cues for syllable perception are contradictory, i.e., the McGurk Effect (Massaro, 1998).

Lipreading/speechreading training methods focus on identification of mouth shapes associated with syllable, word, or phrase production. Practice may be silent, with vocal cues or in background noise. The lipreader is guided to make effective guesses during ongoing speech by attention to redundancies in the language and situational clues. DeFilippo (1990) and Montgomery *et al*. (1984) use techniques combining visual input with the minimal auditory cues available to the hearing-impaired client. Presentation of training materials via computer-assisted interactive video has been shown to be effective (Sims and Gottermeier, 2000).

3. Conversational Fluency Training

Erber (1996) has developed aural rehabilitation therapy based on the concepts of conversational fluency. Working on auditory and visual speech perception is of secondary importance. The main goals are to develop problem-solving skills for recognition of the source of communication breakdown, apply various repair strategies, request assistance from the communication partner, and judge the appropriateness of an applied strategy and evaluate its success. Clients work with their communication partner, and the therapist is an observer. In this way, the conversational role of the partner, and the complex interaction that typically occurs between people, is routinely incorporated into the rehabilitation process. Progress in conversational fluency is determined by such measures as the number of speaking turns taken by each participant, the amount of time during which each participant talked, and the amount of time consumed by conversational breakdown and repair.

I. Summary

Aural rehabilitation for elderly persons with hearing impairments can make the difference between a hearing aid in the dresser drawer and one in daily use. Because aural rehabilitation's goal is effective communication in the face of hearing loss, it can often provide the means for a higher quality of life through maintenance of rich social interactions instead of social isolation and depression. To be successful, it requires commitment from both the therapist and the client for thorough assessment of the full spectrum of hearing and hearing handicaps. Second, all relevant medical diagnosis and treatment must be completed. Finally, multisession individual or group aural rehabilitation should be completed in association with the fitting of a hearing aid, or other assistive devices.

1. Access to Aural Rehabilitation

The current reality of comprehensive aural rehabilitation services leaves much to be desired. Only 16% of audiologists in clinical practice extend aural rehabilitation services beyond routine fitting of hearing aids and a brief orientation to their use (Ross, 1997). There are three main reasons for this situation. First, there is a lack of client knowledge (and therefore, motivation) for comprehensive aural rehabilitation. Audiologists and their services are not well known even though there are over 11,000 audiologists in clinical practice. Second, many audiologists do not possess advanced skills and knowledge about aural rehabilitation, as graduate training is currently dominated by graduate coursework focusing on diagnostic audiology and hearing aids. Third, there are third-party reimbursement problems. In years past, elderly persons might be eligible for aural rehabilitation following hearing aid fitting; however, currently, when available, speech-language pathologists must be the providers working in a clinic, hospital outpatient department or physicians office. The CPT code used is the same code as for speech-language therapy. However, unlike audiologists, speech language pathologists cannot have their own provider number for Medicare, so the therapy must be billed under the physician's Medicare number as an in-office ancillary service. In other words, the physician bills for the therapy as if he/she is providing or supervising it, or it must be billed by the clinic, rehabilitation center, or hospital using the institution's billing number. The Academy of Dispensing Audiologists, The American Academy of Audiology, and The American Speech-Language and Hearing Association have met with the Health Care Finance Administration and

have requested that audiologists be permitted to provide the aural rehabilitation (and vestibular therapy) services using their own provider numbers (Lovenbruck, August 13, 1999, ASHA Forum).

The solutions to these aural rehabilitation access problems are being addressed by professionals in terms of changing the entry level of the audiological profession to the clinical doctorate (AuD). This 3 years (or more) educational experience may enable more opportunities for significant aural rehabilitation skill building. There is the Audiology Awareness Campaign which has as its goal making audiology a household word. In regard to third-party reimbursement issues, the clinician should not assume that elders do not have the ability to pay for aural rehabilitation themselves. Marketing studies have shown that older Americans have more discretionary funds than younger age groups because living expenses have been reduced and retirement income has increased. A last solution for greater utilization of comprehensive aural rehabilitation is a future combination of computer/Web-assisted home study.

References

Alberti, P. W., Pichora-Fuller, M. K., and Riko, K. (1984). Aural rehabilitation in a teaching hospital: Evaluation and results. *Ann. Otol. Rhinol. Laryngol.* **93**, 589–594.

Allum, D. (1996). ''Cochlear Implant Rehabilitation in Children and Adults.'' Singular Publishing Group, San Diego, CA.

American Academy of Audiology (1991). Task force on hearing impairment in aged people. *Audiol. Today* **3**, 7.

Bakin, J., and Weinberger, N. (1990). Classical conditioning induces CS-specific receptive field plasticity in the auditory cortex of the guinea pig. *Brain Res.* **536**, 271–286.

Brooks, D. N. (1979). Counseling and its effect on hearing aid use. *Scand. Audiol.* **8**, 101–107.

Brooks, D. N. (1989). The effect of attitude on benefit obtained from hearing aids. *Br. Soc. Audiol.* **19**, 3–11.

Burkard, R. Trautwein, P., and Salvi, R. (1997). The effects of click level, click rate and level of background masking noise on the inferior colliculus potential (ICP) in the normal and carboplatin-treated chinchilla. *J. Acoust. Soc. Am.* **102**, 3620–3627.

Canlon, B., Borg, E., and Flock, A. (1988). Protection against noise trauma by pre-exposure to a low-level acoustic stimulus. *Hear. Res.* **34**, 197–200.

Carhart, R. (1960). Auditory Training. *In* ''Hearing and Deafness'' (H. Davis, ed.), pp. 276–299. Rinehart, New York.

Caspary, D., Raza, A., Lawhorn Armour, B., Pippin, J., and Arneric, S. (1990). Immunocytochemical and neurochemical evidence for age-related loss of GABA in the inferior colliculus: Implications for neural presbycusis. *J. Neurosci.* **10**, 2363–2372.

Caspary, D., Mildbrandt, J., and Helfert, R. (1995). Central auditory aging: GABA changes in the inferior colliculus. *Exp. Gerontol.* **30**, 349–360.

Clark, J., and Martin, F., eds. (1994). ''Effective Counseling in Audiology: Perspectives and Practice.'' Prentice-Hall, Englewood Cliffs, NJ.

Cole, E. C., and Gregory, H. (1986). Auditory learning. *Volta Rev.* **88** (Suppl.), 5.

Cox, R., and Alexander, G. (1995). The abbreviated profile of hearing aid benefit. *Ear Hear.* **16**, 176–183.

DeFilippo, C. L. (1990). Speechreading training: Believe it or not! *Asha* **32**, 46–48.

Demorest, M., and Erdman, S. (1987). Development of the communication profile for the hearing impaired. *J. Speech Hear. Disord.* **52**, 129–143.

Dillon, H., James, A., and Ginis, J. (1997). Client Oriented Scale of Improvement (COSI) and its relationship to several other measures of benefit and satisfaction provided by hearing aids. *J. Am. Acad.Audiol.* **8**, 27–43.

Erber, N. (1996). ''Communication Therapy for Adults with Sensory Loss,'' 2nd ed., pp. 203–213. Clavis Publ., Melbourne, Australia.

Erdman, S. A., and Demorest, M. E. (1998). Adjustment to hearing impairment II: Audiological and demographic correlates. *J. Speech, Lang. Hear. Res.* **41**, 123–136.

Fujiyoshi, T., Hood, L., and Yoo, T. (1994). Restoration of brainstem auditory-evoked potentials by gene transfer in shiverer mice. *Ann. Otol., Rhinol., Laryngol.* **103**, 449–456.

Garstecki, D. C., and Erler, S. F. (1996). Older adult performance on the communication profile for the hearing impaired. *J. Speech Hear. Res.* **39**, 28–42.

Gates, G. A., Cooper, J. C., Jr., Kannel, W. B., and Miller, N. J. (1990). Hearing in the elderly: The Framingham Cohort, 1983–1985. *Ear Hear.* **11**, 247–256.

Gordon-Salant, S., and Fitzgibbons, P. J. (1993). Temporal factors and speech recognition performance in young and elderly listeners. *J. Speech Hear. Res.* **36**, 1276–1285.

Halonen, T., Pitkanen, A., Saano, V., and Riekkinen, P. (1991). Effects of vigabatrin (γ-vinyl GABA) on neurotransmission-related amino acid and on GABA and benzodiazepine receptor binding in rats. *Epilepsia* **32**, 242–249.

Hight, N., Henderson, D., McFadden, S., Zheng, X., and Ding, D. (1999). The effect of glutathione monoethyl ester (GSH_{ee}) on hearing loss and hair cell loss in chinchillas exposed to impulse noise. *Assoc. Res. Otolaryngol.* Abstr. **602**.

Hu, B., Zheng, X., McFadden, S., Kopke, R., and Henderson, D. (1997). R-phenylisopropyladenosine attenuates noise-induced hearing loss in chinchilla. *Hear. Res.* **113**, 198–206.

Hull, R., ed. (1997). ''Aural Rehabilitation: Serving Children and Adults,'' 3rd ed., pp. 479–482. Singular Publishing Group, San Diego, CA.

Humes, L. E., Watson, B. U., Christensen, L. A., Cokely, G. G., Halling, D. C., and Lee L. (1994). Factors associated with individual differences in clinical measures of speech recognition among the elderly. *J. Speech Hear. Res.* **37**, 465–474.

Jacono, A., Hu, B., Kopke, R., Henderson, D., van de Water, T., and Steinman, H. (1998). Changes in cochlear antioxidant enzyme activity after sound conditioning and noise exposure in the chinchilla. *Hear. Res.* **117**, 31–38.

Jerger, J. (1992). Can age-related decline in speech understanding be explained by peripheral hearing loss? *J. Am. Acad. Audiol.* **3**, 33–38.

Jones, E. (1993). GABAergic neurons and their role in cortical plasticity in primates. *Cereb. Cortex* **3**, 361–372.

Jupiter, T., and DiStasio, D. (1998). An evaluation of the HHIE-S as a screening tool for the elderly homebound population. *J. Acad. Rehab. Audiol.* **31**, 11–21.

Kaltenbach, J., Czaja, J., and Kaplan, C. (1992). Changes in the tonotopic map of the dorsal cochlear nucleus following induction of cochlear lesions by exposure to intense sound. *Hear. Res.* **59**, 213–223.

Karp, A. (1988). Reduced vision and speechreading. *Volta Rev.* **90**, 61–74.

Kilgard, M., and Merzenich, M. (1998). Cortical map reorganization enabled by nucleus basalis activity. *Science* **279**, 1714–1718.

Koch, D. B. (1996). ''Cochlear Implants: A Decade of Clinical Experience in the United States.'' Thieme, New York.

Lesner, S. A., and Kricos, P. B. (1995). Audiologic rehabilitation assessment: A holistic approach. *In* ''Hearing Care for the Older Adult'' (P. Kricos and S. Lesner, eds.). Butterworth-Heinemann, Boston.

Lesner, S. A., Sandridge, S. A., and Kricos, P. B. (1987). Training influences on visual consonant and sentence recognition. *Ear Hear.* **8**, 283–287.

Luterman, D. M. (1996). ''Counseling the Communicatively Disordered and their Families.'' Pro-Ed, Austin, TX.

Massaro, D. W. (1998). ''Perceiving Talking Faces: From Speech Perception to a Behavioral Principle.'' MIT Press, Cambridge, MA.

Mei, Y., Gawai, K., Nie, Z., Ramkumar, V., and Helfert, R. (1999). Age-related reductions in the antioxidant enzymes in the rat inferior colliculus. *Hear. Res.* **135**, 169–180.

Montgomery, A. A., Walden, B., Schwartz, D., and Prosek, R. (1984). Training auditory-visual speech reception in adults with moderate sensorineural hearing loss. *Ear Hear.* **5**, 30–36

Newman, C., and Sandridge, S. (1998). Benefit from, satisfaction with, and cost effectiveness of three different hearing aid technologies. *Am. J. Audiol.* **7**,115–127.

Popelar, J., Syka, J., and Berndt, H. (1987). Effect of noise on auditory evoked responses in awake guinea pigs. *Hear. Res.* **26**, 239–247.

Qiu, C. X., Salvi, R., Ding, D., and Burkard, R. (2000). Inner hair cell loss can lead to enhancement of responses from auditory cortex in unanesthetized chinchillas. *Hear. Res.* (in press).

Recanzone, G., Schreiner, C., and Merzenich, M. (1993). Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. *J. Neurosci.* **13**, 87–103.

Robertson, D., and Irvine, D. (1989). Plasticity of frequency organization in auditory cortex of guinea pigs with partial unilateral deafness. *J. Comp. Neurol.* **282**, 456–471.

Ross, M. (1997). A retrospective look at the future of aural rehabilitation. *J. Acad. Rehab. Audiol.* **30**, 11–28.

Salvi, R., Saunders, S., Gratton, M., Arehole, S., and Powers, N. (1990). Enhanced evoked response amplitudes in the inferior colliculus of the chinchilla following acoustic trauma. *Hear. Res.* **50**, 245–258.

Sandridge, S (1995). Beyond hearing aids: Use of auxiliary aids. *In* ''Hearing Care for the Older Adult'' (P. Kricos and S. Lesner, eds.). Butterworth-Heinemann, Boston.

Sandridge, S., and Lesner, S. (1995). Practical considerations in providing assistive devices. *J. Acad. Rehab. Audiol.* **28**, 68–76

Schuchman, G., Valente, M., Beck, L., and Potts, L. (1999). User satisfaction with an ITE directional hearing instrument. *Hear. Rev.* **6**, 12–22.

Schuknecht, H. F. (1974). ''Pathology of the Ear.'' Harvard University Press, Cambridge, MA.

Sims, D., and Burkard, R. (2000). The auditory brainstem response across click presentation rate: A comparison of normal hearing young and old adults. (To be presented at the 2000 Mid-winter Meeting of the Association for Research in Otolaryngology, St. Petersburg, FL.)

Sims, D., and Gottermeier, L., (2000). Computer applications in aural rehabilitation. *In* ''Rehabilitative Audiology: Children and Adults'' (G. Alpiner and P. McCarthy, eds.). Lippincott Williams & Wilkins, Baltimore, MD.

Souza, P. E., and Turner, C. W. (1994). Masking of speech in young and elderly listeners with hearing loss. *J. Speech Hear. Res.* **37**, 665–661.

Storey, L., Dillon, H., Yeend, I., and Wigney, D. (1998). The National Acoustic Laboratories' procedure for selecting the saturation sound pressure level of hearing aids: Experimental validation. *Ear Hear.* **19**, 267–279.

Takeno, S., Harrison, R., Ibrahim, D., Wake, M., and Mount, R. (1994a). Cochlear function after selective inner hair cell degeneration induced by carboplatin. *Hear. Res.* **75**, 93–102.

Takeno, S., Harrison, R., Mount, R., Wake, M., and Harada, Y. (1994b). Induction of selective inner hair cell damage by carboplatin. *Scanning Microsc*. **8**, 97–106.

Tellal, P., Miller, S., Bedi, G., Wang, X., Hagaranjan, S., Schriener, C., Jenkins, W., and Merzenich, M. (1996). Language comprehension in language-learning impaired children improved with acoustically modified speech. *Science* **271**, 81–84.

Trautwein, P., Hofstetter, P., Wang, J., Salvi, R., and Nostrant, A. (1996). Selective inner hair cell loss does not alter distortion product otoacoustic emissions. *Hear. Res*. **96**, 71–82.

Trychin, S. (1995). Counseling older adults with hearing impairments. *In* ''Hearing Care for the Older Adult'' (P. Kricos and S. Lesner eds.). Butterworth-Heinemann, Boston, MA.

Tye-Murray, N., and Schumm, L. (1994). Conversation training for frequent communication partners. *J. Acad. Rehab. Audiol.* **27**(Suppl.), 209–222.

Ventry, I., and Weinstein, B. (1982). The hearing handicap inventory for the elderly: A new tool. *Ear Hear.* **3**, 128–133.

Ventry, I., and Weinstein, B. (1983). Identification of elderly people with hearing problems. *Am. Soc. Hear. Assoc.* **25**, 37–47.

Wake, M., Takeno, S., Ibrahim, D., Harrison, R., and Mount, R. (1993). Carboplatin ototoxicity: An animal model. *J. Laryngol. Otol.* **107**, 585–589.

Wake, M., Takeno, S., Ibrahim, D., and Harrison, R. (1994). Selective inner hair cell ototoxicity induced by carboplatin. *Laryngoscope* **104**, 488–493.

Walden, B. E., Erdman, S. A., Montgomery, A. A., Schwartz, D. M., and Prosek, R. A. (1981). Some effects of training on speech recognition by hearing impaired adults. *J. Speech Hear. Res.* **24**, 207–216.

Walton, J., Orlando, M., and Burkard, R. (1999). Auditory brainstem response, forward masking, recovery functions are delayed in older humans with normal hearing. *Hear. Res.* **127**, 86–94.

Wang, J., Ding, D., Shulman, A., Stracher, A., and Salvi, R. (1999). Leupeptin protects sensory hair cells from acoustic trauma. *NeuroReport* **10**, 811–816.

Weinberger, N., Javid, R., and Lepan, B. (1993). Long-term retention of learning-induced receptive-field plasticity in the auditory cortex. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 2394–2398.

Weinstein, B. (1986). Validity of a screening protocol for identifying elderly people with hearing problems. *Am. Soc. Hear. Assoc.* **28**, 41–45.

White, J. D., and Regan, M. M. S. (1987). Otologic considerations. *In* ''Communication Disorders in Aging: Assessment and Management'' (H. G. Mueller and V. C. Geoffrey, eds.), pp. 36–70. Gallaudet University Press, Washington, DC.

Willott, J., and Turner, J. (1999). Prolonged exposure to an augmented acoustic environment ameliorates age-related auditory changes in C57BL/6J and DBA/2J mice. *Hear. Res*. **135**, 78–88.

Willott, J., Aitkin, L., and McFadden, S. (1993). Plasticity of auditory cortex associated with sensorineural hearing loss in adult C57BL/6J mice. *J. Comp. Neurol.* **329**, 402–411.

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Richard L. Doty

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Olfaction and Gustation in Normal Aging and Alzheimer's Disease

I. Introduction

The chemical senses of taste and smell are commonly ignored by neurologists, otorhinolaryngologists, and geriatricians, despite their important role in mediating the intake of all environmental nutrients and airborne chemicals required for life and in determining the flavor and palatability of foods and beverages. In addition to purveying aesthetic pleasures, these senses warn us of spoiled foods, leaking natural gas, polluted air, and smoke. Although age-related losses are present in all sensory modalities, the chemical senses—particularly olfaction—seem to be particularly influenced by aging. For example, while less than 2% of the American population is estimated to have olfactory loss (Doty *et al*., 1986; Hoffman *et al*., 1998), between the ages of 65 and 80 years approximately half of the population has major smell loss; over the age of 80 years, three-quarters of the population exhibit such loss (Doty *et al*., 1984). Such loss likely explains why a disproportionate number of elderly persons die from accidental gas poisoning (Chalke *et al*., 1958)[1] and why many elderly report that their food lacks flavor (Doty *et al*., 1984). The latter problem can lead to decreased motivation to eat and, in some cases, nutritional deficiencies.

Although decreased ''taste'' perception during chewing and swallowing reflects, to a large degree, attentuation of olfactory stimulation via the retronasal route (Burdach and Doty, 1987), decrements in true taste function (i.e., sweet, sour, bitter, and salty perception) are present in many elderly persons. Such decrements are particularly evident when small regions of the tongue are evaluated (e.g., Matsuda and Doty, 1995). Additionally, distortions of taste perception often occur in older persons that can be very debilitating and difficult to manage (Cohen and Gitman, 1959).

In this chapter I review, in detail, the age-related perceptual and structural changes known to occur in the olfactory and gustatory systems. In addition, the olfactory losses associated with the age-related disorder of Alzheimer's disease are examined. A brief overview of the anatomy and physiology of the olfactory and gustatory systems is also presented, so as to orient the reader to the design and function of these commonly overlooked sensory systems.

[1]In the United States alone, over 50 million dwellings are supplied with natural gas (Corwin *et al*., 1995).

II. Olfactory and Gustatory System Anatomy

A. Olfactory System

Odorants are detected by cilia projecting into the nasal mucus from the dendritic knobs of approximately 6 million specialized receptor cells of the olfactory neuroepithelium (Fig. 45.1) and, in some cases, by free nerve endings from the ophthalmic and maxillary divisions of the trigeminal nerve distributed throughout the nasal mucosa. Although the olfactory neuroepithelium has been classically located in the region of the cribriform plate, on the superior septum, and on the superior turbinate, recent data suggest that it may extend further anteriorly and ventrally than previously believed (Feron *et al*., 1998). Some airborne stimulants are also sensed within the pharynx and oral cavity via glossopharyngeal and vagus nerves. However, true odor sensations are mediated via the bipolar olfactory receptor cells; the sensations mediated by the other nerves are largely those of the skin senses, such as warmth/coolness, pungency, and irritation (Doty, 1995).

After collecting into bundles of $\sim$200 axons surrounded by ensheathing or Schwann cell mesoaxons, the unmyelinated axons of the olfactory receptor cells traverse the cribriform plate and synapse with dendrites of second order neurons within the glomeruli of the olfactory bulb (Fig. 45.2). Cells containing a given type of receptor project to the same glomerulus or a small set of glomeruli, making the glomeruli, in effect, functional units. The glomeruli also are a major source of convergence within the system, with many more neurons entering than leaving them. Complex interactions occur among glomeruli via periglomerular cells and centrifugal influences from higher centers (Kratskin, 1995).

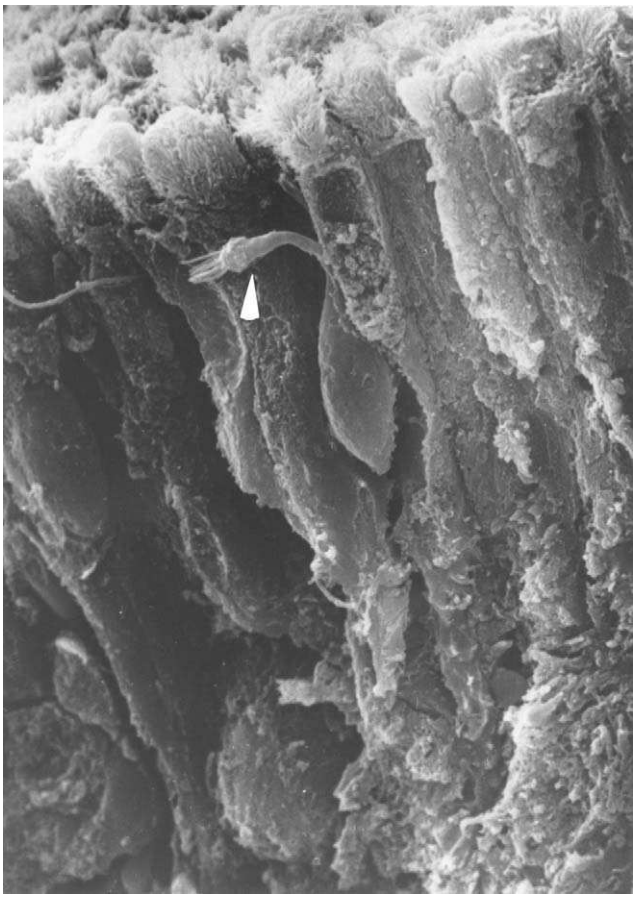

FIG. 45.1. High-magnification freeze-fracture electron photomicrograph of a cross section of the human olfactory neuroepithelium. Microvilli of supporting cells make up the bulk of the surface. Note olfactory receptor cell dissociated from remainder of epithelium (arrowhead) with relatively few cila on the apical end. Photo courtesy of Richard M. Costanzo & Edward E. Morrison, Virginia Commonwealth University—Medical College of Virginia.

Connections between the two olfactory bulbs, as well as limited contralateral bulb and cortexal projections, occur via the anterior olfactory nuclei and anterior commissure. However, the vast majority of olfactory projections from the bulb are ipsilateral. The mitral and tufted cells project directly, without thalamic synapse, to regions of the amygdala and to the more rostral elements of the primary olfactory cortex (e.g., entorhinal cortex) (Schwob and Price, 1984; Price, 1990). No clear point-to-point topography exists between the olfactory bulb regions and cortical projection sites (Price, 1990). Thus, small areas of the bulb can project to large areas of the olfactory cortex and vice versa. Reciprocal connections are present between the olfactory cortex and the orbitofrontal cortex, the mediodorsal and submedial thalamic nuclei, the lateral hypothalamus, the amygdala, and the hippocampus. A major pathway from the olfactory cortex to the orbitofrontal cortex is relayed through the mediodorsal nucleus of the thalamus. Because the complete pattern of higher order olfactory projections is beyond the scope of this chapter, the reader is referred elsewhere for details of this topic (e.g., Schwob and Price, 1984; Price, 1990; Zatorre *et al*., 1992; Carmichael *et al*., 1994; Doty *et al*., 1997).

B. Taste Anatomy

Tastants are detected by cells within taste buds—rosebud-like-looking structures found on the papillae of the tongue and on the soft palate, the uvula, the epiglottis, the rostral esophagus, and the mucous membranes covering the laryngeal cartilages (Fig. 45.3). On the tongue, the circumvallate papillae, which in aggregative are spaced in a chevron-like manner across the tongue near its base, harbor the most taste buds (up to 200); far fewer buds are present on the other types of papilla (Miller, 1995). Indeed, many fungiform papillae contain only one or two buds, and no buds are found on the filliform papillae. Hot peppers and other spicy foods produce stinging, burning, and warming sensations via the trigeminal nerve free nerve endings.

The innervation of the taste buds is shown in Fig. 45.4. The afferent fibers from the taste buds of the fungiform papillae project via the lingual nerve and the chorda tympani branch of the facial nerve and eventually synapse within the nucleus tractus solitarius of the brain stem. The nucleus tractus solitarius extends from the rosterolateral medulla caudally along the ventral border of the vestibular nuclei (Norgren, 1990). The nerves which innervate the taste buds of the foliate and circumvallate papillae travel directly to this nucleus within glossopharyngeal nerve, although the taste bud afferents on the anterior foliate papillae may do so via the facial nerve. The taste buds on the palate are supplied by fibers which travel with the greater superficial petrosal nerve, joining the facial nerve at the geniculate ganglion before reaching the nucleus tractus solitarius. Taste buds of the larynx and esophagus receive projections from the vagus nerve. In humans, the primary cortical taste area is within the parietal operculum and adjacent parainsular cortex, with the secondary cortex being in the orbito frontal lobe (for reviews, see Norgren, 1990; Pritchard, 1991; Small *et al*., 1997).

III. Age-Related Alterations in Olfactory and Gustatory Function

A. Olfaction

As noted in the first paragraph of this chapter, age-related decrements in olfactory function are, on average, quite large. To reiterate the point made earlier, approximately half of the population between the ages of 65 and 80 years have major difficulty in smelling, whereas approximately three quarters over 80 years of age cannot smell. Such losses are apparent on a wide variety of olfactory tests, including tests of odor detection, identification, discrimination, adaptation, and suprathreshold odor intensity perception (Murphy, 1983; Doty, 1989; Gilmore and Murphy, 1989). In addition, age-related declines have been noted in tests sensitive to intranasal irritation and other trigeminally mediated somatosensory sensations (Stevens and Cain, 1986). Although it is not clear whether age declines in a linear or quadratic (or some combination of both) manner over the age span, it is clear that (a) large individual differences in function are present in smell function in older individuals, (b) olfactory dysfunction is greatest after the sixth decade of life, and (c) the age-related loss begins at an earlier age for more men than for women. The age-related changes in odor identification ability, as measured by the widely used and

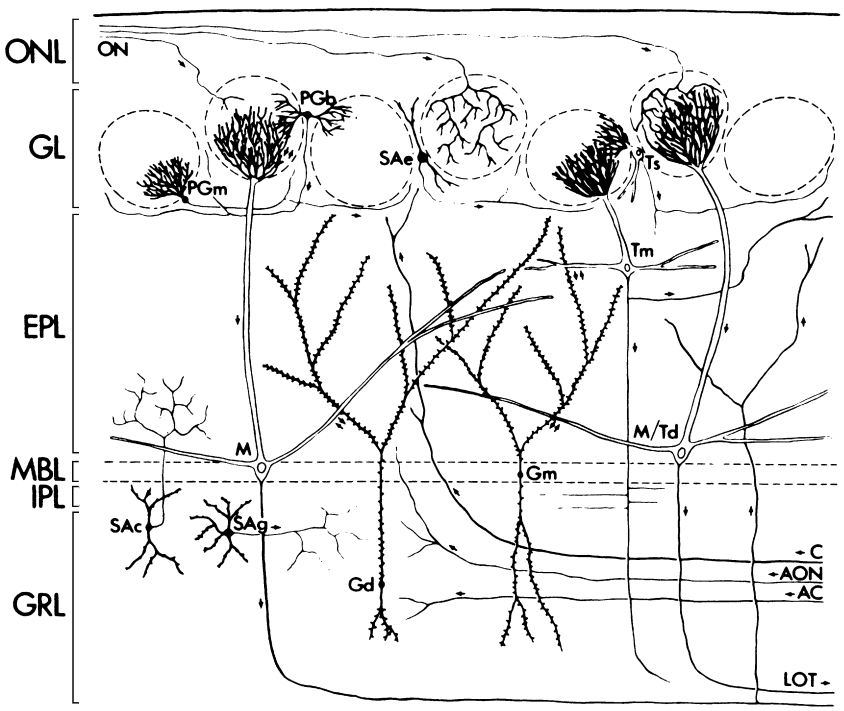

FIG. 45.2. Diagram of major layers and types of olfactory bulb neurons, as based upon Golgi stained material. Main layers are as follows: ONL, olfactory nerve layer; GL, glomerular layer; EPL, external plexiform layer; MBL, mitral body layer; IPL, internal plexiform layer; GRL, granule cell layer. ON, olfactory nerves; PGb, periglomerular cells with biglomerular dendrites; PGm, periglomerular cells with monoglomerular dendrites; M, mitral cell; M/Td, displaced mitral cell or deep tufted cell; Gm, granule cell with cell body in mitral body layer; Gd, granule cell with cell body in deep layers; SAc, short-axon cell of Cajal; SAg, short-axon cell of Golgi; C, centrifugal fibers; AON, fibers from the anterior olfactory nucleus; AC, fibers from anterior commissure; LOT, lateral olfactory tract. Reproduced with permission from Shepherd (1972), ©1973 American Physiological Society.

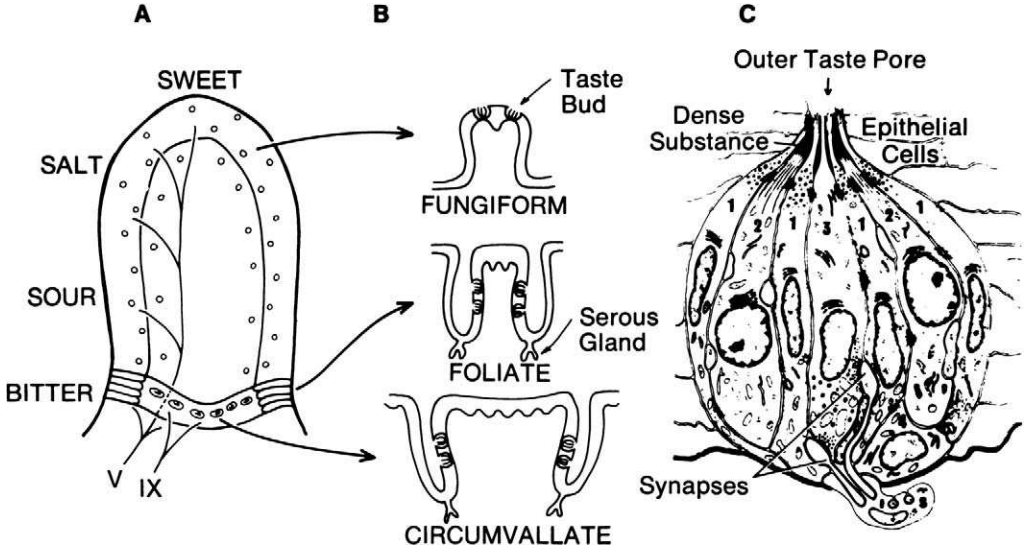

FIG. 45.3. (A and B) Schematic of distribution of taste buds on the human tongue. Taste buds of the fungiform and foliate papillae are innervated by the facial nerve. Those of the circumvallate papillae are invervated by the glossopharyngeal nerve. The trigeminal nerve carries nontaste somatosensory sensations. See text for details. (C) Schematic of fine structure of taste bud. 1 and 2, Presumably supporting cells which secrete materials into the lumen of the bud; 3, a sensory receptor cell; and 4, a basal cell from which the other types arise. Modified from Shepherd (1983).

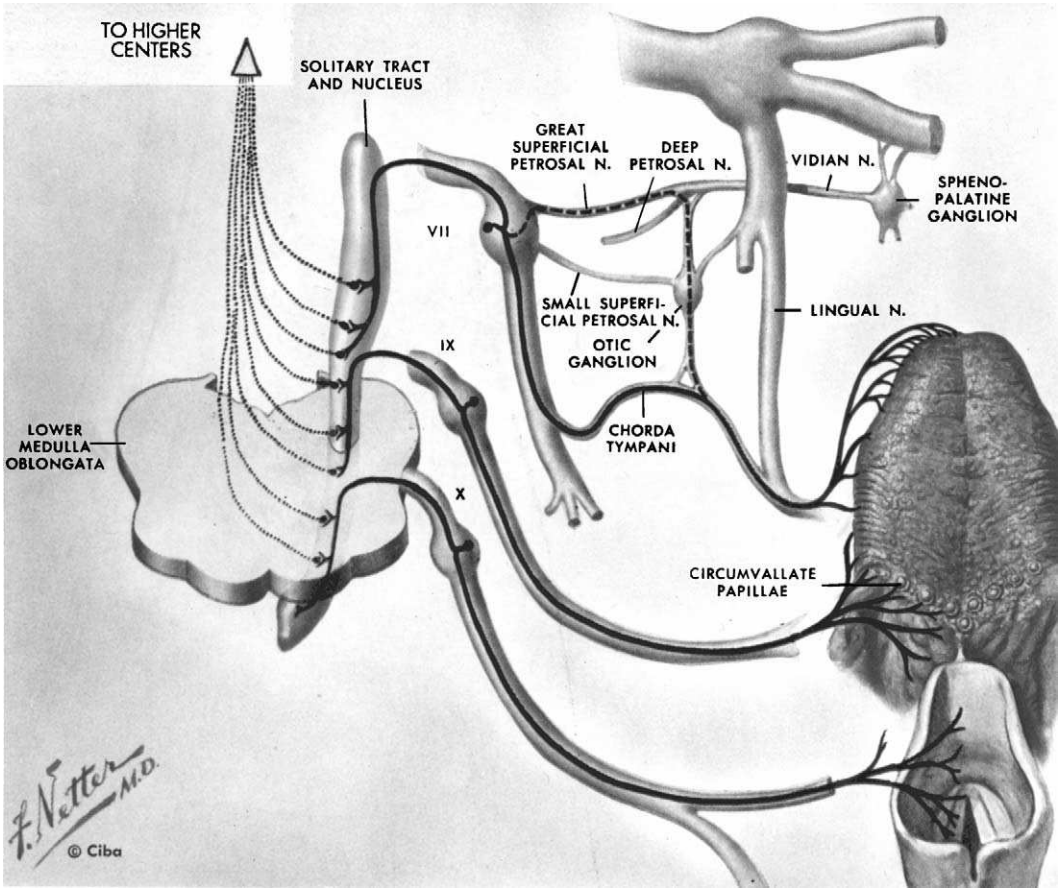

FIG. 45.4. Peripheral pathway of taste fibers from the facial, glossopharyngeal, and vagus nerves. Modified from Netter (1964).

standardized University of Pennsylvania Smell Identification Test, are shown in Fig. 45.5 (Doty *et al*., 1984). These age-related losses are not culture specific, and are much more more pronounced than the influences of gender or smoking on smell function (Doty *et al*., 1984; Frye *et al*., 1990).

In general, decreased sensitivity occurs across a spectrum of odors, although a decline in olfactory function may occur slightly more for some odorants than others (depending upon such factors as the odorant's threshold and the nature of the function relating odorant concentration to perceived intensity). Although the tendency for threshold values of different odorants to be correlated suggests that a ''general olfactory acuity'' factor may exist (analogous to the general intelligence factor derived from items of intelligence tests) (Yoshida, 1984; Doty *et al*., 1994), it should be noted that the thresholds of a number of sensory modalities tend to be correlated with one another, as well as with several cognitive measures (e.g., verbal memory). Hence, a ''general sensory acuity/cognitive'' factor may be operative that encompasses a range of sensory and cognitive entities and subsumes intermodal correlations (Stevens *et al*., 1998).

B. Taste

Age-related decreases in gustatory detection threshold sensitivity occur for both chemical and electrical stimuli (Nilsson, 1979a,b), although, in the case of whole-mouth chemical testing (e.g., ''sip and spit'' testing), the magnitude of the age-related loss is not as marked as that seen in olfaction (Weiffenbach and Bartoshuk, 1992). Decreases in threshold sensitivity have been reported for such tastants as caffeine, citric acid, hydrochloric acid, magnesium sulfate, propylthiourea, quinine, sodium chloride, sucrose, tartaric acid, and a large number of amino acids (see Schiffman *et al*., 1979; Schiffman and Clark, 1980; Cowart, 1981, 1989; Murphy, 1983; Weiffenbach *et al*., 1986; Murphy and Gilmore, 1989; Warwick and Schiffman, 1990; Schiffman, 1997). There is some suggestion, however, that not all taste qualities exhibit the same degree of age-related loss. For example, Weiffenbach *et al*. (1982) reported that, in a study of 81 healthy adults ranging in age from 23 to 88 years (including healthy elderly participants enrolled in the National Institute on Aging's Baltimore Longitudinal Study), sensitivity to sodium chloride and quinine sulfate decreases slightly with age, whereas sensitivity to sucrose and citric acid does not. Methodological factors undoubtly determine to a large degree the extent to which age-related changes occur in any given taste quality. The marked age-related decrement to NaCl when regional assessment is performed is shown in Fig. 45.6.

Age-related changes in suprathreshold taste perception to chemicals have also been observed. For example, flatter functions relating tastant concentrations to perceived intensity have been reported in elderly relative to young adults, at least for some stimuli (e.g., Schiffman and Clark, 1980; Schiffman,

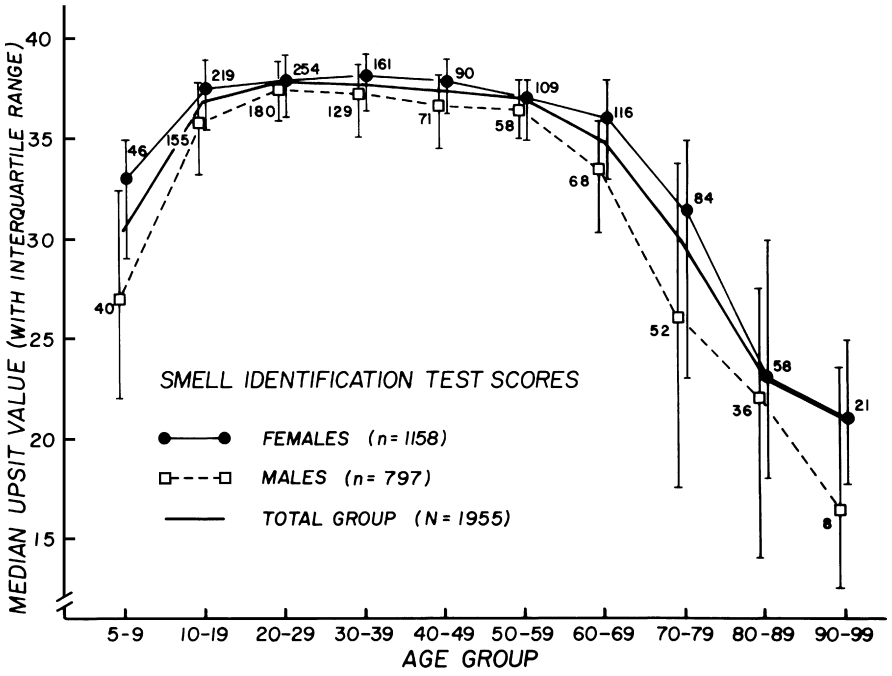

FIG. 45.5. Scores on the University of Pennsylvania Smell Identification Test as a function of age in a large heterogeneous group of subjects. Numbers by data points indicate sample sizes. Reproduced with permission from Doty *et al.* (1984). © 1984 American Association for the Advancement of Science.

1991), although exceptions clearly exist (Chauhan and Hawrysh, 1988). It should be noted, however, that the slope of such functions can be markedly altered by changes in intensity that occur at the extemes of the stimulus continuum, and need not reflect decreased sensitivity across the entire supra-threshold stimulus range. In a manner analogous to what occurs in olfaction (see Fig. 45.5), sex differences in taste function may become more apparent in the later years (Coats, 1974; Fikentscher *et al.*, 1977; Baker *et al.*, 1983; Weisfuse *et al.*, 1986; Lassila *et al.*, 1988).

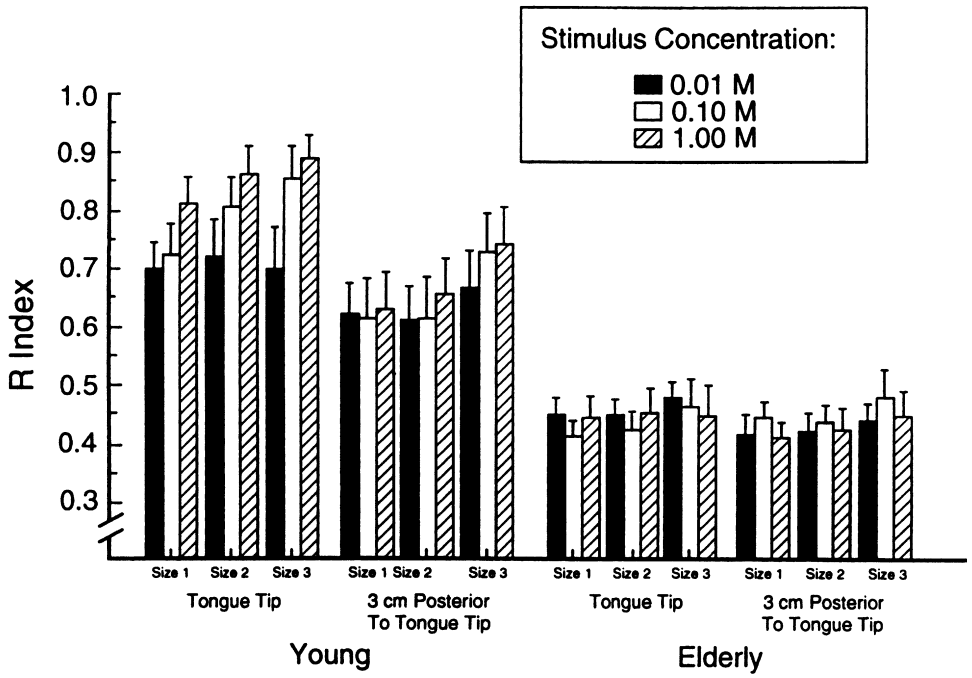

FIG. 45.6. Detection sensitivity to sodium chloride in two age groups for three stimulus concentrations at two tongue loci for three differenze sizes of tongue regions. Size 1, 12.5 mm^2; Size 2, 25 mm^2; Size 3, 50 mm^2. Error bars represent +1 SEM. Reproduced with permission from Matsuda and Doty (1995). © 1995 Oxford University Press.

IV. Changes in Olfaction and Gustation in Alzheimer's Disease

Nearly all studies of olfactory function in patients with Alzheimer's disease report decreased test scores relative to age-matched controls (Doty, 1991; Mesholam *et al*., 1998). Such decrements are observed for tests of odor threshold, discrimination, identification, and memory (Serby *et al*., 1985; Knupfer and Spiegel, 1986; Doty *et al*., 1987; Murphy *et al*., 1990; Moberg *et al*., 1997; Mesholam *et al*., 1998). Although some authors note that olfactory test scores are inversely related to the severity of the dementia (Waldton, 1974; Knupfer and Spiegel, 1986; Murphy *et al*., 1990), such associations are weak ($r_s < 0.40$), and the possible influence of dementia on subtle aspects of nonolfactory components of the tests makes such findings somewhat enigmatic. Earlier notions that detection thresholds are not altered in Alzheimer's disease (AD) and, therefore, by inference, implying that the peripheral olfactory pathways are intact (Serby *et al*., 1991), are not viable in light of the many studies finding threshold deficits in AD (Doty *et al*., 1987; Murphy *et al*., 1990; Mesholam *et al*., 1998) and in patients with central lesions (Doty *et al*., 1997). Although AD-related olfactory dysfunction may reflect, to some degree, the aforementioned physiological changes seemingly associated with normal aging, this is not the whole story. Thus, even very early-stage AD patients with mild dementia consistently score much more poorly on olfactory tests than age-matched controls (Doty *et al*., 1987).

Whether or not patients with Alzheimer's disease similarly exhibit gustatory deficits is not clear. Waldton (1974), in a pioneering study in which apparently the subjects were simply asked after the presentation of a tastant whether or not it was sensed, reported that most of his demented patients exhibited considerable loss of taste function, although no distinction among types of dementia was made. Subsequent studies have generally failed to observe any meaningful taste-related deficits to the basic taste qualities in patients with Alzheimer's disease (Koss *et al*., 1988; Murphy *et al*., 1990). However, Schiffman *et al*. (1990) reported decreased sensitivity to glutamic acid, but not to hydrochloric acid, in AD patients, although this loss was also seen in patients with other forms of dementia as well (e.g., multi-infarct dementia). Given the small numbers of studies on this topic, additional data on this general issue are sorely needed.

V. Causes of Changes in Chemosensory Function in Aging and in Alzheimer's Disease

A. Olfaction

The most common cause of age-related alterations in the ability to smell appears to be damage to the olfactory neuroepithelium from environmental insults (e.g., from viruses, bacteria, toxins, and pollutants). The distal elements of the olfactory receptors are exposed rather directly to the external milieu, thereby being susceptible to nosogenic attack. In general, the olfactory neuroepithelium undergoes cumulative damage throughout life, exhibiting increasing numbers of islands of respiratory-like metaplasia soon after birth (Nakashima *et al*., 1984), decreased epithelial thickness, and decreased numbers of olfactory receptors (Rosli *et al*., 1999). Such damage is likely accelerated in certain environments, such as in the workplace and in some outdoor situations, where increased exposure to chemicals and other predisposing factors exist (Corwin *et al*., 1995). Furthermore, older persons may be more susceptible to epithelial damage, having a less resilient olfactory membrane due to such factors as reduced protein synthesis (as in hypothyroidism) (Beard and Mackay-Sim, 1987; Mackay-Sim and Beard, 1987), loss of neurotrophic factors (Appel, 1981), altered vascularity (Somlyo and Somlyo, 1968), decreased intramucosal blood flow (Hasegawa and Kern, 1977), increased viscosity of the nasal mucus (Koopmann, Jr., 1989), secretory gland and lymphatic atrophy (Koopmann, 1989), and, potentially, decreases in enzyme systems which deactivate xenobiotics within the olfactory mucosa (Dahl and Hadley, 1991).

Interestingly, there is now strong empirical support of Krmpotic-Nemanic's observation (1969) that the number and cross-sectional area of the foramina of the cribriform plate are decreased in the elderly as a result of appositional bone growth. Kalmey *et al*. (1998) examined the posterior regions of the cribriform plate of 57 cribriform plates from 40 skulls of known age and sex. The area of the cribriform plate foramina was determined using a computer analysis of 35 mm pictures of these regions. The mean area of the foramina was 47.3% less in men over the age of 50 years than those under the age of 50 years. The corresponding figure for women was 28.8%. Although the degree to which such occlusion of the foramina contributes to the olfactory loss observed in the elderly is not known, this phenomenon deserves primary consideration.

Even though olfactory receptor cells have the capacity to reconstitute themselves periodically (Graziadei and Graziadei, 1978), this plasticity is likely compromised, at least in some segments of the neuroepithelium, by age-related processes (Breipohl *et al*., 1986; Loo *et al*., 1996). Animal studies suggest that the ratio of dead or dying cells to the number of receptor cells increases with age, implying age-related decreases in mitotic activity. Relative to younger animals, epithelial repair is slower or nonexistent following damage to the receptors by chemical agents such as zinc sulfate or methyl-formiminomethylester (Matulionis, 1982; Breipohl *et al*., 1986), belying an altered neurogenic process.

Destruction of the olfactory receptors leads to degenerative changes within the glomeruli of the olfactory bulb. This observation was capitalized on by in a pioneering study by Smith (1942) to estimate age-related losses of human olfactory receptors. Smith counted the glomeruli within 205 olfactory bulbs of 121 cadavers, and noted that loss of olfactory nerves begins soon after birth and continues throughout life at approximately 1% per year. Smith's findings have been supported by more recent studies. Thus, Meisami *et al*. (1998) measured the number of mitral cells and glomeruli in olfactory bulbs from three young adult women, three adult women of late middle age, and three women in advanced old age. The number of mitral cells and glomeruli decreased steadily with age at an approximate rate of 10% per decade; in the 9th and 10th decades of life, less than 30% of these structures were present. Such decreases presumably explain, in part, the finding from *in vivo* quantita-

tive magnetic resonance imaging studies that human olfactory bulb and tract volumes decrease with age (Yousem *et al*., 1998).

Hinds and McNelly (1977) measured the volume of the glomerular, external plexiform, internal granular, and olfactory nerve layers in Sprague–Dawley rats at 3, 12, 24, 27, and 30 months of age. The size and number of mitral cells were also measured in both the main and accessory bulbs. The volumes of the layers decreased after 2 years, although developmental volumetric increases were noted prior to that time. The decrease in mitral cell layer volume paralleled a sharp decrease in mitral cell number, and an increase in the volume of individual mitral cell body volumes and nucleus sizes, as well as increases in the sizes of mitral cell dendritic trees.

These findings were subsequently observed in the Charles River rat strain (Hinds and McNelly, 1981), although mitral cell loss was not seen in the older animals. Importantly, this study also assessed alterations within the olfactory neuroepithelium. A comparision of regression lines for changes in number of septal olfactory receptors to that of mitral cell body sizes suggested that the decline in receptor number began several months prior to the decline in mitral cell size, implying the bulbar changes followed the epithelial changes. An apprent compensatory increase in synapse number per receptor cell was present in the surviving receptor cell population.

Unlike the epithelium and bulb, the rat piriform cortex shows little age-related change. Thus, Curcio *et al*. (1985) examined the piriform cortex cells and synapses of 3 to 33 month-old rats and found no significant changes in cortical layers Ia and Ib volumes or in the numerical and surface densities of the synaptic apposition zones in layer Ia, which are formed largely by mitral cell axons. Although age-related changes in nuclear volume, soma volume, or numerical density of layer II neurons were not found, the proportion of layer Ia occupied by dendrites and spines was modestly (18%) decreased. An increase in the proportion of glial processes, but not by an alteration in the proportion of axons and terminals, was also noted.

The basis of the olfactory loss observed in AD is poorly understood and it is not yet clear whether such loss simply represents an exaggeration of the olfactory pathology present in normal aging. Histologically, severe changes are observed in the olfactory mucosa (see Fig. 45.7). One postmortem study reported a 40% reduction in the cross-sectional area of the olfactory tracts of AD patients, as well as a 52% loss of myelinated axons in these tracts (Davies *et al*., 1993). Other studies have reported the presence of AD-related neurofibrillary tangles and neuropil treads and Parkinson's disease-related Lewy bodies in all layers of the olfactory bulb, with the exception of the olfactory nerve cell axon layer (Ohm and Braak, 1987). Interestingly, the olfactory bulbs of over 40% of nondemented persons 50 years of age and older also exhibit neurofibrillary tangles (Kishikawa *et al*., 1990).

In AD, central limbic structures that receive olfactory bulb projections preferentially exhibit relatively high numbers of neurofibrillary tangles and neuritic plaques (Hooper and Vogel, 1976; Pearson *et al*., 1985; Reyes *et al*., 1993). Such structures include the hippocampal formation, the periamygdaloid nucleus, the prepiriform cortex, and the entorhinal cortex (Ferreyra-Moyano and Barragan, 1989; Jellinger *et al*., 1991; Braak *et al*., 1996a,b). The most salient and earliest signs of traditional AD neuropathology appear within the transentorhinal cortical regions (Jellinger *et al*., 1991; Braak *et al*., 1996a,b).

Among the more interesting and controversial theories concerning the etiology of the olfactory dysfunction in AD and some other neurodegenerative disorders (e.g., Parkinson's disease), as well as the etiology of the diseases themselves, is the ''olfactory vector hypothesis.'' One form of this theory suggests that the *olfactory loss* results from an environmental toxin, microorganism (e.g., virus, bacterium, or larvae), or other agent that progresses from the nasal cavity into the brain via the olfactory fila (e.g., Roberts, 1986; Ferreyra-Moyano and Barragan, 1989; Doty, 1991, 1997). It has been known for many years that the olfactory nerve is a major route for the entry of numerous viruses (e.g., polio virus, rabies virus, and St. Louis encephalitis virus) and other agents from the external milieu into the central nervous system (Turner and Esmond, 1926; Clark, 1929; Baker, 1995). Indeed, such observations led to the discovery in the 1930s that monkeys could be protected against intranasal inoculation of poliomyelitis virus by cauterizing the olfactory mucosa with picric acid and other caustic chemcials (Schultz and Gebhardt, 1936) and the subsequent prophylactic spraying of children's noses with zinc sulfate in Toronto and other cities in the late 1930s (Schultz and Gebhardt, 1937; Tisdall *et al*., 1937). According to this form of the theory, the olfactory loss need not be ascribed to specific disease pathology (e.g., Lewy bodies, senile plaques, neurofibrillary tangles, neurotransmitter deficiences), even though such pathology may subsequently contribute to the loss, but to damage that preceeds the major development of such pathology. This perspective need not be in opposition to the direction of the development of the subsequent neuropathology within the olfactory system (e.g., central to peripheral), as selective vulnerability of brain regions could be in play as well. Since the arguments for and against various forms of the olfactory vector hypothesis are numerous, the reader is referred elsewhere for further discussion of this topic (Pearson *et al*., 1985; Ferreyra-Moyano and Barragan, 1989; Doty, 1997).

Another intriguing hypothesis, which is not necessarily mutually exclusive of the olfactory vector hypothesis, was put forward by Kurtz *et al*. (1989). The essence of Kurtz's theory is that degeneration of the olfactory system, per se, is the basis of the cognitive decline in AD, since rats who have been anosmic for a period of time have considerable difficulty in learning an active avoidance learning task, unlike rats whose anosmia is of recent origin. To my knowledge, no further assessment has been made of this theory in either animals or humans.

B. Taste

Relative to olfaction, comparatively little research has been done to explain age-related alterations in gustatory function. As with other sensory systems, a relationship between the number of functioning receptor elements and the system's sensitivity would be expected. Three lines of evidence, from young populations, support this notion: first, the perceived intensity of tastants presented to localized regions of the anterior tongue is correlated with the the number of fungiform papillae and, hence, the number of taste buds within the stimu-

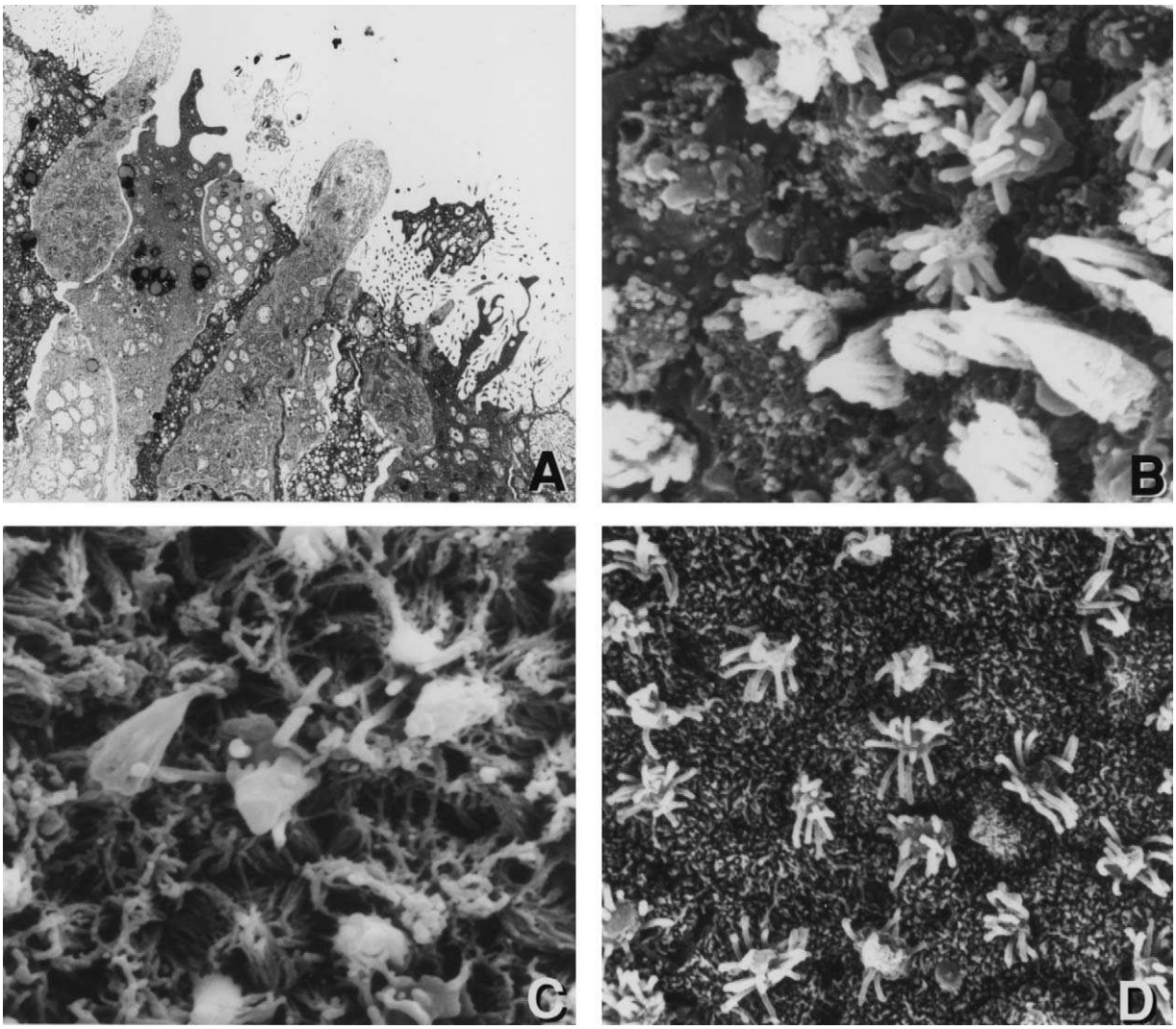

FIG. 45.7. AD-associated changes in the olfactory epithelium. (A) Severe changes in the olfactory epithelium in a 56-year-old patient with presenile dementia. The olfactory cells are highly abnormal, the olfactory knobs appear conical and frequently lack cilia, and the supporting cells are shrunken with a modified apical surface (×12,000). (B–C) Abnormal olfactory epithelium from a 60-year-old AD case (B) and a 56-year-old case with presenile dementia (C). Note the densely packed olfactory knobs, fused and short cilia, and conical transformation (both micrographs, × 11,000). (D) Well-preserved olfactory epithelium from a 66-year-old control person, with evenly distributed olfactory knobs surrounded by supporting cells (×7,000). Transmission and scanning electron micrographs were kindly provided by Drs. D. P. Perl and H. S. H. Choi, Department of Pathology, Mount Sinai School of Medicine, and Bronx VA Medical Center, New York.

lated regions (Smith, 1971); second, subjects with higher densities of taste buds and fungiform papillae (determined using videomicroscopy) rate the intensity of sucrose, NaCl, and 6-*N*-phenylthiouracil (but not citric acid and quinine HCl) higher than subjects with lower densities of taste buds (Miller and Reedy, 1990); and third, the number of stimulus sensations reported during stimulation of individual papillae is correlated with the number of taste buds present in the papillae, as determined from subsequent biopsy (Arvidson, 1979; Arvidson and Friberg, 1980). Surprisingly, however, most recent studies of rodent, monkey, and human tongues suggest that taste bud numbers in the anterior and medial regions are little influenced by age (Mistretta, 1984; Mistretta and Baum, 1984; Bradley *et al*., 1985; Miller, 1988; Miller and Reedy, 1990). For example, the average percentage of fungiform papillae-containing taste buds in Fischer 344 rats ages 4 to 6 months, 20 to 24 months, and 30 to 37 months was reported in one study to be 99.6, 99.3, and 94.7%, respectively (Mistretta and Oakley, 1986). Another study found no statistically meaningful relationship between age and taste bud densities on either the tip or the midregion of tongues from young adults (22–36 years, $n=5$), middle-aged adults (50–63 years, $n=7$), and old adults (70–90 years, $n=6$) (Miller, 1988). Marked variability in the number of taste buds on the tongue at all ages was reported to be much more apparent than any age-related alterations.

The above observations seem, at first glance, to be contradicted by study of tongue biopsies from 200 living persons (Moses *et al*., 1967). In this work, the number of dorsal lingual

fungiform papillae per square inch was found to decrease gradually from birth to 60 years of age. This finding has fallen under criticism, however, by others who make the point that a square inch of tongue surface in a child is not equivalent to a square inch of tongue surface in and adult, since the tongue continues to grow from infancy into adulthood (Mistretta, 1984).

Age-related loss of taste buds have been reported by several authors for human circumvallate papillae. Arey *et al*. (1935), in a relatively extensive study, reported the mean number of cells per circumvallate papilla as follows: from 0 to 20 years of age, 248; from 20 to 70 years, 206; and from 74 to 85 years, 88. Mochizuki (1939) reported the following figures: from 0 to 20 years, 242; from 21 to 60 years, 196; and from 61 to 90 years, 116. Although these pioneering studies have been criticized for both the number and quality of their autopsy specimens and the lack of statistical analysis of their data (Mistretta, 1984), no modern work has apparently attempted to confirm their findings using simlar procedures. A study of taste papillae and taste buds in tongues from rhesus monkeys that ranged in age from 4 to 31 years (a relatively old age for a rhesus monkey) (Bradley *et al*., 1985) reports no meaningful age-related differences in the size or number of fungiform, foliate, or circumvallate papillae, or in the number of taste buds per papilla (Bradley *et al*., 1985). Some of the older monkeys, however, were missing anterior sectors of the tongue, presumably as a result of exposure to outdoor elements or of fighting with cage mates.

Although taste bud numbers appear not to be markedly decreased in old rats (Mistretta and Baum, 1984), McBride and Mistretta (1986) discovered that the chorda tympani nerve of older rats was less responsive, electrophysiologically, to some salts, acids, and sugars. Possible explanations of this phenomena include: decreased intrinsic reactivity of taste buds to taste solutions, decreased multiple neural innervation of taste buds by some taste fibers, alterations in the general structure of the epithelium (which, for example, might impair the movement of stimulus solution into the taste bud pore), and decreased taste nerve responsiveness, per se. It is also possible that some taste buds, although anatomically present, are not fully functional because of altered turnover time or related metabolic events. Since taste buds function in a complex ionic milieu and are bathed with saliva and other secretory products, changes in such products may also undergo age-related changes. In accord with this notion is a hypothesis put forward by Bartoshuk *et al*. (1986). These investigators suggest that the heightened threshold values and flattened suprathreshold psychophysical functions observed in many elderly reflect background tastes which are noticeable at low, but not at moderate or high, stimulus concentrations. Both neural and oral environment changes (e.g., salivary constituents) could contribute to this noise. The observation that improved oral hygiene reduces taste thresholds in some older persons would appear to be congruent with this hypothesis (Langan and Yearick, 1976).

Relatively few studies have examined the structure of the gustatory system of patients with AD. An exception is a recent immunohistochemical study by Yamagishi *et al*. (1995). In this work, the innervation of the foliate and circumvallate taste buds was examined in five AD (three men, two women) and two control (both women) subjects using the following neuronal markers: antisera to protein gene product 9.5, neuron-specific enolase, tyrosine hydroxylase, dopamine beta-hydroxylase, and calcitonin gene-related peptide. Although the mean number of protein gene product 9.5-immunoreactive intragemmal nerve fibers in taste buds of the foliate and circumvallate papillae was reportedly significantly decreased in AD patients, the small number of subjects begs the question as to whether the statistical analyses were, in fact, performed on independent samples. The authors conclude that decreased innervation of the taste buds may account, in part, for decreased taste sensitivity in AD.

VI. Summary and Conclusions

It is clear from the studies reviewed in this chapter that both taste and smell function is lessened in many elderly persons. Such alterations are detected by a variety of sensory tests. Patients with AD exhibit marked declines in olfactory function, although whether they exhibit gustatory dysfunction beyond that of age-matched controls is debatable. In both taste and smell, alterations in peripheral receptive elements may well explain much of the sensory loss that is demonstrable using psychophysical tests. Nevertheless, malfunctioning within higher nervous system structures cannot, at present, be excluded from consideration and it is perhaps prudent to assume that the structural and functional bases of such changes are probably multiple, interacting, and complex.

Whatever the physical basis for altered taste and smell function in older persons, it is clear that such alterations are often profound, subjecting many such persons to risk for food and gas poisoning, and greatly limiting their appreciation of foods, beverages, and aesthetic delights. Physicians caring for the elderly should be aware of these deficiencies so as to better counsel their older patients in ways of maintaining safety and nutritional status.

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References

Appel, S. H. (1981). A unifying hypothesis for the cause of amyotrophic lateral sclerosis, parkinsonism, and Alzheimer's disease. *Ann. Neurol.* **10**, 499–505.

Arey, L. B., Tremaine, M. J., and Monzingo, F. L. (1935). The numerical and topographical relations of taste buds to human circumvallate papillae throughout the life span. *Anat. Rec.* **64**, 9–25.

Arvidson, K. (1979). Location and variation in number of taste buds in human fungiform papillae. *Scand. J. Dent. Res*. **87**, 435–442.

Arvidson, K., and Friberg, U. (1980). Human taste: Response and taste bud number in fungiform papillae. *Science* **209**, 807–808.

Baker, H. (1995). Transport phenomena within the olfactory system. *In* "Handbook of Olfaction and Gustation" (R. L. Doty, ed.), pp. 173–190. Dekker, New York.

Baker, K. A., Didcock, E. A., Kemm, J. R., and Patrick, J. M. (1983). Effect of age, sex and illness on salt taste detection thresholds. *Age Ageing* **12**, 159–165.

Bartoshuk, L. M., Rifkin, B., Marks, L. E., and Bars, P. (1986). Taste and aging. *J. Gerontol*. **41**, 51–57.

Beard, M. D., and Mackay-Sim, A. (1987). Loss of sense of smell in adult, hypothyroid mice. *Brain Res*. **433**, 181–189.

Braak, H., Braak, E., Bohl, J., and Reintjes, R. (1996a). Age, neurofibrillary changes, A β-amyloid and the onset of Alzheimer's disease. *Neurosci. Lett*. **210**, 87–90.

Braak, H., Braak, E., Yilmazer, D., de Vos, R. A. I., Jansen, E. N. H., and Bohl, J. (1996b). Pattern of brain destruction in Parkinson's and Alzheimer's diseases. *J. Neural Transm*. **103**, 455–490.

Bradley, R. M., Stedman, H. M., and Mistretta, C. M. (1985). Age does not affect numbers of taste buds and papillae in adult rhesus monkeys. *Anat. Rec*. **212**, 246–249.

Breipohl, W., Mackay-Sim, A., Grandt, D., Rehn, B., and Darrelmann, C. (1986). Neurogenesis in the vertebrate main olfactory epithelium. *In* "Ontogeny of Olfaction" W. Breipohl, (ed.), pp. 21–33. Springer-Verlag, Berlin.

Burdach, K. J., and Doty, R. L. (1987). The effects of mouth movements, swallowing, and spitting on retronasal odor perception. *Physiol. Behav*. **41**, 353–356.

Carmichael, S. T., Clugnet, M. C., and Price, J. L. (1994). Central olfactory connections in the macaque monkey. *J. Comp. Neurol*. **346**, 403–434.

Chalke, H. D., Dewhurst, J. R., and Ward, C. W., (1958). Loss of smell in old people. *Public Health* **72**, 223–230.

Chauhan, J., and Hawrysh, Z. J. (1988). Suprathreshold sour taste intensity and pleasantness perception with age. *Physiol. Behav*. **43**, 601–607.

Clark, W. E. (1929). Anatomical investigation into the routes by which infections may pass from the nasal cavities into the brain. 1–27. 1929. London. (GENERIC) Ref Type: Serial (Book, Monograph)

Coats, A. C. (1974). Effects of age, sex, and smoking on electrical taste threshold. *Ann. Otol. Rhinol. Laryngol*. **83**, 365–369.

Cohen, T., and Gitman, L. (1959). Oral complaints and taste perception in the elderly. *J. Gerontol*. **14**, 294–298.

Corwin, J., Loury, M., and Gilbert, A. N. (1995). Workplace, age, and sex as mediators of olfactory function: Data from the National Geographic Smell Survey. *J. Gerontol*. **50**, 179–186.

Cowart, B. J. (1981). Development of taste perception in humans: Sensitivity and preference throughout the life span. *Psychol. Bull*. **90**, 43–73.

Cowart, B. J. (1989). Relationships between taste and smell across the adult life span. *Ann. N.Y. Acad. Sci*. **561**, 39–55.

Curcio, C. A., McNelly, N. A., and Hinds, J. W. (1985). Aging in the rat olfactory system: Relative stability of piriform cortex contrasts with changes in olfactory bulb and olfactory epithelium. *J. Comp. Neurol*. **235**, 519–528.

Dahl, A. R., and Hadley, W. M. (1991). Nasal cavity enzymes involved in xenobiotic metabolism: Effects on the toxicity of inhalants. *Crit. Rev. Toxicol*. **21**, 345–372.

Davies, D. C., Brooks, J. W., and Lewis, D. A. (1993). Axonal loss from the olfactory tracts in Alzheimer's disease. *Neurobiol. Aging* **14**, 353–357.

Doty, R. L. (1989). Influence of age and age-related diseases on olfactory function. *Ann. N.Y. Acad. Sci*. **561**, 76–86.

Doty, R. L. (1991). Olfactory dysfunction in neurogenerative disorders. *In* "Smell and Taste in Health and Disease" (T. V. Getchell, R. L. Doty, L. M. Bartoshuk, and J. B. Snow, Jr., eds.), pp. 735–751. Raven Press, New York.

Doty, R. L. (1995). Intranasal trigeminal chemoreception. *In* "Handbook of Olfaction and Gustation" (R. L. Doty, ed.), pp. 821–833. Dekker, New York.

Doty, R. L. (1997). Studies of human olfaction from the University of Pennsylvania Smell and Taste Center. *Chem. Senses*. **22**, 565–586.

Doty, R. L., Shaman, P., Applebaum, S. L., Giberson, R., Siksorski, L., and Rosenberg, L. (1984). Smell identification ability: Changes with age. *Science* **226**, 1441–1443.

Doty, R. L., Gregor, T., and Monroe, C. (1986). Quantitative assessment of olfactory function in an industrial setting. *J. Occup. Med*. **28**, 457–460.

Doty, R. L., Reyes, P. F., and Gregor, T. (1987). Presence of both odor identification and detection deficits in Alzheimer's disease. *Brain Res. Bull*. **18**, 597–600.

Doty, R. L., Smith, R., McKeown, D. A. and Raj, J. (1994). Tests of human olfactory function: Principal components analysis suggests that most measure a common source of variance. *Percept. Psychophys*. **56**. 701–707.

Doty, R. L., Bromley, S. M., Hummel, T. and Moberg, P. J. (1997). Laterality in human nasal chemoreception. *In* "Cerebral Asymmetries in Sensory and Perceptual Processing" (S. Christman, ed.), pp. 497–542. North-Holland Publ. Amsterdam.

Feron, F., Perry, C., McGrath, J. J., and Mackay, S. (1998). New techniques for biopsy and culture of human olfactory epithelial neurons. *Arch. Otolaryngol. Head Neck Surg*. **124**, 861–866.

Ferreyra-Moyano, H., and Barragan, E. (1989). The olfactory system and Alzheimer's disease. *Int. J. Neurosci*. **49**, 157–197.

Fikentscher, R., Roseburg, B., Spinar, H., and Bruchmuller, W. (1977). Loss of taste in the elderly: Sex differences. *Clin. Otolaryngol. Allied Sci*. **2**, 183–189.

Frye, R. E., Schwartz, B. S., and Doty, R. L. (1990). Dose-related effects of cigarette smoking on olfactory function. *JAMA, J. Am. Med. Assoc*. **263**, 1233–1236.

Gilmore, M. M., and Murphy, C. (1989). Aging is associated with increased Weber ratios for caffeine, but not for sucrose. *Percept. Psychophys*. **46**, 555–559.

Graziadei, P. P. C., and Graziadei, G. A. (1978). The olfactory system: A model for the study of neurogenesis and axon regeneration in mammals. *Neuronal Plast*., 131–153.

Hasegawa, M., and Kern, E. B. (1977). The human nasal cycle. *Mayo Clin. Proc*. **52**, 28–34.

Hinds, J. W., and McNelly, N. A. (1977). Aging of the rat olfactory bulb: Growth and atrophy of constituent layers and changes in size and number of mitral cells. *J. Comp. Neurol*. **72**, 345–367.

Hinds, J. W., and McNelly, N. A. (1981). Aging in the rat olfactory system: Correlation of changes in the olfactory epithelium and olfactory bulb. *J. Comp. Neurol*. **203**, 441–453.

Hoffman, H. J., Ishii, E. K., and Macturk, R. H. (1998). Age-related changes in the prevalence of smell/taste problems among the United States population. *Ann. N.Y. Acad. Sci*. **855**, 716–722.

Hooper, M. W., and Vogel, F. S. (1976). The limbic system in Alzheimer's disease. *Am. J. Pathol*. **95**, 1–13.

Jellinger, K., Braak, H., Braak, E., and Fischer, P. (1991). Alzheimer lesions in the entorhinal region and isocortex in Parkinson's and Alzheimer's diseases. *Ann. N.Y. Acad. Sci*. **640**, 203–209.

Kalmey, J. K., Thewissen, J. G., and Dluzen, D. E. (1998). Age-related size reduction of foramina in the cribriform plate. *Anat. Rec*. **251**, 326–329.

Kishikawa, M., Iseki, M., Nishimura, M., Sekine, I., and Fujii, H. (1990). A histopathological study on senile changes in the human olfactory bulb. *Acta. Pathol. Jpn*. **40**, 255–260.

Knupfer, L., and Spiegel, R. (1986). Differences in olfactory test performance between normal aged, Alzheimer and vascular type dementia individuals. *Int. J. Geriatr. Psychiatry* **1**, 3–14.

Koopmann, C. F., Jr. (1989). Effects of aging on nasal structure and function. *Am. J. Rhinol*. **3**, 59–62.

Koss, E., Weiffenbach, J. M., Haxby, J. V., and Friedland, R. P. (1988). Olfactory detection and identification performance are dissociated in early Alzheimer's disease. *Neurology* **38**, 1228–1232.

Kratskin, I. (1995). Functional anatomy, central connections, and neurochemistry of the mammalian olfactory bulb. *In* "Handbook of Olfaction and Gustation" (R. L. Doty, ed.), pp. 103–126. Dekker, New York.

Krmpotic-Nemanic, J. (1969). Presbycusis, presbystasis and presbyosmia as consequences of the analogous biological process. *Acta Oto-Laryngol*. **67**, 217–223.

Kurtz, P., Schuurman, T., and Prinz, H. (1989). Loss of smell leads to dementia in mice: is Alzheimer's disease a degenerative disorder of the olfactory system? *J. Protein Chem*. **8**, 448–451.

Langan, M. J., and Yearick, E. S. (1976). The effects of improved oral hygiene on taste perception and nutrition of the elderly. *J. Gerontol*. **31**, 413–418.

Lassila, V., Sointu, M., Raiha, I., and Lehtonen, A. (1988). Taste thresholds in the elderly. *Proc. Finn. Dent. Soc*. **84**, 305–310.

Loo, A. T., Youngentob, S. L., Kent, P. F., and Schwob, J. E. (1996). The aging olfactory epithelium: Neurogenesis, response to damage, and odorant-induced activity. *Int. J. Dev. Neurosci*. **14**, 881–900.

Mackay-Sim, A., and Beard, M. D. (1987). Hypothyroidism disrupts neural development in the olfactory epithelium of adult mice. *Brain Res*. **433**, 190–198.

Matsuda, T., and Doty, R. L. (1995). Regional taste sensitivity to NaCl: Relationship to subject age, tongue locus and area of stimulation. *Chem. Senses* **20**, 283–290.

Matulionis, D. H. (1982). Effects of the aging process on olfactory neuron plasticity. *In* "Olfaction and Endocrine Regulation" (W. Breipohl, ed.), pp. 299–308. IRL Press, London.

McBride, M. R., and Mistretta, C. M. (1986). Taste responses from the chorda tympani nerve in young and old Fischer rats. *J. Gerontol*. **41**, 306–314.

Meisami, E., Mikhail, L., Baim, D., and Bhatnagar, K. P. (1998). Human olfactory bulb: Aging of glomeruli and mitral cells and a search for the accessory olfactory bulb. *Ann. N.Y. Acad. Sci*. **855**, 708–715.

Mesholam, R. I., Moberg, P. J., Mahr, R. N., and Doty, R. L. (1998). Olfaction in neurodegenerative disease: A meta-analysis of olfactory functioning in Alzheimer's and Parkinson's diseases. *Arch. Neurol. (Chicago)*. **55**, 84–90.

Miller, I. J. (1988). Human taste bud density across adult age groups. *J. Gerontol*. **43**, 826–830.

Miller, I. J. (1995). Anatomy of the peripheral taste system. *In* "Handbook of Olfaction and Gustation" (R. L. Doty, ed.), pp. 521–547. Dekker, New York.

Miller, I. J. J., and Reedy, F. E., Jr. (1990). Variations in human taste bud density and taste intensity perception. *Physiol. Behav*. **47**, 1213–1219.

Mistretta, C. M. (1984). Aging effects on anatomy and neurophysiology of taste and smell. *Gerodontology* **3**, 131–136.

Mistretta, C. M., and Baum, B. J. (1984). Quantitative study of taste buds in fungiform and circumvallate papillae of young and aged rats. *J. Anat*. **138**, 323–332.

Mistretta, C. M., and Oakley, I. A. (1986). Quantitative anatomical study of taste buds in fungiform papillae of young and old Fischer rats. *J. Gerontol*. **41**, 315–318.

Moberg, P. J., Doty, R. L., Mahr, R. N., Mesholam, R. I., Arnold, S. E., Turetsky, B. I., and Gur, R. E. (1997). Olfactory identification in elderly schizophrenia and Alzheimer's disease. *Neurobiol. Aging* **18**, 163– 167.

Mochizuki, Y. (1939). Studies on the papilla foliate of Japanese. 2. The number of taste buds. *Okajimas Folia Anat. Jpn*. **18**, 355–369.

Moses, S. W., Rotem, Y., Jagoda, N., Talmore, N., Eichhorn, F., and Levin, S. (1967). A clinical, genetic and biochemical study of familial dysautonomia in Israel. *Isr. J. Med. Sci*. **3**, 358–371.

Murphy, C. (1983). Age-related effects on the threshold, psychophysical function, and pleasantness of menthol. *J. Gerontol*. **38**, 217–222.

Murphy, C., and Gilmore, M. M. (1989). Quality-specific effects of aging on the human taste system. *Percept. Psychophys*. **45**, 121–128.

Murphy, C., Gilmore, M. M., Seery, C. S., Salmon, D. P., and Lasker, B. R. (1990). Olfactory thresholds are associated with degree of dementia in Alzheimer's disease. *Neurobiol. Aging* **11**, 465–469.

Nakashima, T., Kimmelman, C. P., and Snow, J. B., Jr. (1984). Structure of human fetal and adult olfactory neuroepithelium. *Arch. Otolaryngol* **110**, 641–646.

Netter, F. H. (1964). "The CIBA Collection of Medical Illustrations," Vol. 1. CIBA Pharmaceutical Company, New York.

Nilsson, B. (1979a). Taste acuity of the human palate. II. Studies with electrogustometry on subjects in different age groups. *Acta Odontol. Scand*. **37**, 217–234.

Nilsson, B. (1979b). Taste acuity of the human palate. III. Studies with taste solutions on subjects in different age groups. *Acta Odontol. Scand*. **37**, 235–252.

Norgren, R. (1990). Gustatory system. *In* "The Human Nervous System" (G. Paxinos, ed.), pp. 845–860. Academic Press, San Diego.

Ohm, T. G., and Braak, H. (1987). Olfactory bulb changes in Alzheimer's disease. *Acta Neuropathol*. **73**, 365–369.

Pearson, R. C. A., Esiri, M. M., Hiornes, R. W., Wilcock, G. K., and Powell, T. P. S. (1985). Anatomical correlates of the distribution of the pathological changes in the neocortex in Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A*. **82**, 4531–4534.

Price, J. L. (1990). Olfactory system. *In* "The Human Nervous System" (G. Paxinos, ed.), pp. 979–998. Academic Press, San Diego.

Pritchard, T. C. (1991). The primate gustatory system. *In* "Smell and Taste in Health and Disease" (T. V. Getchell, R. L. Doty, L. M. Bartoshuk, and J. B. Snow, Jr., eds.), pp. 109–125. Raven Press, New York.

Reyes, P. F., Deems, D. A., and Suarez, M. G. (1993). Olfactory-related changes in Alzheimer's disease: A quantitative neuropathologic study. *Brain Res. Bull*. **32**, 1–5.

Roberts, E. (1986). Alzheimer's disease may begin in the nose and may be caused by aluminosilicates. *Neurobiol. Aging* **7**, 561–567.

Rosli, Y., Breckenridge, L. J., and Smith, R. A. (1999). An ultrastructural study of age-related changes in mouse olfactory epithelium. *J. Electron Microsc*. **48**, 77–84.

Schiffman, S. S. (1991). Taste and smell losses with age. *Bol. Assoc. Med. P. R*. **83**, 411–414.

Schiffman, S. S. (1997). Taste and smell losses in normal aging and disease. *JAMA, J. Am. Med. Assoc*. **278**, 1357–1362.

Schiffman, S. S., and Clark, T. B. (1980). Magnitude estimates of amino acids for young and elderly subjects. *Neurobiol. Aging* **1**, 81–91.

Schiffman, S. S., Hornack, K., and Reilly, D. (1979). Increased taste thresholds of amino acids with age. *Am. J. Clin. Nutr*. **32**, 1622–1627.

Schiffman, S. S., Clark, C. M., and Warwick, Z. S. (1990). Gustatory and olfactory dysfunction in dementia: Not specific to Alzheimer's disease. *Neurobiol. Aging*. **11**, 597–600.

Schultz, E. W., and Gebhardt, L. P. (1936). Prevention of intranasally inoculated piliomyelitis in monkeys by previous intranasal irrigation with chemical agents. *Proc. Soc. Exp. Biol. Med*. **34**, 133–135.

Schultz, E. W., and Gebhardt, L. P. (1937). Zinc sulfate prophylaxis in poliomyelitis. *JAMA, J. Am. Med. Assoc*. **108**, 2182–2184.

Schwob, J. E., and Price, J. L. (1984). The development of axonal connections in the central olfactory system of rats. *J. Comp. Neurol*. **223**, 177–202.

Serby, M., Corwin, J., Conrad, P., and Rotrosen, J. (1985). Olfactory dysfunction in Alzheimer's disease and Parkinson's disease. *Am. J. Psychiatry* **142**, 781–782.

Serby, M., Larson, P., and Kalkstein, D. (1991). The nature and course of olfactory deficits in Alzheimer's disease. *Am. J. Psychiatry* **148**, 357–360.

Shepherd, G. M. (1972). Synaptic organization of the mammalian olfactory bulb. *Physiol. Rev*. **52**, 864–917.

Shepherd, G. M. (1983). "Neurobiology." Oxford University Press, New York.

Small, D. M., Jones-Gotman, M., Zatorre, R. J., Petrides, M., and Evans, A. C. (1997). A role for the right anterior temporal lobe in taste quality recognition. *J. Neurosci*. **17**, 5136–5142.

Smith, C. G. (1942). Age incident of atrophy of olfactory nerves in man. *J. Comp. Neurol*. **77**, 589–594.

Smith, D. V. (1971). Taste intensity as a function of area and concentration: Differentiation between compounds. *J. Exp. Psychol*. **87**, 163–171.

Somlyo, A. P., and Somlyo, A. V. (1968). Vascular smooth muscle. I. Normal structure, pathology, biochemistry, and biophysics. *Pharmacol. Rev.* **20**, 197–272.

Stevens, J. C., and Cain, W. S. (1986). Aging and the perception of nasal irritation. *Physiol. Behav.* **37**, 323–328.

Stevens, J. C., Cruz, L. A., Marks, L. E., and Lakatos, S. (1998). A multimodal assessment of sensory thresholds in aging. *J. Gerontol.* **53**, 263–272.

Tisdall, F. F., Brown, A., Defries, R. D., Ross, M. A., and Sellers, A. H. (1937). Nasal spraying as preventive of poliomylitis. *Can. Public Health J.* **28**, 431–434.

Turner, A. L., and Esmond, R. F. (1926). Study of the paths of infection of the brain, meninges and venous blood sinuses from neighboring peripheral foci of inflammation. III. Nasal mucous polypi; intra-nasal operation on the ethmoidal air cells; purulent leptomeningitis; death; autopsy. *J. Laryngol. Otol.* **41**, 717–731.

Waldton, S. (1974). Clinical observations of impaired cranial nerve function in senile dementia. *Acta Psychiatr. Scand.* **50**, 539–547.

Warwick, Z. S., and Schiffman, S. S. (1990). Sensory evaluations of fat-sucrose and fat-salt mixtures: Relationship to age and weight status. *Physiol. Behav.* **48**, 633–636.

Weiffenbach, J. M., and Bartoshuk, L. M. (1992). Taste and smell. *Clin. Geriatr. Med.* **8**, 543–555.

Weiffenbach, J. M., Baum, B. J., and Burghauser, R. (1982). Taste thresholds: quality specific variation with human aging. *J. Gerontol.* **37**, 372–377.

Weiffenbach, J. M., Cowart, B. J., and Baum, B. J. (1986). Taste intensity perception in aging. *J. Gerontol.* **41**, 460–468.

Weisfuse, D., Catalanotto, F. A., and Kamen, S. (1986). Gender differences in suprathreshold taste scaling ability in an older population. *Spec. Care Dentistry* **6**, 25–28.

Yamagishi, M., Takami, S., and Getchell, T. V. (1995). Innervation in human taste buds and its decrease in Alzheimer's disease patients. *Acta Oto-Laryngol.* **115**, 678–684.

Yoshida, M. (1984). Correlation analysis of detection threshold data for ''standard test'' odors. *Bull. Fac. Sci. Eng. Cho Univ.* **27**, 343–353.

Yousem, D. M., Geckle, R. J., Bilker, W. B., and Doty, R. L. (1998). Olfactory bulb and tract and temporal lobe volumes: Normative data across decades. *Ann. N.Y. Acad. Sci.* **855**, 546–555.

Zatorre, R. J., Jones-Gotman, M., Evans, A. C., and Meyer, E. (1992). Functional localization and lateralization of human olfactory cortex. *Nature (London)* **360**, 339–340.

SECTION IV

Locomotion: Basal Ganglia and Muscular Functions

A. Functional Impairments in Humans

(CHAPTERS 46 AND 47)

B. Pathology and Biochemistry of Aging and Disease of Basal Ganglia

(CHAPTERS 48 AND 49)

C. Animal Models

(CHAPTER 50)

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Sharon A. Jubrias and Kevin E. Conley

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Aging Effects on Muscle Properties and Human Performance

Elderly humans show large declines in functional abilities and changes in muscle properties compared to young ones. The purpose of this chapter is to relate the age-related changes in muscle to the reductions in maximum force production and in sustained power output (i.e., endurance) found in the elderly. Muscle atrophy accounts for more than half of the decline in muscle force production. The remainder of this decline can be explained by a reduction in the force per cross-sectional area of muscle fibers. Neither impaired neural recruitment of muscles nor a decrease in the proportion of fast muscle fibers appears to contribute significantly to the loss of force with age. Instead, alterations to both excitation–contraction coupling and the contractile elements are possibilities to account for the loss in force per cross-sectional area with age. Nearly equal contributions by the loss of muscle volume and changes in muscle oxidative properties are responsible for the reduction in sustained muscle power output. The reduction in oxidative capacity of elderly muscle stems from lower mitochondrial volume density and lower oxidative capacity per mitochondrial volume. Thus, loss of muscle size and changes in muscle intrinsic properties largely account for the declines in both force production and sustained power production in the elderly.

I. Introduction

Elderly humans display significant deficits in physical function as compared to the young. There is marked loss of force production by muscle starting in the sixth decade of life and continuing thereafter (Larsson *et al*., 1979). Similarly, endurance performance peaks in the second decade of life and drops with age thereafter as shown by losses in both muscle power output and oxygen uptake at the aerobic limit (Astrand and Rodahl, 1986). These changes in muscle function have important effects on physical activities of daily living in the elderly. The reduction in the capacity to generate force limits the ability of a person to rise from a chair, get in and out of a bathtub, place groceries in a cabinet, etc. Similarly, the drop in muscle endurance hinders simple tasks such as walking through a shopping mall, mowing the lawn, or vacuuming the floor. Thus, significant reductions in muscle strength and endurance compromise the ability of the elderly to lead an independent life.

There are similarities between the reductions in force production and endurance with age. In each case, the reductions reflect two main factors: the loss of muscle size and changes in intrinsic properties of the muscle cell. A number of reviews have documented changes in muscle properties with age (Brooks and Faulkner, 1994b; Larsson and Ansved, 1995; Holloszy and Kohrt, 1996). Of particular interest to the neurobiologist is Larsson's review of the effects of aging on the motor unit of the rat (Larsson and Ansved, 1995). Our goal is to identify the changes in muscle properties that are important to the decline of muscle performance in elderly humans.

II. Strength Changes with Age

A. What Determines Muscle Strength and Why Is It Reduced with Age?

The ability of human skeletal muscle to produce force declines steadily after about 60 years of age (Larsson *et al*., 1979; Vandervoort and McComas, 1986; Narici *et al*., 1991). The loss of force production in the elderly is substantial, with elderly subjects producing only about 65% of the force generated by young subjects in knee extension (Fig. 46.1) (Borges, 1989; Overend *et al*., 1992a). In one group of subjects ages 65–80 years, force production by the quadriceps muscle during isokinetic knee extension dropped an average of 10 N/year, resulting in a 39% decline in force between 65 and 80 years of age (Jubrias *et al*., 1997). A reduction of force production with age has been shown for muscles of the upper and lower extremities (Bemben *et al*., 1991; Narici *et al*., 1991), for voluntary and electrically stimulated contractions (Harridge *et al*., 1995; Vandervoort and McComas, 1986), and for isometric and shortening contractions (Davies *et al*., 1987; Frontera *et al*., 1991). Although the extent of the decline

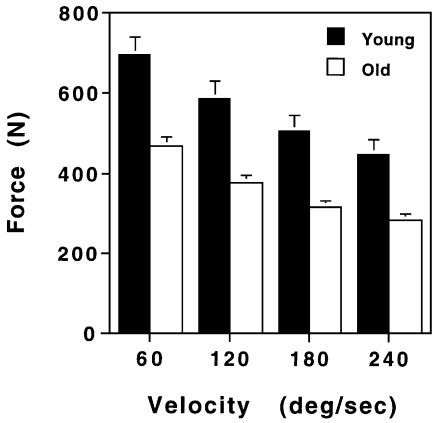

FIG. 46.1. Force production as a function of angular velocity during isokinetic knee extension in young (35 years) and old (70 years) subjects.

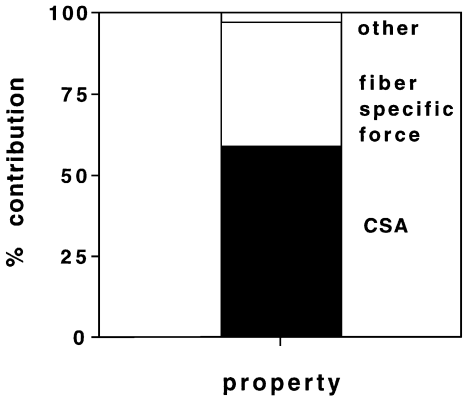

FIG. 46.3. Relative contributions of muscle cross-sectional area (CSA), muscle fiber-specific force, and nonmuscle properties (other) to the decline of force production with age by muscle *in vivo*.

appears to vary among muscles (McDonagh *et al*., 1984; Bemben *et al*., 1991), it is clear that this phenomenon represents a significant change in muscle functional capacity. A decline in muscle strength correlates with an increased risk of falls and loss of independence in daily activities (Pendergast *et al*., 1993; Sonn, 1996), so the reduction in muscle strength with age has a significant impact on both an individual's health and independence.

Muscle force production is governed by three main factors: muscle size, intrinsic properties of the muscle, and neural activation. Figure 46.2 illustrates the relationship of these factors to force production by the muscle. The combination of a muscle's size (cross-sectional area) and the specific force (force per cross-sectional area) of its fibers determines the force production capacity of that muscle. The actual force that is produced by a muscle *in vivo* results from both its capacity for force generation and its recruitment by motorneurons. Reductions in force with age must stem from changes in some or all of these factors.

Our approach in this chapter is to examine what is known about changes with age in each of these factors and from this make a reasonable estimate of the contribution each makes to the reduction of force production capacity. Figure 46.3 shows that changes in the size and intrinsic properties of the muscle largely account for the drop in force output by muscle in the elderly. Muscle cross-sectional area, which reflects the number and size of individual fibers, is the factor most responsible for declining force production with age. The specific force of muscle fibers, which is determined by such properties as contractile protein content and calcium handling and sensitivity, is lower in old age (Larsson *et al*., 1997) and accounts for the balance of the force reduction. Although a gradual loss of fast fiber content in elderly muscle is often proposed as a factor in lower force production by the elderly, we show that this is unlikely to have a significant role. The same is true for alterations in muscle recruitment. In the sections that follow, we examine the changes with age seen for each of these major factors and the mechanisms that may underlie these changes. It will be apparent that despite numerous studies describing the reduction of force with age, there are still significant gaps in our knowledge of the detailed mechanisms responsible for this decrease in muscle function.

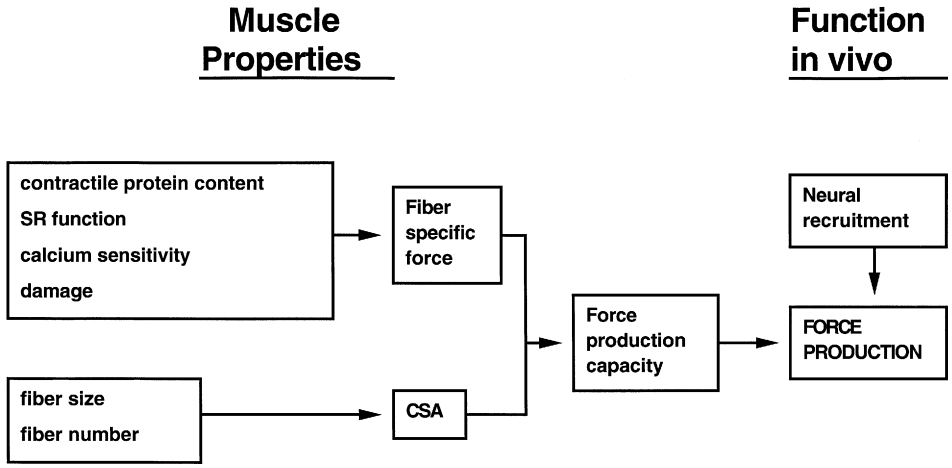

FIG. 46.2. Diagram of the relationship between muscle properties and muscle force production *in vivo*.

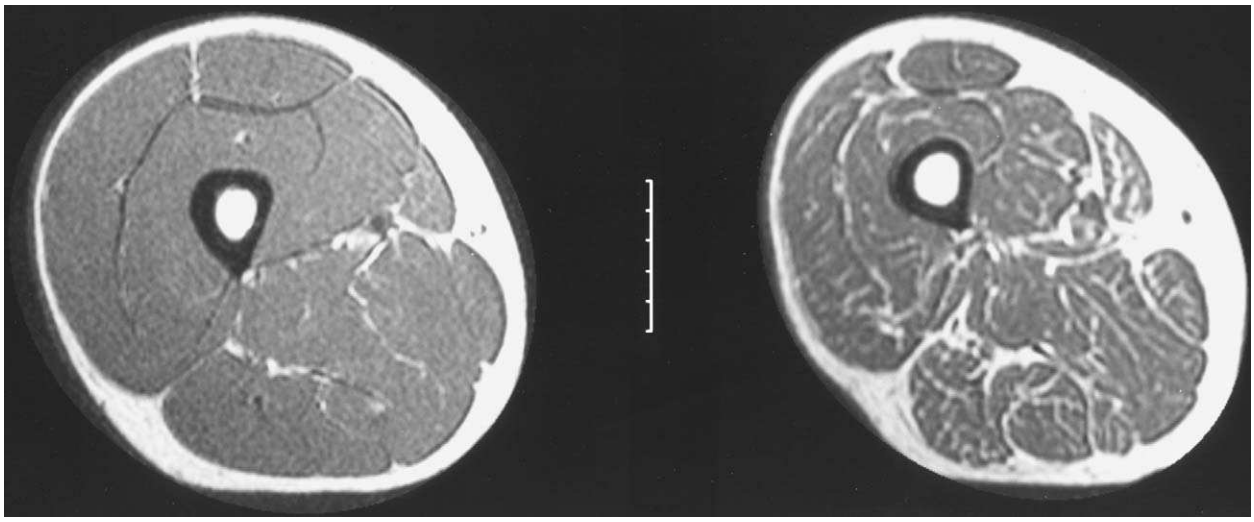

FIG. 46.4. Magnetic resonance images taken at the midthigh level in 2 subjects with similar body mass and height: left, 25-year-old male; right, 65-year-old male. Note the lesser muscle (gray) area and greater fat (white) area in the older subject.

B. Much of the Decline in Force with Age Is Explained by the Reduction of Muscle Cross-Sectional Area

1. Force versus Cross-Sectional Area

We now know that the loss of muscle mass with age is substantial and explains more than half of the reduction in force seen with aging. Magnetic resonance (MR) and computed tomography (CT) images taken *in vivo* show that elderly subjects have only about 75–80% of the quadriceps area of young subjects (Vandervoort and McComas, 1986; Overend *et al*., 1992a,b; Welle *et al*., 1996), and we found that the cross-sectional area of the quadriceps declines by about 20% between the ages of 65 and 80 years (Jubrias *et al*., 1997). Postmortem measurements show an even larger decline with age of 40% (Lexell *et al*., 1988). These numbers exceed estimates (Larsson *et al*., 1979) from anthropomorphic measurements *in vivo* that were unable to account accurately for changes in thigh composition. For example, Overend and associates (1992b) demonstrated that although the total area of the thigh was no different with age, elderly men had 26% and 18% less quadriceps and hamstring cross-sectional area, respectively, and 60% more nonmuscle tissue than did young men. Figure 46.4 shows MR images of the mid-thigh of a young and an elderly subject. This image clearly demonstrates the reduction of muscle area and increase in fat and connective tissue that accompanies aging in normal subjects. Several studies (Young *et al*., 1985; Vandervoort and McComas, 1986; Overend *et al*., 1992a) have measured both force and cross-sectional area in their subjects, and the 20–25% drop in muscle area that parallels the 35–40% drop in muscle force production suggests that the loss of muscle size can explain one-half to two-thirds of the loss of force with age.

Is this apparent connection between muscle size and force based on physical properties rather than coincidence? Figure 46.5 shows the relationship between maximum muscle force output and muscle cross-sectional area. This relationship results because the amount of force that can be generated by muscle depends in part on the number of myosin–actin cross-bridges present. Since a larger cross-sectional area contains more myofibrillar proteins, they generate more force than smaller muscles when activated similarly. Thus, the loss of muscle area with age results in lower force generation (Fig. 46.1) and accounts for up to two-thirds of the loss in force seen in aging.

2. Why Is Muscle Cross-Sectional Area Lower in the Elderly?

The number and size of individual fibers within a muscle determine the muscle's cross-sectional area, and both of these are reduced with age. In humans there is a pronounced loss of muscle fibers after about 50 years of age, but this process may begin even earlier (Lexell *et al*., 1988). These authors examined cross sections of whole human vastus lateralis muscle and found that 80-year-olds have almost 40% fewer muscle fibers in the quadriceps than do 20-year-olds. Type 1 (slow)

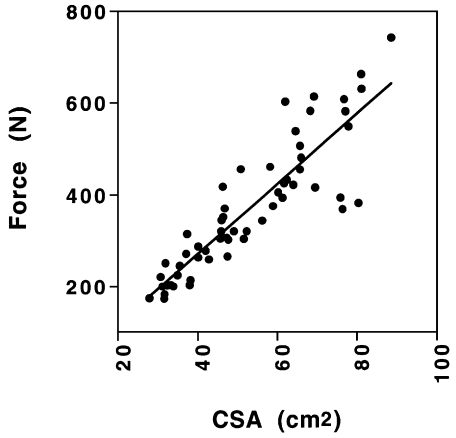

FIG. 46.5. Relationship between force output and cross-sectional area (CSA) of the quadriceps muscles, $r^2 = 0.79$.

and type 2 (fast) fibers were lost in roughly equal numbers. Muscle fiber loss is due mainly to the denervation of fibers followed by only partial reinnervation and subsequent atrophy and degeneration of the remaining denervated fibers (Larsson and Ansved, 1995; Lexell, 1995). Whole muscle cross-sectional area is also affected by a reduction of mean fiber area in the elderly. In contrast to the relatively equal loss of slow and fast fibers, fiber atrophy mainly affects fast fibers. Both biopsy (Larsson *et al*., 1979; Stalberg *et al*., 1989; Coggan *et al*., 1992) and whole cross-section (Lexell *et al*., 1988) data show a significant loss of type 2 fiber area, with the reduction ranging from 15 to 40%. This fast fiber atrophy may result from the drop in physical activity levels often seen as humans age, the denervation/reinnervation process, and lower synthetic rates of muscle proteins (Welle *et al*., 1993; Balagopal *et al*., 1997) found in older subjects. It is clear that processes both internal and external to the muscle result in the loss of muscle fiber number and area with age, leading ultimately to a decrease in the total muscle available for force production.

A comparison of the differences between the young and the old in force output and muscle cross-sectional area shows that the drop in force usually exceeds the amount of muscle atrophy (Young *et al*., 1985; Vandervoort and McComas, 1986; Overend *et al*., 1992a), with atrophy explaining about two-thirds of the force decrement. Thus, elderly muscle *in vivo* produces less force per muscle area (specific force) than does young muscle. This is also true of the continued loss of force seen after about age 60. For example, we found that between the ages of 65 and 80, muscle force production by the quadriceps decreased by about 40%, while the quadriceps's cross-sectional area dropped by only about 20% (Jubrias *et al*., 1997). Muscle atrophy is a major factor in the loss of force with age, but changes in other muscle properties or in neural input must account for the lower specific force observed *in vivo*. How can we determine which of these other factors are important in the loss of force seen in the elderly?

C. Changes in Muscle Intrinsic Factors Also Result in Lower Force Production with Age

1. Is the Specific Force of Single Muscle Fibers Reduced?

Evidence from isolated single muscle fibers strongly suggests that changes within the muscle fibers themselves are responsible for the remainder of the force deficit not explained by muscle atrophy. Studying isolated single fibers provides a way of examining the behavior of muscle without neural input or consideration of the relative fiber type proportions within the muscle, and so allows us to see whether individual fiber types of the elderly are less able to produce force than those of the young. Larsson and associates (1997) demonstrated that the specific force of fibers containing either myosin heavy chain (MHC) I or IIa isoforms is lower in elderly humans by about 10 and 25%, respectively. Although the Larsson study had a small number of subjects (4 young and 4 old), similar findings have been reported for some strains of rats and mice (Brooks and Faulkner, 1988; Degens *et al*., 1995; Li and Larsson, 1996). Since elderly human quadriceps muscle has a somewhat greater content of slow than fast muscle fibers, we can use 15% (the weighted average of 10% and 25%) as an estimate of the reduction in specific force of elderly fibers. This value is about half the size of the force deficit *in vivo* that is not explained by muscle atrophy. Thus, the combination of reduced muscle size and a lower specific force of muscle fibers in the elderly accounts for much of the observed force reduction *in vivo*.

2. What Accounts for Lower Fiber-Specific Force?

The reduced specific force of muscle fibers may result from altered calcium handling and sensitivity, a reduction in myofibrillar protein content, or lower resistance to ultrastructural damage. Unlike the ample evidence of change in muscle size and its consequences, alterations in these other muscle properties and their impact on force production are not as thoroughly researched. Thus, although it is clear that intrinsic muscle properties change, the relative contributions of these altered properties to lower fiber specific force has not been determined. Despite this, it is still possible to make an estimate of the relative importance of these factors to reduced fiber-specific force.

3. What Is the Role of Calcium in the Reduction of Specific Force?

Changes in calcium handling or the sensitivity to calcium of the contractile apparatus appear to play a significant role in the reduction of force production by elderly muscle. Calcium is important to force production since it triggers the contractile apparatus. The amount of force generated by a fiber is directly related to the concentration of calcium in the cytosol. Under normal physiological conditions in the young, enough calcium is released by the sarcoplasmic reticulum during muscle activation to saturate troponin fully and allow maximum force generation. If calcium release by the sarcoplasmic reticulum is reduced significantly the force generated by the fiber will be lower. Delbono and colleagues (1995) examined isolated human fast fibers and showed that calcium release by the sarcoplasmic reticulum is reduced by about 33% relative to young subjects. Delbono *et al*. (1997) also reported that this lower calcium release is accompanied by reduced specific force in those fibers, although the magnitude of the drop in specific force has not yet been published. However, if we use published values for the force-calcium relationship in isolated human fast fibers (Ruff, 1989), we can estimate that a decrease in calcium release as reported by Delbono *et al*. (1995) would result in about 23% less force. This approximates the 25% drop in specific force of fast fibers from the elderly reported by Larsson *et al*. (1997). The lower calcium release may be due to reduced synthesis of sarcoplasmic reticulum proteins in the elderly, which results in proteins with reduced function (Ferrington *et al*., 1998).

In addition to decrements in calcium release by the sarcoplasmic reticulum, a change in the sensitivity of the contractile apparatus to calcium would also lead to altered force production by muscle. Brooks and Faulkner (1994a) demonstrated a reduced calcium sensitivity in old vs young mice. In the old mice, saturating levels of calcium produced normal force, but lower levels of calcium produced less force than did simi-

lar levels of calcium in the young. Using the change in calcium sensitivity found in the Brooks and Faulkner paper (1994a), we calculate that a 33% drop in maximum calcium release as reported by Delbono *et al*. (1995) for older humans would result in about a 10% reduction in specific force. Thus, if the contractile apparatus of elderly humans also has a reduced calcium sensitivity, this could intensify the effect of the lower calcium release demonstrated by Delbono *et al*. (1995). Although the calcium sensitivity for elderly human fibers has not yet been reported to our knowledge, it is possible that the combination of reduced calcium release and reduced calcium sensitivity can account for the drop in fiber-specific force reported for the elderly.

4. Does a Relative Loss of Contractile Protein Contribute to Lower Specific Force?

The force generated by a muscle fiber depends on the number of actin–myosin cross-bridges formed during contraction, so a decrease in the amount of contractile protein per fiber area would also result in a lower specific force. Several papers have reported less contractile and more connective tissue in muscle fibers of old animals (Alnaqeeb *et al*., 1984; Kovanen, 1989). A drop in the contractile protein content (per volume) of fibers leading to a 40% reduction in specific force has also been demonstrated in human subjects who undergo lengthy bedrest (Larsson *et al*., 1996). It is unlikely that age alone results in the profound contractile protein loss that accompanies bed rest; however, the lower levels of physical activity commonly associated with aging might reduce the contractile protein content of elderly muscles. The importance of this mechanism to the loss of force per cross-sectional area awaits measurement of the contractile protein loss with age.

5. Is Elderly Muscle-Specific Force Lower Due to Muscle Damage?

A final intrinsic mechanism for lower force production by elderly muscle is an increased susceptibility to muscle damage coupled with a reduced capacity for regeneration. The muscle of old mice sustains more damage during eccentric contractions than does young muscle (see Brooks and Faulkner, 1994b). This also appears to be true for elderly humans as suggested by indirect measures of damage (Manfredi *et al*., 1991), but no ultrastructural studies of elderly human muscle after exercise have been reported. Elderly humans (Dedrick and Clarkson, 1990), rats (McBride *et al*., 1995), and mice (Brooks and Faulkner, 1994b) show a diminished capacity for regeneration after injury, with a prolongation of the force deficit associated with damaging contractions. An impaired ability to repair damage may stem from the lower rates of myofibrillar protein synthesis found in the elderly (Welle *et al*., 1993; Balagopal *et al*., 1997) or from reduced activation of satellite cells (Barton-Davis *et al*., 1998). The low rates of protein synthesis may also lead to a poorer quality of contractile proteins since the ability to repair routine damage from oxygen free radicals would be blunted. Barton-Davis and coworkers (1998) showed that overexpression of insulin-like growth factor in mouse muscle evoked by injection of a recombinant adeno-associated virus prevented the age-related drop in specific forces and this was apparently mediated by satellite cell activation. It is clear that muscle damage could be a factor in the lower specific force seen in the elderly, but this conclusion must await ultrastructural analysis of elderly muscle.

D. Other Proposed Mechanisms Are Unlikely to Be of Major Significance

We have shown that the reduction of force output by older subjects relative to the young can be explained by the measured reductions in muscle area and fiber-specific force. However, this explanation does not address two mechanisms that are often suggested as potential factors in the force deficit of elderly muscle: fiber type changes and altered recruitment. In this section, we examine these factors and show why neither is likely to play a significant role in the loss of force production with age.

1. Does a Decline of Fast Fiber Content Cause a Drop in Force?

Many studies report a loss of fast fiber and increase in slow fiber content with age (Larsson *et al*., 1979; Stalberg *et al*., 1989; Klitgaard *et al*., 1990). Lexell and Downham (1992) examined whole cross sections of human vastus lateralis muscle and found that the relative area of type 2 fibers is about 40% in 70-year-olds and about 50% in 30-year-olds. There are reports that muscle force production *in vivo* increases with a greater content of fast fibers (Inbar *et al*., 1981; Ivy *et al*., 1981; Young, 1984). Higher specific force for fast vs slow muscle fibers has been measured in isolated human fibers from the quadriceps, with fast fiber force per cross-sectional area about 25% higher than slow (Larsson *et al*., 1997). However, the data from single-fiber studies are conflicting, with the specific fiber preparation technique used playing a major role in determining whether a difference is seen between fast and slow fibers. We can estimate the impact of changing fiber type on force production with the figures given above. If fast fibers generate 25% more force per cross-sectional area than slow fibers and there is 10% less relative fast fiber area with age, then this drop in fast fiber content would result in a 2.5% loss of force. This reduction in force that may result from a shift from fast to slow fibers is small compared to the roughly 35% decline seen with age (Borges, 1989; Overend *et al*., 1992a). Thus, in contrast to the important influence of muscle atrophy and reduced specific force of both fast and slow types, a change in the fiber composition of elderly muscles is not a significant factor in the lower force production in this population.

2. Is Muscle Recruitment a Problem for the Elderly?

Altered neural recruitment is another potential mechanism for the decline in the force production of whole muscle with age. Neural adaptations have an important role in the strength gains seen following resistance exercise training in the young and the old (see Enoka, 1997), suggesting that neural mechanisms may also play a role in age-related losses of muscle force

production. There are two main neural mechanisms with the potential to reduce the measured force of whole human muscle: an inability to fully activate the agonist muscle and increased recruitment of antagonist muscles.

It is unlikely that an inability to recruit all motor units of a muscle at optimal contraction rates plays a major role in the loss of strength in the elderly. Studies of leg (Vandervoort and McComas, 1986; van Schaik *et al*., 1994), arm (Brown *et al*., 1990; De Serres and Enoka, 1998), and hand (Phillips *et al*., 1992) muscles indicate that older subjects are able to recruit all motor units with voluntary activation. In contrast, Kent-Braun and LeBlanc (1996) reported that four of five elderly subjects showed an additional force increment when 50 Hz electrical stimulation of the dorsiflexors was superimposed on a maximum voluntary contraction, but they did not indicate the size of the additional force. This observation was interpreted as an inability of the elderly to activate muscles at sufficient firing frequencies to achieve maximum force. In support of this, Kamen and associates (1995) have shown that elderly subjects have lower motorneuron firing rates than younger subjects. However, these authors point out that due to the increased twitch contraction time in elderly muscle, lower firing frequencies may be adequate to achieve tetanus in these muscles. De Serres and Enoka (1998) question the functional significance of the small differences in force seen with imposed high-frequency pulse trains. There is thus no compelling evidence to suggest that inadequate recruitment contributes to the reduction of force output with age.

3. Does Antagonist Muscle Coactivation Reduce Force in the Elderly?

Very little attention has been given to the role of antagonist muscle coactivation in the reduction of measured muscle force in the elderly, but this mechanism may be a contributor. Hakkinen *et al*. (1998) reported that in untrained subjects, hamstring coactivation during quadriceps contraction was greater in the elderly than in the young, during both isometric and dynamic contractions. Coactivation is in part related to training status (Osternig *et al*., 1986; Carolan and Cafarelli, 1992), and older subjects reduced the coactivation of their antagonists to levels similar to the young following training of the agonist movement (Hakkinen *et al*., 1998). The recruitment of antagonist muscles *in vivo* produces a force in opposition to that of the agonists such that the effective force output of the agonist muscle is reduced. In addition, activation of antagonist muscles reduces the neural drive to the agonists by reciprocal inhibition (Sale, 1988). Reduced coactivation of antagonists accounts for about a third of the increase in force production in the early stages of strength training in the young (Carolan and Cafarelli, 1992), so it is clear that the recruitment of antagonist muscles can have an important effect on resulting force. Taken together, these studies suggest that coactivation of antagonist muscles may contribute to the drop in force production *in vivo* seen in the elderly. We demonstrated earlier that muscle atrophy and the reduced specific force of muscle fibers are sufficient to explain the force deficit with age, but it may be that coactivation also has an important role in individuals who show very large force deficits.

III. Endurance Performance and Age

Two characteristics important to maximal force production—muscle size and intrinsic properties—are also key to endurance performance. The intrinsic properties important to strength relate primarily to the contractile elements. In contrast, the intrinsic properties important to endurance relate primarily to the supply and demand for ATP. This is apparent in the decline in endurance performance with age, which involves changes in both maximal oxygen uptake and muscle power output (Astrand and Rodahl, 1986). The goal of this section is to determine how muscle properties related to ATP supply and demand change with age, what effect these changes have on cellular energetics, and how these changes are reflected in the loss of muscle performance in the elderly.

A. Sources and Sinks for ATP in Active Muscle

Aging has an effect on each of the ATP supply and demand properties. Our goal is to evaluate the consequences of the age-related changes in the integrated energetics of the muscle cell to exercise performance at the muscle and whole-body level. To this end, let us first consider ATP supply and demand in active muscle. The integrated nature of the intracellular properties involved in the balance between energy demand and supply necessary for muscle contraction (Conley, 1994) is shown in Fig. 46.6. The primary consumers of ATP in the muscle are the ion and myosin ATPases (Rall, 1985), while the primary source of ATP for sustained contractions is the mitochondria, which use oxygen and carbon fuels in the process of oxidative phosphorylation. A secondary source of ATP—but primary source of carbon fuel—is glycolysis. This pathway is also the source of H^+ and lactate, which accumulate at high work levels and can inhibit sustained muscle contraction.

B. Balancing ATP Supply to Demand

1. Does Aging Affect ATP Supply and Demand?

We can directly measure contractile ATP demand and ATP supply using noninvasive magnetic resonance methods that characterize a whole-muscle rather than a muscle sample from a biopsy (Blei *et al*., 1993a,b; Conley *et al*., 1998, 1999b).

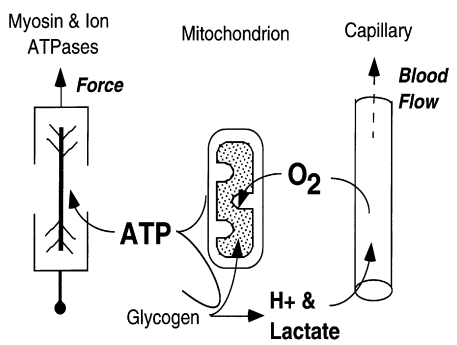

FIG. 46.6. Diagram of the mass and energy balances involved in ATP supply and demand in muscle.

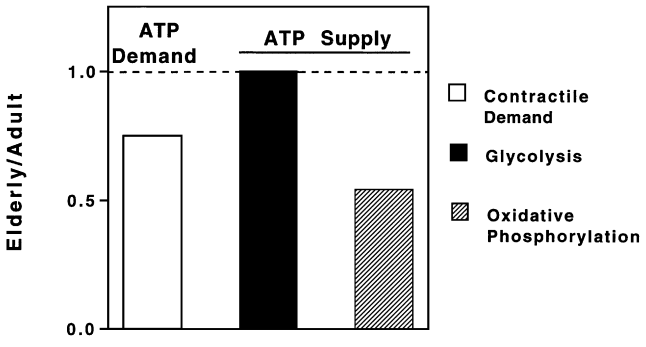

FIG. 46.7. Ratios of ATP demand and supply properties of vastus lateralis muscle in elderly (mean age, 68 years) vs adult (mean age, 38 years).

Figure 46.7 contains data from our laboratory showing that both contractile demand and oxidative capacity of elderly (mean age, 68 years) vastus lateralis are lower than in younger adults (mean age, 38 years). Contractile costs are not routinely determined on muscle, so it is unclear whether this decline between the third and the sixth decade of life is typical of aging muscle. However, the reduction in oxidative capacity has been reported in previous MR studies and supported by similar reductions in oxidative enzyme activity (Coggan *et al*., 1993; McCully *et al*., 1993). Thus elderly muscle shows reductions in both the supply and the demand for ATP compared with that seen in younger adults.

2. What Accounts for Lower ATP Demand?

Changes in contractile properties or excitation–contraction coupling could be the basis of the reduced contractile cost in elderly muscle. The basis of these changes in muscle properties may lie in an ongoing denervation and reinnervation process in elderly muscle (Larsson *et al*., 1997). Fiber type grouping and reduction in the number of functioning motor units are indicative of such a process (Doherty and Brown, 1997; Lexell, 1995). The resultant shift in myosin heavy chain to the slower type I fibers is often cited as the cause of the drop in contractile speed and ATP utilization, but there appears to be only a small fiber type change during this denervation and reinnervation process (Lexell, 1995). Thus the frequently noted change in fiber type properties with age has only a small effect on contractile costs.

An age-related change that is more important to energetics, however, is the finding of reduced contraction velocity for a given fiber type (Larsson *et al*., 1997). Several causes for this reduced velocity of contraction with age are possible. First, expression of additional, but undetected, myosin heavy chain isoforms may be responsible for the slowing of muscle with age (Larsson *et al*., 1997; Thompson and Brown, 1999). A second possibility is contractile protein changes, such as myofibrillar loss or increased spacing of myofilaments, which have also been proposed to account for the loss of specific force in elderly muscle (Larsson *et al*., 1997). A third possibility is the uncoupling of excitation from calcium release resulting in reduced calcium available for activation in elderly compared to young human muscle mentioned earlier (Delbono *et al*., 1995). Reduced calcium activation could account for the lower specific force found *in vivo* as well as the reduced cost per twitch. Lower calcium cycling itself would reduce contractile costs. Finally, the reduced regenerative capacity of elderly muscle may make it more susceptible to injury, which impairs function (Brooks and Faulkner, 1994b). An increased ''wear and tear'' as the basis of reduced function is bolstered by the Barton-Davis *et al*. (1998) finding that stimulation of muscle regeneration via activation of satellite cells by insulin-like growth factor I eliminated age-related specific force loss. The end result is a lower mechanical performance of aged human muscle fibers, which is consistent with our measurement of lower contractile ATP use.

3. What Accounts for the Lower ATP Supply?

The 50% difference in oxidative capacity per muscle mass between elderly and adult muscle shown in Fig. 46.7 is slightly larger than the 30% difference in oxidative enzyme activity reported by McCully *et al*. (1993) for groups similar in age to ours. These workers found that oxidative enzyme activity was lower in proportion to the oxidative properties measured by MR. We have found that the loss in oxidative capacity with age was due primarily to a lower mitochondrial volume but also to a reduced oxidative capacity per mitochondrial volume. Papa (1996) has shown a steady age-related decline in mitochondrial oxidative enzymes. Our finding of a 20% loss of oxidative capacity per mitochondrial volume between the fourth and seventh decade of life is similar to the loss of mitochondrial respiratory enzyme activity reported by Papa (Conley *et al*., 1999b). Both increased DNA mutations in the mitochondrial and nuclear genomes coding for mitochondrial respiratory enzymes and the direct action of oxygen free radicals appear to be the causative agents of this decline in oxidative enzyme activity (Ozawa, 1997). Thus two factors appear to contribute to this lower oxidative capacity per muscle mass in the elderly: reducedmitochondrial content and reduced activity of mitochondrial enzymes. These reductions mean a lower capacity for oxidative ATP synthesis in elderly muscle. The consequences of the lower oxidative capacity to muscle performance are discussed in section III.D.2.

4. What Age-Related Factors Account for Muscle Changes?

Nonathletic youth have lower oxidative capacity and lower muscle mass compared to endurance athletes (Proctor *et al*., 1995). Is the lower level of chronic activity with age responsible for the observed age-related changes in muscle properties? To separate those changes related to reduction in activity vs those due to aging in general, we can examine elderly subjects who have maintained a high level of activity, specifically, master athletes. These athletes show a loss of fat-free mass in general and quadriceps muscle mass in specific compared to young athletes (Coggan *et al*., 1990; Proctor and Joyner, 1997; Proctor *et al*., 1998). A reduced maximum oxygen consumption (VO_2max) per fat-free mass indicates lower oxidative capacity of the underlying muscle. The lack of difference in the activity of oxidative enzymes of these master athletes compared to young athletes suggests that histochemical determinations of single enzyme activities may not be sensitive to the age-related

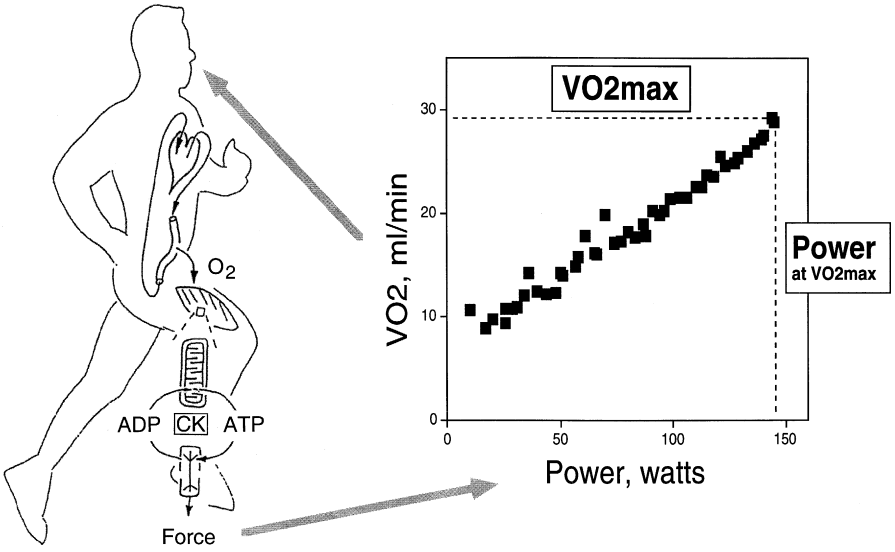

FIG. 46.8. Oxygen uptake as a function of leg power output from rest to the aerobic capacity (VO_2max) in an exercising human.

changes in the oxidative pathway. Similarly, contractile properties are slower in elderly athletes, as shown by a longer time to peak tension in a twitch (Harridge *et al*., 1997). The reduction in muscle mass, oxidative enzyme activity, and slower contractile properties in the trained master athletes indicate that these changes are an inevitable part of aging and not due to inactivity *per se*. How are the age-related changes in muscle reflected in physical performance?

C. How Human Performance Reflects the Effects of Aging on Muscle Properties

1. How Do We Measure Performance?

Typically aerobic fitness is measured as the highest oxygen consumption achieved by a subject on a treadmill or cycle ergometer. Figure 46.8 shows that O_2 uptake increases with leg power output to the limit of uptake at VO_2max. This power output by the legs represents a direct measure of aerobic exercise performance that can be related to the muscle properties governing the demand and supply of ATP. Our focus is on how these muscle properties determine aerobic leg performance; specifically, how muscles generate and use ATP in power production and how the changes in muscle properties with age affect muscle power production.

2. How Do Muscle Properties Determine Performance?

Two classes of properties are critical to muscle performance across ages: muscle mass and energetic properties. We showed previously that maximal isokinetic leg force is directly related to muscle cross-sectional area (Jubrias *et al*., 1997). The combination of muscle size and energetic properties is important to sustained muscle performance because together they determine the capacity for ATP supply and demand. This section focuses on how the intracellular properties involved in ATP supply and demand determine the ability to sustain this power output.

The key properties governing ATP flux and the role of these properties in determining muscle power output are illustrated in Fig. 46.9. This scheme is derived from a comprehensive study of the role of these properties in human endurance performance recently reviewed by Coyle (1995). Figure 46.9 connects the individual muscle properties discussed earlier to muscle power output based on the effect of these properties on ATP supply and demand. The key property in this scheme is the mitochondrial volume, which determines O_2 consumption and the majority of ATP supply. A secondary source of ATP–but a major pathway for substrate supply to the mitochondria—is glycolysis (Connett and Sahlin, 1996; Conley *et al*., 1997, 1998). This pathway may also generate H^+ during aerobic exercise, which can inhibit oxidative metabolism (as indicated by the minus sign in Fig. 46.9) and excitation–contraction coupling, thereby limiting sustained performance (Coyle, 1995). The final key muscle property is the fiber type, which is energetically important because it determines the efficiency of conversion of ATP into power output by the muscle. Muscles capable of high levels of sustained performance often have a high proportion of the more efficient slow twitch (type I) fibers, which use less ATP to generate a given power production (Coyle, 1995). To what extent can these findings help us understand the loss of performance in the elderly?

D. Maximum Aerobic ATP Supply

1. Mitochondria and O_2 Demand

The first step in understanding how the decline in muscle properties relates to the decline in muscle performance is to ask how muscle properties determine performance in young, healthy muscle. The capacity for oxidative ATP supply that underlies sustained performance depends on the mitochondrial content of the muscle. As the sites for O_2 uptake and aerobic ATP generation, mitochondria depend on the cardiovascular system for O_2 delivery to sustain ATP supply. Does this O_2 delivery ever limit the capacity of the mitochondria? Recent work indicates that there is no limitation in untrained young subjects (Cardus *et al*., 1998), but that a small cardiovascular limitation may restrict the measured VO_2 to less than the mito-

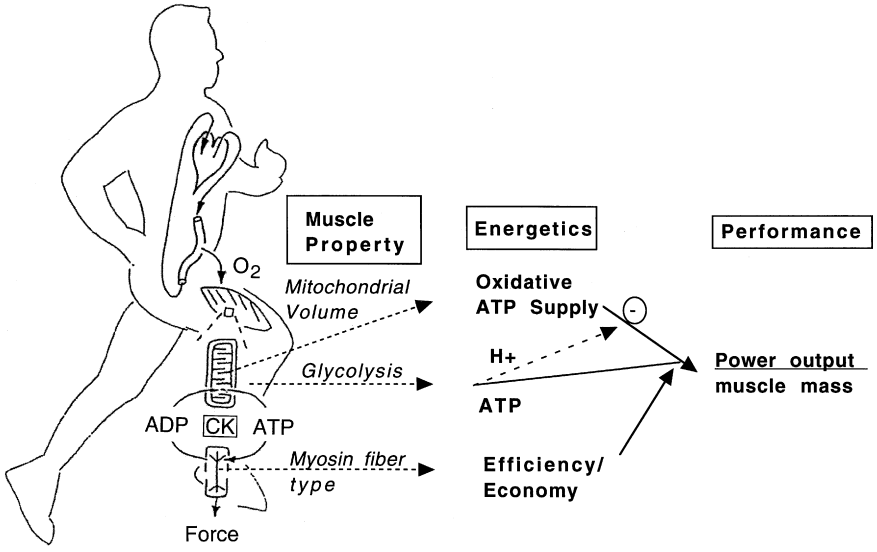

FIG. 46.9. Diagram of the links between muscle properties, energetics, and leg power output per muscle mass.

chondrial capacity in athletic individuals (Richardson *et al.*, 1999). The elderly have a greater O_2 extraction and a lower blood flow rate compared to young at the same submaximal work level (Proctor *et al.*, 1998), but there is no direct measure of an O_2 delivery limitation to sustained performance or at VO_2max in the elderly to our knowledge.

2. Muscle Oxidative Properties Are Critical to Sustained Performance

Coyle (1995) found that oxidative enzyme activity is a major determinant of leg power output and performance time of athletes. Another measure of the importance of muscle oxidative properties in determining O_2 flux is the close relation between maximum oxygen consumption per body mass and mitochondrial content of the vastus lateralis in a wide range of young subjects (Hoppeler *et al.*, 1973). A similar proportionality between VO_2max and muscle oxidative properties in elderly subjects was found by McCully *et al.* (1993). These workers found that the lower VO_2max in elderly subjects was accompanied by a lower muscle oxidative enzyme activity.

3. How Is This Reduced VO_2max Related to Power Output?

A good demonstration of the link between muscle power output and oxidative capacity was shown by Hoppeler *et al.* (1985) in young subjects. They showed that endurance training raised mitochondrial volume and leg power output. The oxygen consumption of the legs estimated from the rise in power output (estimated using a typical cycling efficiency of 25%) matched the elevation of VO_2max with training. This calculation indicated that the majority of the increase in VO_2max occurred in the leg muscles. Since the legs consume about 85% of the oxygen during cycling at VO_2max (Poole *et al.*, 1992), this correspondence between the predicted rise in VO_2 based on leg power output and the measured VO_2max makes sense. These results demonstrate that the increased VO_2 of the quadriceps generated a higher ATP flux and, in turn, supported the higher leg power output found after training.

The importance of muscle size to leg power output is evident in the comparison of elderly vs young subjects and in untrained vs trained elderly (Coggan *et al.*, 1993). The lower power output in untrained elderly reflected both a reduced muscle size as well as a lower oxidative capacity per muscle volume compared to trained elderly or the young. The difference in oxidative capacity between the untrained elderly and the trained youth resulted from primarily a lower oxidative enzyme activity but also a smaller muscle volume. These results are similar to those of Conley *et al.* (1999a) who found that the combination of lower muscle mass (65% of young adult muscle) and reduced oxidative capacity per muscle (50% of young adult muscle) accounted for the reduction of VO_2max in elderly muscle to 33% (65% $\times$ 50% = 33%) of the adult value. Thus both the size and oxidative properties of muscle need to be taken into account in understanding the oxidative capacity and power output of muscle.

4. How Does Maximal Aerobic Performance Relate to Elderly Activity?

Muscles can work to VO_2max, but this is a transient exercise that cannot be maintained. It does allow one to measure the maximal aerobic performance and determine the factors that set this aerobic limit. It is quite clear that oxidative capacity is critical to submaximal performance as well. Many endurance events occur at about 70% of VO_2max, but despite the submaximal level, performance is closely related to VO_2max (Coyle, 1995). For example, the preferred walking speed in elderly subjects elicits VO_2 well below the aerobic capacity, but speed is nonetheless related to VO_2max (Conley *et al.*, 1995; Buchner *et al.*, 1996). Similarly, Coyle (1995) reported that performance in a number of athletic events elicits submaximal sustained activity that is proportional to VO_2max. Thus, exercise for young and old alike may never reach levels that elicit VO_2max, but this aerobic capacity affects the levels of

exercise that can be sustained. How is sustained performance related to VO_2max?

E. Setting the Limit to Sustained ATP Supply

1. Glycolysis and Sustained Performance

The highest sustainable level of performance in muscles working alone and in whole-body performance is associated with accumulation of glycolytic byproducts. Glycolysis provides the majority of the substrate supply for oxidative phosphorylation during exercise. However, pyruvate production may exceed oxidation even under sustained exercise conditions, causing inhibitory byproducts, such as H^+ and lactate, to accumulate (Conley *et al*., 1997). Muscles can sustain work until H^+ begins to accumulate and intracellular pH drops; at higher work levels force production declines and work cannot be maintained. Chilibeck *et al*. (1998) found that this pH threshold occurred at a lower percentage of peak aerobic power during ankle plantar flexion in elderly (60%) vs young subjects (70%).

2. Are the Muscle and Whole-Body Thresholds for Sustained Activity Related?

The work rate at which lactate accumulates in the blood is termed the lactate threshold and typically occurs at the limit to sustained whole body activity (Coyle, 1995). Syström *et al*. (1990) found that the muscle intracellular pH decline corresponded with the threshold for lactate accumulation in the blood. Since muscle generates lactate under fully aerobic conditions (Conley *et al*., 1998) and whole-body VO_2 can increase well above the lactate threshold, the pH decline in the muscle and lactate accumulation in the blood does not represent an oxygen limitation to respiration. Instead, it reflects a glycolytic flux that exceeds the capacity to oxidize pyruvate (see Connett and Sahlin, 1996). This correspondence of the drop in muscle pH and blood lactate accumulation suggests a link between muscle fatigue and the limit to sustained performance. For the elderly, lactate accumulation in the blood is reported to occur at a lower percentage of VO_2max in sedentary compared with highly trained cyclists but is similar among well-trained athletes regardless of age (Masse-Biron *et al*., 1992). Thus, the percentage of the oxidative capacity at which the limit to sustained performance occurs may not be a strict function of age, but rather may be more related to the training state of the individual.

3. Can the Limit to Sustained Activity Be Increased?

Endurance exercise training in the elderly has been shown to raise the limit to sustained activity in two ways (Marsh *et al*., 1993). First, exercise training increases the VO_2max and thereby the capacity for ATP synthesis. Second, the lactate threshold occurs at a higher fraction of VO_2max. This elevated threshold could result from both a reduction in glycolytic flux and a higher oxidation of this flux during exercise. Thus endurance training in the elderly can increase not only the ATP supply capacity of the muscle but also the fraction of this capacity that can be sustained to support aerobic work. The resultant elevated lactate threshold and increased oxidative capacity means a higher limit to sustained ATP supply and greater sustained muscle power output.

4. Sustained Performance: Interaction of Glycolysis, Oxidative Phosphorylation, and Contractile Properties

The limit to sustained performance is a good example of the integrative nature of muscle energetics. As shown in Fig. 46.9, oxidative phosphorylation may set the limit to aerobic ATP supply, but the extent of glycolytic H^+ and lactate production will determine the fraction of the aerobic limit that can be sustained for more than a few minutes. Further, the nature of the muscle fiber type determines the efficiency of the conversion of ATP into force production. Thus there is a clear interaction between glycolysis, oxidative phosphorylation, and contractile properties in setting the limit to sustained performance. Endurance training has the potential to vary not only the aerobic limit but also the fraction of that limit that can be sustained and the muscle fiber type. Typical training studies focus on the small change achieved in VO_2max (usually <15%), which is not the only factor determining elderly performance. Perhaps it is now time to evaluate the effect of training on activities that are relevant to the functioning of the elderly in everyday life, such as sustained performance in vacuuming or carrying groceries (Conley *et al*., 1995). These sustained activities may well depend on the limit to sustained performance—and the interaction of glycolysis, oxidative phosphorylation and contractile properties—rather than on the aerobic capacity alone.

IV. Conclusions

A great deal of progress has been made in cataloging the gross changes in muscle properties and function that occur with age. It is now clear that we can account for a significant fraction of the loss of physical performance in the elderly based on the age-related changes in muscle properties. Loss of muscle size is responsible for more than half the drop in force production and sustained power output with age. This smaller muscle size reduces the cross-sectional area for force production and the volume for sustained power output. The balance of the reduction in muscle performance is primarily due to the intrinsic properties of muscle. Elderly muscle has a lower force per cross-sectional area and a smaller mitochondrial volume density for sustained ATP supply. The end result is a drop in the ability to generate and sustain force production in the elderly that greatly exceeds the loss expected from muscle mass alone. Some of this functional loss can be regained through the use of strength and endurance exercise training programs. However, studies of master athletes show that the loss of muscle mass is an inevitable part of the aging process. A focus on the mechanisms underlying the loss of muscle mass and change in properties may lead to therapies that can either reverse or avoid these apparently inevitable changes in muscle properties and the loss of physical performance with age.

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References

Alnaqeeb, M. A., Al Zaid, N. S., and Goldspink, G. (1984). Connective tissue changes and physical properties of developing and ageing skeletal muscle. *J. Anat*. **139**, 677–689.

Åstrand, P.-O., and Rodahl, K. (1986). "Textbook of Work Physiology." McGraw-Hill, New York.

Balagopal, P., Rooyackers, O. E., Adey, D. B., Ades, P. A., and Nair, K. S. (1997). Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. *Am. J. Physiol*. **273**, E790–E800.

Barton-Davis, E. R., Shoturma, D. I., Musaro, A., Rosenthal, N., and Sweeney, H. L. (1998). Viral mediated expression of insulin-like growth factor I blocks the aging-related loss of skeletal muscle function. *Proc. Natl. Acad. Sci. U.S.A*. **95**, 15603–15607.

Bemben, M. G., Massey, B. H., Bemben, D. A., Misner, J. E., and Boileau, R. A. (1991). Isometric muscle force production as a function of age in healthy 20- to 74-yr-old men. *Med. Sci. Sports Exercise* **23**, 1302–1310.

Blei, M., Conley, K., and Kushmerick, M. (1993a). Separate measures of ATP utilization and recovery in human skeletal muscle. *J. Physiol. (London)* **465**, 203–222.

Blei, M., Conley, K., Odderson, I., Esselman, P., and Kushmerick, M. (1993b). Individual variation in contractile cost and recovery in a human skeletal muscle. *Proc. Natl. Acad. Sci. U.S.A*. **90**, 7396–7400.

Borges, O. (1989). Isometric and isokinetic knee extension and flexion torque in men and women aged 20-70. *Scand. J. Rehabil. Med*. **21**, 45–53.

Brooks, S. V., and Faulkner, J. A. (1988). Contractile properties of skeletal muscles from young, adult and aged mice. *J. Physiol. (London)* **404**, 71–82.

Brooks, S. V., and Faulkner, J. A. (1994a). Isometric, shortening, and lengthening contractions of muscle fiber segments from adult and old mice. *Am. J. Physiol*. **267**, C507–C513.

Brooks, S. V., and Faulkner, J. A. (1994b). Skeletal muscle weakness in old age: Underlying mechanisms. *Med. Sci. Sports Exercise* **26**, 432–439.

Brown, A. B., McCartney, N., and Sale, D. G. (1990). Positive adaptations to weight-lifting training in the elderly. *J. Appl. Physiol*. **69**, 1725–1733.

Buchner, D. M., Cress, M. E., Esselman, P. C., Margherita, A. J., de Lateur, B. J., Campbell, A. J., and Wagner, E. H., (1996). Factors associated with changes in gait speed in older adults. *J. Gerontol*. **51**, M297–M302.

Cardus, J., Marrades, R. M., Roca, J., Barbera, J. A., Diaz, O., Masclans, J. R., Rodriguez-Roisin, R., and Wagner, P. D. (1998). Effects of F_IO_2 on leg VO_2 during cycle ergometry in sedentary subjects. *Med. Sci. Sports Exercise* **30**, 697–703.

Carolan, B., and Cafarelli, E. (1992). Adaptations in coactivation after isometric resistance training. *J. Appl. Physiol*. **73**, 911–917.

Chilibeck, P. D., McCreary, C. R., Marsh, G. D., Paterson, D. H., Noble, E. G., Taylor, A. W. and Thompson, R. T. (1998). Evaluation of muscle oxidative potential by ^{31}P-MRS during incremental exercise in old and young humans. *Eur. J. Appl. Physiol*. **78**, 460–465.

Coggan, A. R., Spina, R. J., Rogers, M. A., King, D. S., Brown, M., Nemeth, P. M., and Holloszy, J. O. (1990). Histochemical and enzymatic characteristics of skeletal muscle in master athletes. *J. Appl. Physiol*. **68**, 1896-1901.

Coggan, A. R., Spina, R. J., King, D. S., Rogers, M. A., Brown, M., Nemeth, P. M, and Holloszy, J. O. (1992). Histochemical and enzymatic comparison of the gastrocnemius muscle of young and elderly men and women. *J. Gerontol*. **47**, B71–B76.

Coggan, A. R., Abduljalil, A. M., Swanson, S. C., Earle, M. S., Farris, J. W., Mendenhall, L. A., and Robitaille, P. M. (1993). Muscle metabolism during exercise in young and older untrained and endurance-trained men. *J. Appl. Physiol*. **75**, 2125–2133.

Conley, K. (1994). Cellular energetics during exercise. *Adv. Vet. Sci. Comp. Med*. **38A**, 1–39.

Conley, K., Cress, M., Jubrias, S., Esselman, P., and Odderson, I. (1995). From muscle properties to human performance using magnetic resonance. *J. Gerontol*. **50**, 35–40.

Conley, K. E., Blei, M. L., Richards, T. L., Kushmerick, M. J., and Jubrias, S. A. (1997). Activation of glycolysis in human muscle *in vivo*. *Am. J. Physiol*. **273**, C306–C315.

Conley, K. E., Kushmerick, M. J., and Jubrias, S. A. (1998). Glycolysis is independent of oxygenation state in stimulated human skeletal muscle in vivo. *J. Physiol. (London)* **511**, 935–945.

Conley, K. E., Esselman, P. E., Jubrias, S. A., Cress, M. E., Inglin, B., Mogadam, C., and Schoene, R. B. (2000). Ageing, muscle properties and maximal O_2 uptake rate in humans. *J. Physiol. (London)* **526**, 211–217.

Conley, K. E., Jubrias, S. A., and Esselman, P. E. (2000). Oxidative capacity and ageing in human muscle. *J. Physiol. (London)* **526**, 203–210.

Connett, R. J., and Sahlin, K. (1996). Control of glycolysis and glycogen metabolism. "Exercise: Regulation and Integration of Multiple Systems" (L. B. Rowell and J. T. Shepherd, eds.), pp. 870–911. Oxford University Press, New York.

Coyle, E. F. (1995). Integration of the physiological factors determining endurance performance ability. *Exercise Sport Sci. Rev*. **23**, 25–63.

Davies, C. T. M., Thomas, D. O., and White, M. J. (1987). Mechanical properties of young and elderly human muscle. *Acta Med Scand., Suppl*. **711**, 219–226.

Dedrick, M. E., and Clarkson, P. M. (1990). The effects of eccentric exercise on motor performance in young and older women. *Eur. J. Appl. Physiol*. **60**, 183–186.

Degens, H., Hoofd, L., and Binkhorst, R. A. (1995). Specific force of the rat plantaris muscle changes with age, but not with overload. *Mech. Ageing Dev*. **78**, 215–219.

Delbono, O., O'Rourke, K. S., and Ettinger, W. H. (1995). Excitation-calcium release uncoupling in aged single human skeletal muscle fibers. *J. Membr. Biol*. **148**, 211–222.

Delbono, O., Renganathan, M., and Messi, M. L. (1997). Excitation-$Ca2^+$ release-contraction coupling in single aged human skeletal muscle fiber. *Muscle Nerve* (Suppl. 5), S88–S92.

De Serres, S. J., and Enoka, R. M. (1998). Older adults can maximally activate the biceps brachii muscle by voluntary command. *J. Appl. Physiol*. **84**, 284–291.

Doherty, T. J., and Brown, W. F. (1997). Age-related changes in the twitch contractile properties of human thenar motor units. *J. Appl. Physiol*. **82**, 93–101.

Enoka, R. M. (1997). Neural adaptations with chronic physical activity. *J. Biomech*. **30**, 447–455.

Ferrington, D. A., Krainev, A. G., and Bigelow, D. J. (1998). Altered turnover of calcium regulatory proteins of the sarcoplasmic reticulum in aged skeletal muscle. *J. Biol. Chem*. **273**, 5885–5891.

Frontera, W. R., Hughes, V. A., Lutz, K. J., and Evans, W. J. (1991). A cross-sectional study of muscle strength and mass in 45- to 78-yr-old men and women. *J. Appl. Physiol*. **71**, 644–650.

Hakkinen, K., Kallinen, M., Izquierdo, M., Jokelainen, K., Lassila, H., Malkia, E., Kraemer, W. J., Newton, R. U., and Alen, M. (1998). Changes in agonist-antagonist EMG, muscle CSA, and force during

strength training in middle-aged and older people. *J. Appl. Physiol.* **84**, 1341–1349.

Harridge, S., Magnusson, G., and Saltin, B. (1997). Life-long endurance-trained elderly men have high aerobic power, but have similar muscle strength to non-active elderly men. *Aging*. **9**, 80–87.

Harridge, S. D., White, M. J., Carrington, C. A., Goodman, M., and Cummins, P. (1995). Electrically evoked torque-velocity characteristics and isomyosin composition of the triceps surae in young and elderly men. *Acta Physiol. Scand.* **154**, 469–477.

Holloszy, J. O., and Kohrt, W. M. (1996). Exercise. ''Handbook of Physiology: Aging,'' pp. 633–666. Oxford University Press, New York.

Hoppeler, H., Lüthi, P., Claassen, H., Weibel, E., and Howald, H. (1973). The ultrastructure of the normal human skeletal muscle. *Pflügers Arch.* **344**, 217–232.

Hoppeler, H., Howald, H., Conley, K., Lindstedt, S. L., Claassen, H., Vock, P., and Weibel, E. R. (1985). Endurance training in humans: Aerobic capacity and structure of skeletal muscle. *J. Appl. Physiol.* **59**, 320–327.

Houmard, J. A., Weidner, M. L., Gavigan, K. E., Tyndall, G. L., Hickey, M. S., and Alshami, A. (1998). Fiber type and citrate synthase activity in the human gastrocnemius and vastus lateralis with aging. *J. Appl. Physiol.* **85**, 1337–1341.

Inbar, O., Kaiser, P., and Tesch, P. (1981). Relationships between leg muscle fiber type distribution and leg exercise performance. *Int. J. Sports Med.* **2**, 154–159.

Ivy, J. L., Withers, R. T., Brose, G., Maxwell, B. D., and Costill, D. L. (1981). Isokinetic contractile properties of the quadriceps with relation to fiber type. *Eur. J. Appl. Physiol.* **47**, 247–255.

Jubrias, S. A., Odderson, I. R., Esselman, P. E., and Conley, K. E. (1997). Decline in isokinetic force with age: Muscle area and specific force. *Pflügers Arch.* **434**, 246–253.

Kamen, G., Sison, S. V., Du, C. C. D., and Patten, C. (1995). Motor unit discharge behavior in older adults during maximal-effort contractions. *J. Appl. Physiol.* **79**, 1908–1913.

Kent-Braun, J. A., and LeBlanc, R. (1996). Quantitation of central activation failure during maximal voluntary contractions in humans. *Muscle Nerve* **19**, 861–869.

Klitgaard, H., Mantoni, M., Schiaffino, S., Ausoni, S., Gorza, L., Laurent-Winter, C., Schnohr, P., and Saltin, B. (1990). Function, morphology and protein expression of ageing skeletal muscle: A cross-sectional study of elderly men with different training backgrounds. *Acta Physiol. Scand.* **140**, 41–54.

Kovanen, V. (1989). Effects of ageing and physical training on rat skeletal muscle. An experimental study on the properties of collagen, laminin, and fibre types in muscles serving different functions. *Acta Physiol. Scand.* **577** (Suppl.), 1–56.

Larsson, L., and Ansved, T. (1995). Effects of aging on the motor unit. *Prog. Neurobiol.* **45**, 397–458

Larsson, L., Grimby, G., and Karlsson, J. (1979). Muscle strength and speed of movement in relation to age and muscle morphology. *J. Appl. Physiol.* **46**, 451–456.

Larsson, L., Li, X., Berg, H., and Frontera, W. R. (1996). Effects of removal of weight-bearing function on contractility and myosin isoform composition in single human skeletal muscle cells. *Pflügers Arch.* **432**, 320–328.

Larsson, L., Li, X., and Frontera, W. R. (1997). Effects of aging on shortening velocity and myosin isoform composition in single human skeletal muscle cells. *Am. J. Physiol.* **272**, C638–C649.

Lexell, J. (1995). Human aging, muscle mass, and fiber type composition. *J. Gerontol.* **50**, 11–16.

Lexell, J., and Downham, D. (1992). What is the effect of ageing on type 2 muscle fibers? *J. Neurol. Sci.* **107**, 250–251.

Lexell, J., Taylor, C. C., and S. M. (1988). What is the cause of aging atrophy? Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year-old men. *J. Neurol. Sci.* **84**, 275–294.

Li, X., and Larsson, L. (1996). Maximum shortening velocity and myosin isoforms in single muscle fibers from young and old rats. *Am. J. Physiol.* **270**, C352–C360.

Manfredi, T. G., Fielding, R. A., O'Reilly, K. P., Meredith, C. N., Lee, H. Y., and Evans, W. J. (1991). Plasma creatine kinase activity and exercise-induced muscle damage in older men. *Med. Sci. Sports Exercise* **23**, 1028–1034.

Marsh, G. D., Paterson, D. H., Potwarka, J. J., and Thompson, R. T. (1993). Transient changes in muscle high-energy phosphates during moderate exercise. *J. Appl. Physiol.* **75**, 648–656.

Masse-Biron, J., Mercier, J., Collomp, K., Hardy, J. M., and Prefaut, C. (1992). Age and training effects on the lactate kinetics of master athletes during maximal exercise. *Eur. J. Appl. Physiol.* **65**, 311–315.

McBride, T. A., Gorin, F. A., and Carlsen, R. C. (1995). Prolonged recovery and reduced adaptation in aged rat muscle following eccentric exercise. *Mech. Ageing Dev.* **83**, 185–200.

McCully, K. K., Fielding, R. A., Evans, W. J., Leigh, J. S., and Posner, J. D. (1993). Relationship between in vivo and in vitro measurements of metabolism in young and old human calf muscles. *J. Appl. Physiol.* **75**, 813–819.

McDonagh, M. J. N., White, M. J., and Davies, C. T. M. (1984). Different effects of aging on the mechanical properties of human arm and leg muscles. *Gerontology* **30**, 49–54.

Narici, M. V., Bordini, M., and Cerretelli, P. (1991). Effect of aging on human adductor pollicis muscle function. *J. Appl. Physiol.* **71**, 1277–1281.

Osternig, L. R., Hamill, J., Lander, J. E., and Robertson, R. (1986). Co-activation of sprinter and distance runner muscles in isokinetic exercise. *Med. Sci. Sports Exercise* **18**, 431–435.

Overend, T. J., Cunningham, D. A., Kramer, J. F., Lefcoe, M. S., and Paterson, D. H. (1992a). Knee extensor and knee flexor strength: Cross-sectional area ratios in young and elderly men. *J. Gerontol.* **47**, M204–M210.

Overend, T. J., Cunningham, D. A., Paterson, D. H., and Lefcoe, M. S. (1992b). Thigh composition in young and elderly men determined by computed tomography. *Clin. Physiol.* **12**, 629–640.

Ozawa, T. (1997). Genetic and functional changes in mitochondria associated with aging. *Physiol. Rev.* **77**, 425–464.

Papa, S. (1996). Mitochondrial oxidative phosphorylation changes in the life span. Molecular aspects and physiopathological implications. *Biochim. Biophys. Acta* **1276**, 87–105.

Pendergast, D. R., Fisher, N. M., and Calkins, E. (1993). Cardiovascular, neuromuscular, and metabolic alterations with age leading to frailty. *J. Gerontol.* **48**, M61–M67.

Phillips, S. K., Bruce, S. A., Newton, D., and Woledge, R. C. (1992). The weakness of old age is not due to failure of muscle activation. *J. Gerontol.* **47**, M45–M49.

Poole, D., Gaesser, G., Hogan, M., Knight, D., and Wagner, P. (1992). Pulmonary and leg VO2 during submaximal exercise: Implications for muscular efficiency. *J. Appl. Physiol.* **72**, 805–810.

Proctor, D. N., and Joyner, M. J. (1997). Skeletal muscle mass and the reduction of VO_2max in trained older subjects. *J. Appl. Physiol.* **82**, 1411–1415.

Proctor, D. N., Sinning, W. E., Walro, J. M., Sieck, G. C., and Lemon, P. W. (1995). Oxidative capacity of human muscle fiber types: Effects of age and training status. *J. Appl. Physiol.* **78**, 2033–2038.

Proctor, D. N., Shen, P. H., Dietz, N. M., Eickhoff, T. J., Lawler, L. A., Ebersold, E. J., Loeffler, D. L., and Joyner, M. J. (1998). Reduced leg blood flow during dynamic exercise in older endurance-trained men. *J. Appl. Physiol.* **85**, 68–75.

Rall, J. (1985). Energetic aspects of skeletal muscle contraction: Implications of fiber types. *Exercise Sport Sci Rev.* **13**, 33–74.

Richardson, R. S., Grassi, B., Gavin, T. P., Haseler, L. J., Tagore, K., Roca, J., and Wagner, P. D. (1999). Evidence of O_2 supply-dependent VO_2 max in the exercise-trained human quadriceps. *J. Appl. Physiol.* **86**, 1048–1053.

Ruff, R. L. (1989). Calcium sensitivity of fast- and slow-twitch human muscle fibers. *Muscle Nerve* **12**, 32–37.

Sale, D. G. (1988). Neural adaptation to resistance training. *Med. Sci. Sports Exercise* **20**, S135–S145.

Sonn, U. (1996). Longitudinal studies of dependence in daily life activities among elderly persons. *Scand. J. Rehabil. Med.* **34** (Suppl.), 1–35.

Stalberg, E., Borges, O., Ericsson, M., Essen-Gustavsson, B., Fawcett, P. R. W., Nordesjo, L. O., Nordgren, B., and Uhlin, R. (1989). The quadriceps femoris muscle in 20-70 year-old subjects: Relationship between knee extension torque, electrophysiological parameters, and muscle fiber characteristics. *Muscle Nerve* **12**, 382–389.

Syström, D. M., Kanarek, D. J., Kohler, S. J., and Kazemi, H. (1990). ^{31}P nuclear magnetic resonance spectroscopy study of the anaerobic threshold in humans. *J. Appl. Physiol.* **68**, 2060–2066.

Thompson, L. V., and Brown, M. (1999). Age-related changes in contractile properties of single skeletal fibers from the soleus muscle. *J. Appl. Physiol.* **86**, 881–886.

Vandervoort, A. A., and McComas, A. J. (1986). Contractile changes in opposing muscles of the human ankle joint with aging. *J. Appl. Physiol.* **61**, 361–367.

van Schaik, C. S., Hicks, A. L., and McCartney, N. (1994). An evaluation of the length-tension relationship in elderly human ankle dorsiflexors. *J. Gerontol.* **49**, B121–B127.

Welle, S., Thornton, C., Jozefowicz, R., and Statt, M. (1993). Myofibrillar protein synthesis in young and old men. *Am. J. Physiol.* **264**, E693–E698.

Welle, S., Totterman, S., and Thornton, C. (1996). Effect of age on muscle hypertrophy induced by resistance training. *J Gerontol.* **51**, M270–M275.

Young, A. (1984). The relative isometric strength of type I and type II muscle fibres in the human quadriceps. *Clin. Physiol.* **4**, 23–32.

Young, A., Stokes, M., and Crowe, M. (1985). The size and strength of the quadriceps muscles of old and young men. *Clin. Physiol.* **5**, 145–154.

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Parkinson's Disease: Symptoms and Age Dependency

I. Epidemiology of Parkinson's Disease

Parkinson's disease is one of the most frequent neurodegenerative diseases, which mainly affects the elderly. To estimate incidence and prevalence of Parkinson's disease, however, some problems need to be mentioned. There exists no antemortem diagnostic test for Parkinson's disease and the most reliable diagnostic method is expert neurologic examination at regular time intervals. In autopsy studies, the diagnosis of Parkinson's disease before death has been found to be incorrect in about 24% of cases (Hughes *et al*., 1993). Essential tremor may account for 10–40% of the false-positive diagnoses of Parkinson's disease. In contrast, Parkinson's disease may be misdiagnosed as depression or, in the very elderly, ''normal'' aging. Other neurodegenerative disorders, like progressive nuclear palsy or multiple system atrophy, may not be distinguished easily from Parkinson's disease early in the course of the disease. Many ''atypical'' parkinsonian syndromes were recognized only in the past several decades and probably have been classified as Parkinson's disease in early reports.

A. Incidence and Prevalence of Parkinson's Disease

Several studies have been performed to estimate the frequency of Parkinson's disease. The overall incidence is estimated to be 20/100,000 per year, and raises to about 1% of persons over 50 years of age (Gilmore, 1984; Schapira, 1999), and even higher in older populations (Conley and Kirchner, 1999). Prevalence of Parkinson's disease varies from 10 to 405 per 100,000 population. This variation may be due to differences in case-finding procedures, in diagnostic criteria, in accessibility of medical services, and in the age distribution of populations. Most frequently, prevalence is about 100–187 per 100,000 (Mayeux *et al*., 1992). As for incidence, prevalence rises almost exponentially after age 50. By the eighth decade, prevalence in Europe and North America is estimated to be between 1000 and 3000 per 100,000 persons (Tanner and Ben Shlomo, 1999). This is confirmed by the Rotterdam Study: prevalence figures were 0.3% for those ages 55 to 64 years, 1.0% for those ages 65 to 74 years, 3.1% for those ages 75 to 84 years, and 4.3% for those ages 85 to 94 years. Among women ages 95 to 99 years, prevalence was 5.0% (de Rijk *et al*., 1995). In some studies an apparent decrease in late life is seen. This is probably due to ascertainment and diagnosis difficulties in this population, rather than an actual decline in disease frequency. In fact the only unequivocal risk factor for Parkinson's disease is increasing age.

B. Mortality

In the Hoehn and Yahr series, mortality ratio in patients with primary parkinsonism was 2.9 times that expected in the age-matched population (Hoehn and Yahr, 1967). In a recent study of parkinsonian patients, the overall risk for death, adjusted for age and sex, was 2.0 times that of persons without parkinsonism (Bennett *et al*., 1996). Since the introduction of more effective antiparkinsonian medication, especially levodopa, mortality has decreased in younger parkinsonian patients. However, in older parkinsonian patients an increase during the past few decades in mortality is found. In the United States between 1962 and 1984, mortality decreased for persons younger than age 70, but increased for persons of 75 years and older (Kurtzke and Goldberg, 1988). This is confirmed by Morens *et al*. (1996), who found that after age 60, Parkinson's disease associated mortality rates appeared to increase logarithmically. This increase in mortality rate was not attributable to age alone. Increased age-related Parkinson's disease mortality was associated with both absolute age and duration of illness longer than 10 years. Between 70 and 89 years of age, parkinsonian patients had a two- to threefold increase in the risk of dying, corresponding to a mortality ratio of 2.5 (Morens *et al*., 1996). In a recent study of Louis, Marder, and Côté risk of mortality was higher in parkinsonian patients (rate ratio = 2.7) after adjusting for baseline age, years of education, sex, ethnicity, and smoking status. It was even higher when Parkinson's disease was combined with dementia (rate

ratio = 4.9). In fact, dementia is a significant predictor of death in Parkinson's disease (Marder *et al*., 1991). A high base-line score of extrapyramidal signs was most associated with increased risk of mortality among the patients with Parkinson's disease. After subanalysis of the different extrapyramidal signs, severe bradykinesia was the motor manifestation that most highly correlated with increased mortality (Louis *et al*., 1997). The Sydney multicenter study found increased mortality risk among parkinsonian men, whereas this was not significantly different for women, compared to the general Australian population. Predictors of mortality, according to this study, are age at onset and prestudy progression rate. Several studies have shown that the effects of levodopa on mortality are apparent in the early years of the disease. In contrast, despite levodopa therapy, mortality is rising in a later stage of the disease (Curtis *et al*., 1980; Diamond *et al*., 1987). Data from the DATATOP cohort suggests that carefully selected patients with early Parkinson's disease without comorbidity have normal life expectancy when adequately treated and frequently seen by consulting physicians (Anonymous, 1998). This is confirmed by Tanner and Ben-Shlomo (1999). For persons with Parkinson's disease diagnosed before the age of 60 they found a relative survival similar to that of the general population, in contrast to people with an older age at diagnosis, who showed a lower relative survival (Tanner and Ben Shlomo, 1999).

Pneumonia is the most common cause of death, probably due to immobility and increased risk of aspiration (Anonymous, 1998). Death from cerebrovascular disease is increased as well (Gorell *et al*., 1994). Some studies have suggested a reduced risk of death from cancer in parkinsonian patients (Jansson and Jankovic, 1985; Gorell *et al*., 1994), but other studies did not confirm this (Wermuth *et al*., 1995).

C. Regional and Racial Variation

Estimates of prevalence vary widely depending on geographical location. A northwest to southeast gradient is suggested (Kurtzke and Goldberg, 1988) and Parkinson's disease prevalence appears to be highest in Europe and North America, whereas rates in Japan, China, and Africa are markedly lower. In the United States Parkinson's disease prevalence is much lower among African-Americans. Already in 1972, Kessler (1972a,b) found a higher frequency of Parkinson's disease for Caucasians then for African-Americans. In a survey of Parkinson's disease in New York, age-adjusted prevalence rates were lower for African-Americans than for Caucasians and Hispanics. Surprisingly, however, incidence rates were highest among African-American men, but these incidence rates were otherwise comparable across sex and ethnic groups. By ethnic group, the cumulative incidence was higher for African-Americans than for Caucasians and Hispanics, but more deaths occurred among incident African-American patients. These findings could result from a delay in diagnosis due to limited access to appropriate health services among these people (Mayeux *et al*., 1995). Some studies, however, show similar rates for African-American, Asian-American, and European-American subjects (Morens *et al*., 1996). In a study among a cohort of American men of Japanese or Okinawan ancestry, epidemiological data are in general in accord with those from Europe and the United States. Incidence data are 5- to 10-fold higher at each age stratum than age-specific incidence figures from China. These findings most likely cannot be explained by methodological differences between Chinese and Western studies alone. However, this possibility cannot be ruled out, as the Chinese data have not been verified yet by other studies. Similar findings have been observed in a study in Mississippi, where African-American men and women have Parkinson's disease prevalence rates more comparable to Caucasian men and women than African men and women in Nigeria (Schoenberg *et al*., 1985, 1988; Osuntokun *et al*., 1987). These data suggest that risk of developing Parkinson's disease is more a function of environmental factors than racial ones. So different distributions of Parkinson's disease causing factors across populations may contribute to geographic differences in epidemiological findings. These factors could be differences in exposure to causative and protective influences, but also genetic differences in susceptibility to disease. It could be that an environmental agent might only act in genetically susceptible people.

D. Gender Differences

Males tend to have a modestly increased age-adjusted Parkinson's disease prevalence (Diamond *et al*., 1990). Male-to-female ratio range from 0.86 (in Japan) to 3.7 (Chinese studies). In a study in New York, age-adjusted prevalence was lower for women compared with men across all ethnic groups. However, in another study, age-adjusted incidence did not differ between men and women in all ethnic groups (Mayeux *et al*., 1995). This is confirmed by the Rotterdam Study in which no significant gender differences in prevalence were found (de Rijk *et al*., 1995). Gender-specific differences show more variability worldwide than association with increasing age.

II. SYMPTOMS

A. Motor Symptoms

The classic triad of symptoms of Parkinson's disease consists of tremor, rigidity, and hypokinesia. Postural impairment has been called the fourth major symptom of Parkinson's disease. The disease is a slowly progressive disorder and signs and symptoms develop usually over several years. In the early stages of the disease the signs and symptoms may be vague and nonspecific in such a way that a reliable diagnosis cannot yet be made.

The typical parkinsonian tremor is a 3 to 6 Hz distal resting tremor. It consists of alternate contractions of agonist and antagonist muscles, including flexors, extensors, pronators and supinators of the wrists and arms during rest. This may result in the ''pill rolling'' movement of the hand. Often the typical parkinsonian resting tremor starts on one side of the body. In most patients the signs will develop in due course on both sides, but asymmetry will usually persist throughout the disease. The legs or lower jaw may also be involved. The tremor tends to disappear with action. Resting tremor is found in 79–90% of patients with Parkinson's disease in clinical studies and in 76–100% in autopsy-proven studies. Some patients have little or no resting tremor but a predominate action or postural tremor. Resting tremor can be found in other diseases as

well, like multiple system atrophy or progressive nuclear palsy. Tremor at rest may also be induced by neuroleptic agents.

Rigidity can be present in all four limbs and in the trunk, but mainly affects the arms and is often of the cogwheel type. The increase in tone is fairly equal in flexors and extensors, but slightly more in flexors. It can be diagnosed in 89–99% of parkinsonian patients.

Bradykinesia means slowness of movements, whereas hypokinesia stands for poverty of movement. Hypokinetic features include facial hypomimia, reduced eye blinking, hypophonic speech, and micrographia. There is difficulty in initiating movements, resulting in start hesitation. Rapid repetitive movements are impaired. Bradykinesia is present in 77 to 98% of patients with Parkinson's disease, but it is not unique to Parkinson's disease. It can also occur as a result of other extrapyramidal disorders, such as progressive nuclear palsy, multiple system atrophy, corticobasal degeneration, and normal aging.

Symptoms can be gravitated by contralateral activation or concentration on mental or physical tasks. Each one of these features can be present for a long time, before others develop. Symptoms usually begin unilaterally or asymmetrically. Later they are bilateral or generalized.

Gait is impaired in patients with Parkinson's disease as well. The patients walk slowly with small shuffling steps. Parkinsonian patients move in a rigid manner and turn en bloc. Their posture is stooped, because of flexion of the shoulders, neck, and trunk. In Parkinson's disease the center of gravity is shifted forward. Walking can be hampered by stutter steps, resulting in start and turn hesitation, and sudden ''freezing.'' This phenomenon refers to the patient's feet stuck to the ground while walking, rendering the patient unable to move with the lower body. It especially happens on turns and in elevators or doorways. Freezing and related phenomena are called motor blocks. It occurs in 32% of parkinsonian patients (Giladi *et al*., 1992). Retropulsion refers to the phenomenon that the standing patient, if pushed backward, is able to regain his balance slowly by small and slow steps or even fails to do so and falls. When walking, patients may have problems stopping and legs are preceded by the flexed trunk, resulting in frequent little short steps or propulsive gait. This pattern is characteristic of advanced Parkinson's disease. In the beginning of the disease symptoms are unilateral, affecting only one side. Patients appear to drag a leg when walking and arm swing is decreased at the affected side. In a later stage, when the opposite side is affected as well, steps are short and the feet barely clear the floor. Usually symptoms stay asymmetrical, in contrast to normal aging. At later stages parkinsonian patients may experience problems of autonomic dysfunction such as constipation, incontinence, hypotension, and impotence. If this occurs in an early stage of Parkinson's disease, the diagnosis should be questioned (Gelb *et al*., 1999).

Not only motor symptoms in PD occur. An olfactory disorder is sometimes also present and manifested as an increase of the olfactory detection threshold (Potagas *et al*., 1998), which is probably due to the presence of Lewy bodies in the olfactory bulb and neuronal loss in the anterior olfactory nucleus (Daniel and Hawkes, 1992; Pearce *et al*., 1995). Other features include increased saliva production and increased sweating.

None of the three major symptoms has enough sensitivity or specificity to diagnose Parkinson's disease. For this reason a scheme has been developed for diagnostic classification: The UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria (see list below; Daniel and Lees, 1993).

Step 1: Diagnosis of parkinsonian syndrome:
- Bradykinesia (slowness of initiation of voluntary movement with progressive reduction in speed and amplitude of repetitive actions)
- At least one of the following:
  - 4- to 6-Hz rest tremor
  - Rigidity
  - Postural instability not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction

Step 2: Exclusion criteria for Parkinson's disease:
- History of repeated strokes with stepwise progression of parkinsonian features
- History of repeated head injury
- History of definite encephalitis
- Oculogyric crises
- Neuroleptic treatment at onset of symptoms
- More than one affected relative
- Sustained remission
- strictly unilateral features after 3 years
- Supranuclear gaze palsy
- Cerebellar signs
- Early severe autonomic involvement
- Early severe dementia with disturbances of memory, language, and praxis
- Babinski sign
- Presence of cerebral tumor or communicating hydrocephalus on CT scan
- Negative response to large doses of levodopa (if malabsorption is excluded)
- MPTP exposure

Step 3: Supportive prospective positive criteria for Parkinson's disease (three or more required for diagnosis of definite Parkinson's disease):
- Unilateral onset
- Rest tremor present
- Progressive disorder
- Persistent asymmetry affecting side of onset most
- Excellent response (70–100%) to levodopa
- Severe levodopa-induced chorea
- Levodopa response for 5 years or more
- Clinical course of 10 years or more

B. Nonmotor Symptoms

Several other nonmotor clinical features occur in Parkinson's disease, such as dementia, depression, and psychotic features. In 20 to 40% of patients with Parkinson's disease, cognitive impairment develops and the risk of dementia in nondemented Parkinson's disease patients is almost twice that of age-matched nondemented elderly controls. Prevalence of dementia in Parkinson's disease is estimated to be 10.9% (Mayeux *et al*., 1988). Parkinson's disease patients with dementia have a shorter life expectancy. Dementia in Parkinson's disease is characterized by a severe dysexecutive syndrome without instrumental disorders like aphasia, apraxia, or agnosia. In several studies, the influence of age at onset on the presentation and course of Parkinson's disease has

been demonstrated. Young onset patients have little cognitive impairment even after a disease duration of over 20 years (Quinn *et al*., 1987). Dementia mainly develops in patients with late onset, after 70 years. In this cohort of patients, the prevalence of dementia is more than twice that of younger patients (Mayeux *et al*., 1988). Usually it develops after several years of disease progression. This in contrast to diffuse Lewy body disease, where cognitive deficits occur very early (less than 2 years) or even precede motor symptoms (Dubois and Pillon, 1999). Other risk factors for developing dementia in Parkinson's disease are lack of education and severe motor deficits (UPDRS motor scores above 24) (Marder *et al*., 1990). Cognitive deficits, however, are usually less prominent than motor symptoms.

Depression is estimated to occur in 30–60% of patients with Parkinson's disease at some point during the disease. It has been suggested that there is a natural tendency for chronic, disabling diseases to induce depression. However, it appears that the prevalence of depression in patients with Parkinson's disease is higher than in other chronic disorders. In addition, the depression is unrelated to the severity of motor symptoms and depression can continue despite improvement in motor symptoms after L-dopa therapy. It is possible that depression precedes the motor symptoms of Parkinson's disease. There are some similarities between clinical and biochemical changes in Parkinson's disease and depression. Clinical similarities include akinesia and psychomotor retardation, while biochemical similarities include dysfunction of the dopaminergic, noradrenergic and serotonergic systems. Reduced serotonergic function is associated with psychomotor retardation, reduced noradrenergic function with bradyphrenia and reduced dopaminergic function with extrapyramidal symptoms, cognitive slowing, and more severe symptoms of depression (Leonard, 1999).

Psychotic symptoms may either be a manifestation of the disease itself, or it may be the result of therapy with dopaminergic agents (Douglas *et al*., 1999). Psychosis may also occur as a reaction to the disease and functional impairment (''reactive psychosis''), although this is probably a rare condition. About 30% of parkinsonian patients, treated with levodopa, have experienced psychotic symptoms and the lifetime prevalence may approach 50%. Visual hallucinations are most common and the images are usually full formed human or animal figures. Usually insight is preserved (Aarsland and Larsen, 1999).

III. Pathologic Findings

The most prominent lesion in Parkinson's disease is the degeneration of neuromelanin-containing neurons in the pars compacta of the substantia nigra, which at postmortem inspection is visibly pale. Also selected monoaminergic brain-stem nuclei (catecholaminergic and serotoninergic), the cholinergic nucleus basalis of Meynert, hypothalamic neurons, small cortical neurons (particularly in the cingulate gyrus and entorhinal cortex), olfactory bulb, sympathetic ganglia, and parasympathetic neurons may be involved in the progressive degenerative process of Parkinson's disease.

Not all dopaminergic neurons are equally susceptible. Within the substantia nigra pars compacta, neuronal loss appears to be most severe in the ventrolateral part, followed by the medial part and the dorsal part (Fearnley and Lees, 1991; Damier *et al*., 1999). It results in a regional loss of striatal dopamine (Lang and Lozano, 1998). The nigrostriatal dopaminergic neurons which project to the putamen are more affected than those which project to the caudate nucleus and nucleus accumbens and whose dysfunction is believed to be responsible for akinesia and rigidity. This pattern of cell loss seems to be unique to Parkinson's disease and is different of the pattern seen in normal aging. Neuronal loss of the medial nigral cells, with enhanced involvement of projections to the caudate nucleus, could result in more cognitive symptoms. Another possible clinicopathological correlation may be based on degenerative changes of the olfactory bulb, causing anosmia. Autonomic dysfunction may be the result of lesions in the sympathetic and parasympathetic ganglia or degeneration in the intermediolateral columns of the spinal cord (Rajput and Rozdilsky, 1976). Some believe that dementia in Parkinson's disease may be the result of cell loss in the nucleus basalis of Meynert (Rogers *et al*., 1985).

The surviving, but dying, catecholaminergic neurons may contain Lewy bodies, an important pathological feature. Lewy bodies are spherical, eosinophilic cytoplasmic inclusions with a dense core and peripheral halo, found in pigmented cells. In Parkinson's disease the most important anatomical sites where these bodies are located are the substantia nigra and locus coeruleus. They are not specific to Parkinson's disease. They are also present in other neurodegenerative disorders, in particular in Alzheimer's disease (AD). AD, however, is associated with cortical Lewy bodies, particularly in frontal, temporal, anterior cingulate, and insular regions, whereas Parkinson's disease is associated with subcortical Lewy bodies, mainly in the substantia nigra. Lewy bodies are also seen as an incidental finding in about 10% of people older than 60 years. Gibb and Lees discovered that the age-specific prevalence of Lewy bodies in the brains of persons without clinical Parkinson's disease increased from 3.8 to 12.8% between the sixth and ninth decade of life. It has been suggested that the presence of incidental Lewy bodies actually constitutes a presymptomatic stage of Parkinson's disease (Gibb and Lees, 1988a). McKeith, (1999) however, suggests a relationship between this ''incidental'' pathology and dementia with Lewy bodies.

The mechanisms of cell death in Parkinson's disease are still unknown. Several factors have been mentioned to play a role in neuronal degeneration in Parkinson's disease, such as mitochondrial dysfunction, oxidative stress and excitotoxity, and free radical production. It is believed that the neuronal death in the pars compacta of the substantia nigra in apoptotic (Burke, 1998; Burke and Kholodilov, 1998), but this is not universally accepted and necrosis has been suggested as well.

A. Number of Melanized Neurons in Substantia Nigra Pars Compacta

As described above, incidence and prevalence is highest among Caucasian people. The prevalence of Parkinson's disease probably is lowest in India, according to the results of several

studies. Prevalence ranges from 14 (Razdan *et al*., 1994) to 27 (Gourie-Devi *et al*., 1996) per 100,000 persons, whereas the prevalence in Western countries usually is above 100 per 100,000. The question arises which factors could play a role in the pathogenesis of Parkinson's disease. The pathological diagnosis of Parkinson's disease is based on the demonstration of loss melanized dopaminergic neurons in the pars compacta of the substantia nigra. In studies performed on Western populations, the amount of melanized neurons decreases with advancing age and losses of 30% (McGeer *et al*., 1977) and 48% (Fearnley and Lees, 1991) have been reported. These findings supported the role of age in the pathogenesis of Parkinson's disease. However, Muthane *et al*., (1998) found in normal Indian brains, in comparison with age-matched brain from Western populations, a significantly lower number of melanized nigral neurons, with differences up to 40%. Moreover, with advancing age, there was no significant loss of melanized neurons in the pars compacta of the substantia nigra. This finding is supported by a few studies of Caucasian people, which found no increased loss of melanized neurons with advancing age (Pakkenberg *et al*., 1991). However, in India, as elsewhere in the world, the prevalence of Parkinson's disease raises with increasing age. So probably age by itself may not be the factor responsible for the loss of nigral neurons in Parkinson's disease. Perhaps protective factors responsible for slowing or stopping Parkinson's disease are less sufficient with advancing age, thereby resulting in an increase in the prevalence among elderly individuals (Muthane *et al*., 1998).

IV. Other Movement Disorders in Elderly Patients

Aging is the only unequivocal risk factor for Parkinson's disease (Tanner and Goldman, 1996). It is also a major risk factor for several other movement disorders and neurodegenerative diseases. There are several causes of movement or gait disorders in the elderly, of which some can be easily mistaken for Parkinson's disease. The many characteristics and patterns of gait disorders may appear difficult to evaluate and require special expertise, but simple observation of the patients and their gait yield valuable information. The patient history may reveal stepwise progression, suggesting vascular disease. Pain with walking usually excludes a neurological cause of the gait disorder. Magnetic resonance imaging (MRI) may be used for screening for hydrocephalus or multiple infarcts. Positron emission tomography (PET) scans of the brain may help for diagnosing Parkinson's disease, multiple system atrophy and progressive nuclear palsy. Gait disorders in the elderly, irrespective their underlying pathology, contribute to the risk of falling and fractures (Tinetti *et al*., 1986). The higher risk of falling limits elders in their mobility and independence, also because of the fear of falling itself. Imms and Edholm surveyed in 1981 a group of older people (mean age, 78 years) and found that half of them reduced their activity because of their concerns about falling.

Many signs of gait disorders, slowing down and stiffening, are accepted as being part of aging. However, most of these signs may signal an underlying disease. Critchley (1931) warned that "an abnormal gait in the aged is frequently the result of disease outside the nervous system." In many cases gait disorders are of orthopedic (osteoarthrosis, osteomalacia, unsuspected fractures), endocrinological (hypothyroidism), psychological (depressive state, fear of falling), or general (general muscle weakness) origin (Cunha, 1998). Circulatory or respiratory systems may play a role as well in determining gait velocity because of the need to minimize energy expenditure (Elble, 1997). The incidence of subtle extrapyramidal signs on neurological examination of elderly persons with no known neurologic or psychiatric disorder is high. Bennett *et al*. (1996) showed that 35% of people over 65 years old had these subtle changes, while around 3% in similarly aged persons had Parkinson's disease. In a community study in North Carolina, 15% of adults over 60 years of age had some degree of difficulty with ambulation (Newman *et al*., 1960). In Western Europe 20–25% of people ages 80 or older, use mechanical aids for walking (Lundgren-Lindquist *et al*., 1983). A review of the variety of conditions, which can cause gait disorders or may be confused with Parkinson's disease, will be given here.

A. Essential Tremor

One of the most common causes of misdiagnosis of Parkinson's disease is essential tremor also known as senile tremor, which is a misnomer. It is inherited as an autosomal dominant disorder with incomplete penetrance. A familiar incidence is common in about one-third to one-half of cases. In the elderly, however, it is often sporadic (Gilmore, 1984). The incidence of essential tremor increases with climbing age. Considering prevalence, the Rautakorpi's *et al*. study (1982) reported a prevalence of 12.5% in his population. In a study of a population ages 65 and older, Louis *et al*. (1995) found a prevalence of 4%. A higher prevalence of 23% in a population of people older than 70 years has been observed by Elble (1998).

Essential tremor is an action with a frequency of 8 to 12 Hz (the resting tremor in Parkinson's disease is 4 to 6 Hz). It may sometimes be present at rest as well, but generally increases with activity. The amplitude increases with age. According to Marttila and Rinne (1976), essential tremor accounted for 26% of cases of presumed Parkinson's disease. This misdiagnosing can occur because of many reasons. Both conditions frequently occur in the elderly. Parkinsonian patients sometimes suffer from a postural action tremor of the hands, as seen in essential tremor. Patients with essential tremor may have some bradykinesia and rigidity, which could be normal in the elderly. Furthermore, some hallmarks of Parkinson's disease, such as asymmetric manifestation and a resting component of the tremor, can also be found in cases of essential tremor (Tolosa and Balaguer, 1989). There are, however, some striking differences between these two disorders. Essential tremor frequently involves the head in contrast to tremor in Parkinson's disease. Conversely, a resting tremor of the leg or slow vertical jaw tremor is often seen in Parkinson's disease but rarely in essential tremor. Secondary cogwheeling may be present in essential tremor, but no lead pipe rigidity and no akinesia are seen as in Parkinson's disease.

B. Vascular Parkinsonism

Vascular parkinsonism results from multiple small cerebral infarcts, located predominantly in the basal ganglia secondary

to hypertensive cerebrovascular disease. Some patients with vascular parkinsonism present with a progressive gait disorder without a medical history of strokes (Sudarsky, 1997). Chronic hypertension leads to fibrinoid necrosis and occlusion of arterioles supplying the basal ganglia. An akinetic rigid syndrome with urinary incontinence, dysarthria, abulia, and dementia may occur. The extrapyramidal signs may coexist without pyramidal dysfunction, including weakness, hyperreflexia, spasticity, pseudobulbar palsy, emotional lability, and Babinski's signs. These pyramidal signs may be important in differentiating this disorder from Parkinson's disease. The patient is slow and walks wide-based (unlike in Parkinson's disease) with short, shuffling, somewhat irregular footsteps. Postural stability may be impaired. As in Parkinson's disease, freezing and start hesitation may occur.

However, the upper body may show little or no parkinsonian features; thus facial hypomimia may occur. (Another term used for this disorder is lower body parkinsonism.) Another striking difference with Parkinson's disease is usually the absence of tremor and no fatigue or decrement of rapid alternating movements. In vascular parkinsonism symptoms are symmetric and contrast to Parkinson's disease. Parkinsonian patients usually are younger than those with vascular parkinsonism. However, if signs and symptoms are not clear-cut, the differential diagnosis may be difficult.

Computerized tomography shows multiple infarcts but sometimes misses small ones. Magnetic resonance imaging demonstrates infarctions in the deep gray matter structures and ischemic changes in periventricular white matter. According to Sudarsky, vascular parkinsonism accounts for 15–16% of the gait disorders among elderly patients (Sudarsky and Ronthal, 1983; Sudarsky, 1997).

C. Multiple System Atrophy

Multiple system atrophy refers to a sporadic, gradually progressive, idiopathic neurodegenerative process of adult onset characterized by varying proportions of cerebellar dysfunction, autonomic failure, pyramidal signs and parkinsonism, that is poorly responsive to L-dopa therapy. Cell loss and gliosis (without Lewy bodies) are not only present in substantia nigra, but also in multiple other structures, including striatum, olives, pons, cerebellum, intermediolateral cell columns and Onuf's nucleus in the spinal cord. Most commonly, multiple system atrophy begins early in the sixth decade of life, progresses more rapidly than Parkinson's disease, and has a lower life expectancy (median survival = 9.3 years). No case of multiple system atrophy has been reported before the age of 30.

Parkinsonism occurs in 90% of patients with multiple system atrophy and is the dominant motor disorder in 80% of these patients. In contrast to Parkinson's disease, the parkinsonism in multiple system atrophy is usually bilateral. Unlike Parkinson's disease, tremor is often not the classical rest tremor, but a postural or action tremor. Cerebellar dysfunction and pyramidal signs both occur in about half of patients (Quinn, 1989; Wenning *et al*., 1994). Cerebellar dysfunction as the dominant symptom occurs in about 20% of patients (Quinn, 1995). Autonomic failure appears in almost all patients with multiple system atrophy. In Parkinson's disease patients it may occur as well, mostly in a late stage of disease, whereas in multiple system atrophy it usually occurs earlier and more severely (Magalhaes *et al*., 1995). Indeed, in multiple system atrophy autonomic failure may precede the motor symptoms by months or even years. Frequently, the first symptom is impotence in men and incontinence in both men and women (Parkinson's disease usually causes just frequency increase and urgency due to hyperreflexia of the detrusor muscle). Postural hypotension is another common feature in multiple system atrophy, which may appear in Parkinson's disease as well, though less severely. Inspiratory stridor is present in about 30% of patients with multiple system atrophy. When combined with parkinsonism it is highly suggestive of the diagnosis of multiple system atrophy (Quinn, 1995). Other signs of autonomic failure are thermoregulation disturbances, gastro-intestinal problems and phenomena of Raynaud.

The response to levodopa of patients with multiple system atrophy is usually absent or poor. However, in about 30% of patients an initial response has been reported, usually temporary. Levodopa induced dyskinesias are usually absent in multiple system atrophy, so high doses of levodopa can be administered. Differentiating multiple system atrophy from Parkinson's disease may be very difficult, especially in early stages of disease. Some key features suggest nonidiopathic parkinsonism including: poor or no respons to levodopa therapy; cerebellar, pyramidal, or autonomic signs; symmetric start of symptoms; absence of classic resting tremor; early instability or falls; and rapid clinical progression.

Additional diagnostic tests may include external urethral (or anal) sphincter EMG, standard tests of cardiovascular autonomic function, and imaging of the brain. Computed tomography or magnetic resonance imaging sometimes shows cerebellar or brain-stem atrophy. Fluorodeoxyglucose positron emission tomography (PET) scanning may be useful as well.

D. Progressive Supranuclear Palsy

Progressive supranuclear palsy, or Steele-Richardson-Olszewski syndrome, is an idiopathic degenerative disease, not uncommon in the elderly, which mimics Parkinson's disease. It occurs at a rate of 0.3 per 100,000 per year (Golbe, 1993) and its prevalence is 1.46 per 100,000. Pathologic investigation shows cell loss and neurofibrillary tangles, mainly in the brain stem, globus pallidus, subthalamic nucleus, and dentates nucleus. The clinical presentation includes the tetrad of supranuclear gaze paralysis, axial rigidity, dementia, and pseudobulbar palsy. It is associated with bradykinesia, severe postural disorder and frequent falls. Supranuclear gaze paralysis affects vertical more than horizontal gaze. Voluntary downward gaze is slow and usually incomplete, but when the oculocephalic reflex is performed, full downward gaze is obtained. Pseudobulbar palsy is characterized by dysphagia and dysarthria. Dementia is progressive and consists of slowing of cognition, memory deficits, and personality changes suggestive of frontal lobe dysfunction (Litvan *et al*., 1996). Progressive nuclear palsy may be distinguished from Parkinson's disease by the absence of rest tremor, the extended neck posture, rigidity more truncal than in limbs, and abnormal eye movements. Progressive nuclear palsy patients have a stiff, broad-based gait with ataxia. In contrast to Parkinson's disease patients, they do

not turn en bloc, but pivot. During pivoting they also tend to fall backward.

E. Corticobasal Degeneration

Corticobasal degeneration presents with a unique pattern of progressive impairment. It appears in mid to late adult life, usually beginning after age 60. The duration of the illness until death is about 6 to 8 years. Pathologic and histologic evaluation reveal frontoparietal atrophy and neuronal loss, gliosis, and swelling of the cell body with resistance to staining methods (achromasia) (Kompoliti *et al*., 1998). Prototypic findings are combined parkinsonian signs, other movement disorder, and higher cortical dysfunction, with marked asymmetry of involvement. The most common extrapyramidal sign is rigidity, followed by bradykinesia. Sometimes tremor is also present but does not resemble the parkinsonian tremor. Tremor in corticobasal degeneration is more rapid (6–8 Hz), is mainly present during action, and varies in amplitude. Other movement disorders include myoclonus and dystonia. Higher cortical dysfunction includes dyspraxia, involving the limbs and ocular and orofacial muscles, cortical sensory loss, dementia (which usually occurs late in the disease), and aphasia. A striking feature of corticobasal degeneration is the ''alien hand/limb'' phenomenon (Stacy and Jankovic, 1992).

F. Normal-Pressure Hydrocephalus

Normal-pressure hydrocephalus is often idiopathic, but has also been associated with many neurological diseases. The classic triad of symptoms includes frontal dementia, urinary incontinence, and gait disorder with unsteadiness. It is associated with enlargement of the cerebral ventricles on CT or MRI and a cerebrospinal fluid pressure of 180 mm H_2O or less. A dynamic test is necessary to confirm the diagnosis of true (opposed to *ex vacuo*) hydrocephalus (Borgesen and Gjerris, 1982). The removal and 50 ml of cerebrospinal fluid may improve the symptoms of gait disorder (Sudarsky and Simon, 1987). Mental dysfunction improves less than gait after a shunt. The patient walks wide-based with small steps, feet ''glued to the floor,'' marked imbalance, and difficulty initiating walking. Postural instability with frequent falling may occur. Clinical signs may include hyperreflexia, extensor plantar responses and extrapyramidal signs, including hypokinesia and freezing during walking. Diagnosing normal-pressure hydrocephalus may be difficult since the three cardinal symptoms are common in the elderly. Moreover, gait disorder may precede other symptoms for several years and can be the only symptom for a long period. Normal-pressure hydrocephalus is a common cause of gait disorders in the elderly, while in dementia it accounts for only 0–5% of persons with dementia (Fisher, 1982). It should account for 4–6.7% of the gait disorders in the elderly (Sudarsky and Ronthal, 1983; and Sudarsky, 1997).

G. Metabolic and Endocrine Disorders

Some of these disorders may produce akinetic–rigid syndromes. Hypothyroidism may cause parkinsonism with motor slowing. Hypoparathyroidism, resulting in calcifications of the basal ganglia, may produce a clinical syndrome consisting of parkinsonism, chorea, cerebellar dysfunction or a mixed extrapyramidal–pyramidal syndrome, resembling the lacunar state (Kovacs *et al*., 1993). Metabolic and toxic disorders causes gait disorders in 2.5% of the elderly (Sudarsky and Ronthal, 1983; Sudarsky, 1997).

H. Drug-Induced Parkinsonism

Many drugs commonly prescribed in the geriatric practice can affect gait. It can be caused by drugs that deplete presynaptic dopamine stores (such as reserpine or tetrabenazine); neuroleptic drugs, such as phenothiazines (chlorpromazine), butyrophenones (haloperidol), thioxanthines (flupenthixol), and substituted benzamides (sulpiride); tricyclic antidepressants; prochlorperazine, used for motion sickness, vertigo, or unsteadiness; metoclopramide for gastrointestinal symptoms or migraine; the atypical calcium-blocking drug cinnarizine and flunarizine for vestibular disorders or hypertension; and benzodiazepines, chronic intoxication characterized by disorientation, sedation, or agitation and a progressive deterioration of gait.

Drug-induced parkinsonism results in bradykinesia and rigidity with facial amimia, dysarthria, and diminished or disappearing arm swing. Tremor is less common, but can be identical to the classic resting tremor of Parkinson's disease. Moreover, symptoms of drug-induced parkinsonism usually are asymmetrical, as in Parkinson's disease. Drug-induced parkinsonism often resolves quickly within weeks after stopping medication, although it may take months, especially if depot neuroleptic medications were used.

I. Senile Gait

Senile gait is defined as a gait abnormality of unknown etiology occurring in individuals older than 60 years (Koller *et al*., 1983). The clinical presentation is variable and not precisely defined, but can best be described as the gradual appearance of a broad-based gait with small steps associated with diminished arm swing, stooped posture, flexion of the hips and knees, uncertainty and stiffness in turning, occasional difficulty initiating steps, or a tendency toward falling (Critchley, 1948). They have difficulty in initiating walk, often shuffle, and their feet seem to be stuck to the floor. According to Sudarski and Ronthal (1983), this accounts for 16% of undiagnosed gait disorders in the elderly. Bloem *et al*. (1997) found a comparable percentage of 10% in a population of people over age 85. The underlying cause of this disorder remains unclear. Clinical and laboratory examinations fail to reveal any cause of this gait disturbance and some investigators state that it is a consequence of normal aging. Some believe that it is unlikely that these symptoms represent a true clinical entity (Elble *et al*., 1992). It is unclear whether there are multiple types of senile gait or whether it is a single progressive disease. It could also be a manifestation of extrapyramidal dysfunction (Critchley, 1948), frontal lobe disease (Meyer and Merrow, 1960), cerebellar dysfunction, hydrocephalus, or secondary to sensory abnormalities. It may be difficult to distinguish this type of gait disturbance from gait in Parkinson's disease. Symmetrical

signs, absence of tremor, and poor response to levodopa and the main differences between these entities.

V. Changes in Gait with Normal Aging

It is difficult to define age-associated changes in gait, because most of these changes can be attributed to several diseases commonly seen in elders. Some of the gait disturbances may reflect orthopedic, vascular, circulatory, respiratory, or other problems. However, some age-associated changes in posture and locomotion are nonspecific and mild. A short review of these changes is given below (Elble, 1997). These changes associated with normal aging may be difficult to distinguish from symptoms related to Parkinson's disease.

A. Gait Initiation

Gait initiation is a purposeful, stereotyped sequence of postural shifts that propels the body culminating in a forward step (Elble *et al*., 1994). Normal postural control and the integration of posture and movement are clearly involved. Normal old people initiate gait in nearly the same manner as normal younger people (Elble *et al*., 1994), but the older people generate smaller ankle moments of force during initiation of gait, resulting in a diminished moment of force propelling the body forward, probably due to weaker muscles or a desire to limit forward acceleration, thereby increasing the postural stability. In Parkinson's disease patients have great difficulty with taking the first step (start-hesitation). It occurs in 86% of Parkinson's disease patients. External cueing may be helpful in this condition.

B. Sitting and Standing

When preparing for standing while sitting, knees are flexed to bring the feet closer to the chair, resulting in a shorter distance that the center of mass must move forward. During the transition from sitting to standing and vice versa, there is a moment of postural instability, which may lead to falls (Schultz *et al*., 1992). In older people, reduced of motion in hips, knees, and spine is common. This may impede the shift of the total body center of mass over the feet when standing and over the chair when sitting. Weakness in the hips and knees may reduce moments of force as well, resulting in the necessity to use upper extremities or additional assistance (Elble, 1997). Parkinsonian patients have problems with rising from a chair, because they omit the above-mentioned preparation. They push their body up from the armrests of the seat and sometimes tend to fall back, thus requiring more than one attempt. In later stages they need help to get up from a chair.

C. Walking

Normal gait depends on normal functions of many systems, including neuromuscular, skeletal, circulatory, and respiratory system (Downton, 1993) and the differential diagnosis of gait disorders is extensive (see above). Older people walk more slowly and exhibit a shorter stride. As a result older people have a faster cadence (steps per minute) for a given speed of walking. Average velocity of normal walking is 20% lower than in younger people and average velocity of fast walking is 17% lower in older people (Elble *et al*., 1991). Walking speed declines with increasing age with a small decline of less than 1% per year (Bendall *et al*., 1989). Many older people show an increased toe-floor clearance during the ''swing-phase'' of walking. This may be regarded as a compensation to a greater risk of falling. They spend longer time in stance phases and shorter time in swing phases than young subjects. All these changes result in longer walking cycle duration. No differences in cadence have been noticed between younger and older people. In addition the hip rotation and knee flexion during swing phase is less and the ankle extension at the end of stance phase is less in older people. Postural sway when standing still is increased in the elderly. Also the posture differs from younger people: there is a tendency to stoop, probably because of dorsal kyphosis. Hips, knees, and ankles are in some degree of flexion. Some authors believe that this posture is due to balance difficulties, but there is little agreement about this (Cunha *et al*., 1987). Unlike Parkinson's disease, symptoms are usually bilateral and there is no ''freezing.''

D. Balance

In the control of posture and movement somatosensory, visual and vestibular feedback is necessary. Mild disturbances in any two of these modalities may result in significant functional impairment.

In older people, multiple sensory deficits are common. Pain and thermal sensitivity decrease with age (Prakash and Stern, 1973). Decrements in tactile sensitivity, joint position, stereognosis have been demonstrated with age (Skinner *et al*., 1984). There is a major loss of vibration sensation in the elderly, especially in the feet. About two-thirds of old persons show diminished vibratory function (Prakash and Stern, 1973). This might be due to age-related changes in receptor function, but could also be the result of underlying diseases which mainly affect elderly people, such as arthritic joint disease or diabetes mellitus.

Visual input is important for automatic balance responses, necessary for responses to uneven floors or other hazards. Age-related visual impairment is common and may be caused by several factors, such as presbyopia (due to decreasing accomodation) and cataracts. Other visual changes include macular degeneration and diabetic retinopathy.

Vestibular function is essential for the postural responses. A reduction in the number of vestibular hair cell loss and nerve cells is evident in aging and particularly prominent after age 60 (Richter, 1980). The number of hair cells in aged mice, for example, is about 80% of that in young adult mice (Konrad *et al*., 1999). Age-related changes in hearing function start in young adult life but become significant after age 65. Especially speech and loudness discrimination are affected, usually involving high frequencies.

The central integration of visual with somatosensory and vestibular information is, according to Teasdale *et al*. (1991), slower in older people, who tend to have reduced visual perception of motion, as demonstrated by Gilmore *et al*. (1992). With aging, a certain degree of cell loss occurs in the cerebellum which might contribute to locomotor impair-

ment in the elderly (Coleman and Flood, 1987). Finally, older people are more prone to adverse effects of medications (long-acting benzodiazepines), resulting in impaired balance. It remains unclear whether this is due to actual changes in receptor physiology or to a slower rate of excretion or metabolism. So postural support responses are slowed in older subjects and there is a change in the capacity to integrate sensory information. Patients with Parkinson's disease show postural instability. Usually, this occurs late in the disease, and is a prominent feature of stage III of Hoehn and Yahr. When pulling, retropulsion may occur as well as backward falls. In later stages patients may fall spontaneously. Analysis of compensatory body movements during standing on a moving treadmill indicated that the timing and amplitudes of adjustments were inappropriate in Parkinson's disease patients in comparison to healthy subjects (Dietz *et al*., 1993).

VI. Subclassification of Parkinson's Disease

The variability in expression of the clinical syndrome of Parkinson's disease is considerable and suggests the existence of subtypes with distinct clinical patterns, especially concerning the age of symptom onset, rate of disease progression, and clinical manifestation. This clinical heterogeneity probably reflects a broad spectrum of manifestations of one pathological event: the loss of pigmented neurons in the substantia nigra pars compacta and the presence of Lewy bodies. Several studies have tried to define clinical subgroups on the basis of distinguishing features, including family history of Parkinson's disease, variable progression, and age of onset of symptoms of disease.

A. Young-Onset versus Old-Onset Parkinson's Disease

Friedman has compared clinical expression of patients with onset age of symptoms above 70 years with patients with onset age below 45 years (Friedman, 1994). In this study there were some striking differences between these groups. The DATATOP (depreny1 and tocopherol antioxidative therapy of parkinsonism) study has investigated the variability in clinical expression by comparing several factors, like early versus late onset of disease, benign versus malign status, Hoehn-Yahr stage I versus Hoehn-Yahr stage II and tremor versus postural instability and gait disorder type (Jankovic *et al*., 1990). This study suggests that there are probably two different subtypes of Parkinson's disease: early onset and late onset of disease. Patients with early-onset Parkinson's disease progress at a slower rate, which has been confirmed by others (Goetz *et al*., 1988). These patients are more sensitive to levodopa and to levodopa-induced dyskinesias, and dyskinesias seem to appear earlier in those patients. Not only will they develop focal dystonia early in the course of illness, but also end-of-dose phenomenon is more often observed in patients with early onset of the disease (Quinn *et al*., 1987; Gibb and Lees, 1988b). Similarly, motor fluctuations, such as the wearing-off effect, tend to occur earlier in young-onset patients (Kostic *et al*., 1991). Initial symptom in this group is usually tremor. This is in contrast to Friedman's (1994) results which found that early-onset patients usually start with paresthesia and have bradykinesia as the dominant symptom. They tend to perform better on neuropsychological tests, but this may be explained by their younger age.

Patients with late onset of disease have a more aggressive form of Parkinson's disease with faster progression and more severe motor disability. They have as initial symptoms bradykinesia, postural instability, rigidity, and less often tremor, according to the DATATOP study. This study also suggests that motor deterioration in Parkinson's disease does not necessarily parallel cognitive decline and it is postulated that cognitive impairment in Parkinson's disease results mainly from a deficit correlated to dopaminergic dysfunction. According to Friedman (1994), late-onset patients with Parkinson's disease more often have tremor as both presenting and dominant symptom. Late-onset parkinsonian patients more frequently develop psychotic complications and less frequent dyskinesia as complication of levodopa treatment than young-onset ones. Friedman found a striking difference in symptomatology of psychotic complications. Whereas late-onset patients used to have simple, mostly visual hallucinations with preserved insight, early-onset ones tend to develop paranoid behavior without preserved insight. The number of early-onset patients with psychotic complications, however, was very low in this study. There was no striking difference in overall functioning based on activities of daily living between those two groups. According to Friedman (1994), the lesion in early-onset Parkinson's disease concerns predominantly the dopaminergic system. This monosystemic lesion may explain the greater susceptibility to dyskinesia (and the fact that bradykinesia is the dominant symptom of this disease). Lower susceptibility to dyskinesia in late-onset parkinsonian patients could be the result of age-related decline in the number of dopaminergic receptors in striatum. In contrast late-onset patients are more susceptible to developing psychotic complications, probably due to more widespread lesions of the central nervous system resulting from aging. Older people are in general more likely to develop psychotic reactions to various external stimuli (such as infections, intoxications) (Koponen, *et al*., 1989). Age-related brain atrophy involving also other neurotransmitter systems is probably the reason for the different symptoms in Parkinson's disease between old-onset and early-onset patients (Friedman, 1994). Some studies have observed a higher frequency of a first-degree relative with Parkinson's disease in early-onset patients (Barbeau and Pourcher, 1982; Stern *et al*., 1991), although other studies found no difference in the number of affected relatives among early- and late-onset cases (Quinn *et al*., 1987; Gibb and Lees, 1988b). Others have noted an increased exposure to farming and rural living in patients with early-onset of disease. Despite the differences in clinical manifestation, the cellular morphology and frequency of Lewy bodies in the substantia nigra are identical in early- and late-onset cases (Gibb and Lees, 1988b).

B. Other Subtypes of Parkinson's Disease

The existence of possible subgroups of Parkinson's disease, have also been investigated, including early-onset, tremor dominant, postural instability/gait disorder predominant, benign, and malignant forms. Comparing tremor-dominant to

postural instability/gait disorder-dominant Parkinson's disease revealed more severe motor disability in the postural instability/gait disorder group. Usually, patients with tremor as dominant symptom did not only have more severe tremor at rest, but also more severe action and postural tremor. Postural instability/gait disorder patients had, in addition to their greater postural and gait difficulties, more severe bradykinesia and rigidity (Jankovic *et al*., 1990).

Some authors have divided patients into benign and malignant groups based on duration of symptoms and stage of disease. Patients were arbitrarily considered to have a benign form of Parkinson's disease if they were Hoehn and Yahr stage 2 or less, combined with parkinsonian symptoms for 4 years or less. In contrast, patients with malignant Parkinson's disease were those with early postural imbalance, and disease duration less than 1 year. Patients with a benign form of Parkinson's disease usually were younger at onset of disease and had tremor as dominant symptom. Indeed, benign tremulous parkinsonism is a well-recognized subgroup of patients with a relatively nonprogressive, long-term course of disease. The benign group had an earlier onset than the malignant one and the latter one performed slightly worse on the Mini Mental State Examination, although this difference was not statistically significant after adjusting for age. To distinguish the benign form from the malignant one, other factors may be important as well, like response to medication, genetic factors, and cognitive impairment (Stern and Koller, 1993). A recent study shows that patients with a benign form of Parkinson's disease have mild dyskinesia, if present, and that none of these patients had dementia or hallucinations. All of them are responsive to drugs (Hely *et al*., 1999).

Recently Graham and Sagar (1999) have investigated the heterogeneity in Parkinson's disease using a data-driven approach. They suggested the existence of three distinct subtypes: a motor only subtype, without intellectual impairment; a motor and cognitive subtype; and a rapid progressive subtype.

The latter group is characterized by an older age of disease onset than the other subtypes, rapidly progressive motor and cognitive disability, and more orthostasis. Due to the rapid progression, patients belonging to this subtype have a shorter life expectancy than the other groups. This was confirmed by Louis *et al*. (1997), who found that mortality increases with age the development of dementia, and the severity of motor disability. Gender may be associated with a differential progression of Parkinson's disease. According to the Sydney multicenter study of Parkinson's disease, dyskinesia develops earlier in women during the first 10 years of disease. After 10 years there is no significant difference in the prevalence of dyskinesia, but women tend to have a higher score in Hoehn and Yahr stage (Hely *et al*., 1999).

VII. Brain Metabolism in Aging and Parkinson's Disease

During development and aging, the brain will change anatomically and physiologically to support the behavior of normal adults. Chugani *et al*. (1987) have investigated the maturation of the brain during the first years of life by using positron emission tomography (PET) and 2-deoxy-[^{18}F]-fluoro-D-glucose (FDG). They discovered that in the first 4 weeks of life (the neonatal period), the most important region of metabolic activity is the primary sensorimotor are, and that high activity is also found in the thalamus, brain stem, and cerebellar vermis (see Fig. 47.1). During the second postnatal month a small

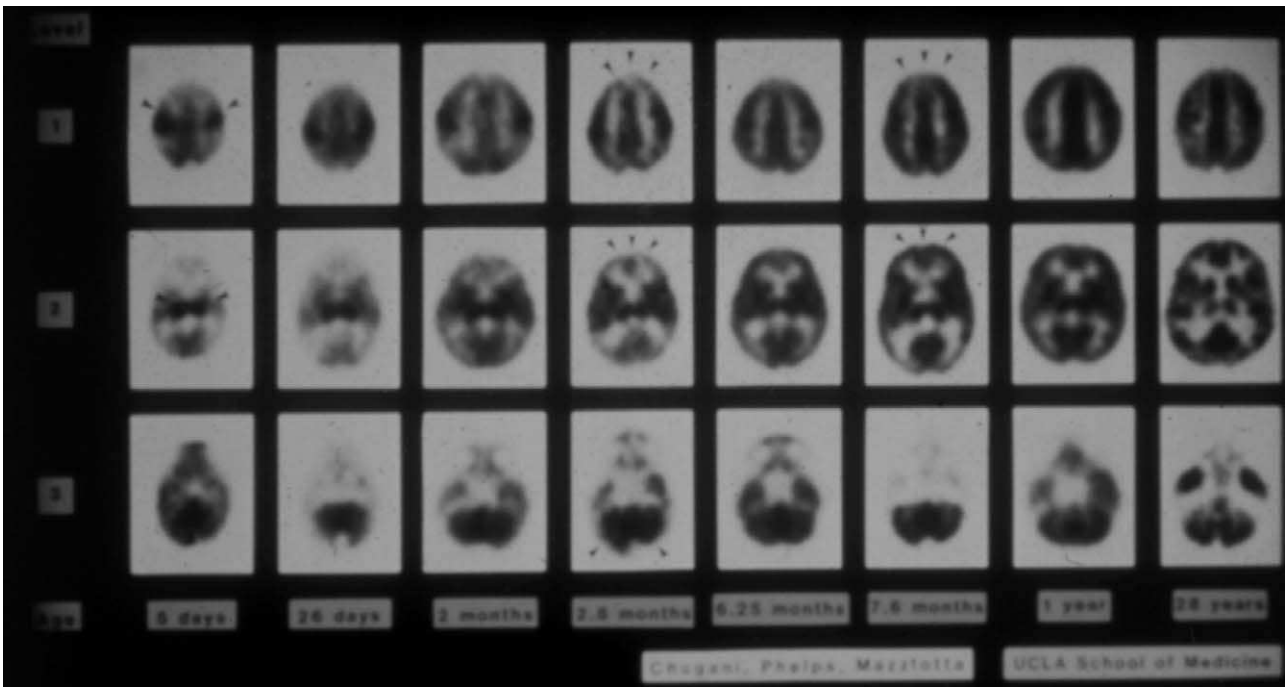

FIG. 47.1. PET study of human brain functional development (Chugani *et al*., 1987).

increase in metabolism in calcarine and temporal cortices can be seen. Approximately 3 months after birth, considerable rises in metabolic activity in the anterior parietal, temporal, and calcarine cortices as well as in basal ganglia and cerebellar cortex are found. By 1 year, the metabolic pattern resembles that in adults, but absolute values are lower than adult ones. Adult rates are reached by 2 years of age. Metabolic rates continue to rise until 3–4 years, so values, exceed those of adults by a factor of approximately 2. These high values are maintained until age 8–9. At this age, they begin to decline and by the end of the second decade adult values are reached. The highest increases in metabolic activity were observed in the cerebral cortices (Chugani *et al*., 1987).

Aging is associated with the degeneration of specific neural systems. Normal aging is predominantly characterized by metabolic changes in the prefrontal cortex. By using FDG-PET scans metabolism of these systems can be investigated. In middle age there is a trend in favor of frontal hypermetabolism. Moeller and co-workers (1996) have tried to explore the metabolic topography of aging and found various topographic profiles. One was characterized by relative frontal hypometabolism associated with convariate metabolic increases in the parietooccipital association areas, basal ganglia, midbrain and cerebellum. Another one revealed relative basal ganglia hypermetabolism associated with covariate decreases in frontal premotor cortex (Moeller *et al*., 1996) (Fig. 47.2, see color insert). In a recent study, Mielke *et al*. (1998) also found a decline of regional cerebral glucose metabolism in frontal areas (Mielke *et al*., 1998).

Although the most important lesion in Parkinson's disease is located in the substantia nigra and its dopaminergic projections, lesions of the presynaptic nigrostriatal dopamine system result in widespread abnormalities in regional brain metabolism. To define the metabolic topography of parkinsonism the same topographic strategy has been used. According to the results of Eidelberg and co-workers (1994) the metabolic profile of Parkinson's disease is characterized by increased activity in the lentiform nucleus, thalamus, pons, and cerebellum, whereas activity is decreased in the lateral frontal, paracentral, and parietal association areas (Fig. 47.3, see color insert). Increase in activity in the lentiform nucleus and thalamus in Parkinson's disease is consistent with experimental animal studies (Palombo *et al*., 1990; Mitchell *et al*., 1992). Increased metabolism in the ventral thalamus has been confirmed by others (Crossman *et al*., 1985; Palombo *et al*., 1990). The subject scores for the metabolic profile in Parkinson's disease correlated with the individual Hoehn and Yahr score and with rigidity and bradykinesia ratings, but not with tremor (Eidelberg *et al*., 1994). The reproducibility of this unique pattern of regional metabolic covariation in patients with Parkinson's disease has recently been assessed by Moeller *et al*. (1999). This topography appears to be highly reproducible across patient populations.

References

Aarsland, D., and Larsen, J. (1999). Psychosis in basal ganglia disorders: epidemiology. *In* "Mental Dysfunction in Parkinson's Disease II" (E. C. Wolters, P. Scheltens and H. W. Berendse, eds.), pp. 218–236. Academic Pharmaceutical Productions, Utrecht.

Anonymous. (1998). Mortality in DATATOP: A multicenter trial in early Parkinson's disease. Parkinson Study Group. *Ann. Neurol.* **43**, 318–325.

Barbeau, A., and Pourcher, E., (1982). New data on the genetics of Parkinson's disease. *Can. J. Neurol. Sci.* **9**, 53–60.

Bendall, M. J., Bassey, E. J., and Pearson, M. B. (1989). Factors affection walking speed of elderly people. *Age Ageing* **18**, 327–332.

Bennett, D. A., Beckett, L. A., Murray, A. M., Shannon, K. M., Goetz, C. G., Pilgrim, D. M., and Evans, D. A. (1996). Prevalence of parkinsonian signs and associated mortality in a community population of older people *N. Engl. J. Med.* **334**, 71–76.

Bloem, B. R., Boers, I., Lagaay, A. M., Haan, J., Wintzen, A. R., and Roos, R. A. (1997). Gait impairment in the oldest old. *Tijdschr. Gerontol. Geriatr.* **28**, 76–81.

Borgesen, S. E., and Gjerris, F. (1982). The predictive value of conductance to outflow of CSF in normal pressure hydrocephalus. *Brain* **105**, 65–86.

Burke, R. E. (1998). Programmed cell death and Parkinson's disease. *Mov. Disord.* **13** (Suppl. 1), 17–23.

Burke, R. E., and Kholodilov, N. G. (1998). Programmed cell death: Does it play a role in Parkinson's disease? *Ann. Neurol.* **44**, S126–S133.

Chugani, H. T., Phelps, M. E., and Mazziotta, J. C. (1987). Positron emission tomography study of human brain functional development. *Ann. Neurol.* **22**, 487–497.

Coleman, P. D., and Flood, D. G. (1987). Neuron numbers and dendritic extent in normal aging and Alzheimer's disease. *Neurobiol. Aging* **8**, 521–545.

Conley, S. C., and Kirchner, J. T. (1999). Parkinson's disease—the shaking palsy. Underlying factors, diagnostic considerations, and clinical course. *Postgrad. Med.* **106**, 39–42,45–46,49–50.

Critchley, M. (1931). The neurology of old age. *Lancet* **1**, 1221–1230.

Critchley, P. (1948). Senile disorders of gait. *Geriatrics* **3**, 364–370.

Crossman, A. R., Mitchell, I. J., and Sambrook, M. A. (1985). Regional brain uptake of 2-deoxyglucose in N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism in the macaque monkey. *Neuropharmacology* **24**, 587–591.

Cunha, U., Leduc, M., Nayak, U. S., and Isaacs, B. (1987). Why do old people stoop? *Arch. Gerontol. Geriatr.* **6**, 363–369.

Cunha, U. V. (1988). Differential diagnosis of gait disorders in the elderly. *Geriatrics* **43**, 33–38, 42.

Curtis, L., Lees, A. J., Stern, G. M., and Marmot, M. G. (1984). Effect of L-dopa on course of Parkinson's disease. *Lancet* **2**, 211–212.

Damier, P., Hirsch, E. C., Agid, Y., and Graybiel, A. M. (1999). The substantia nigra of the human brain. II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. *Brain* **122**, 1437–1448.

Daniel, S. E., and Hawkes, C. H. (1992). Preliminary diagnosis of Parkinson's disease by olfactory bulb pathology. *Lancet* **340**, 186.

Daniel, S. E., and Lees, A. J. (1993). Parkinson's Disease Society Brain Bank, London: Overview and research. *J. Neural Transm.* **39** (Suppl.), 165–172.

de Rijk, M. C., Breteler, M. M., Graveland, G. A., Ott, A., Grobbee, D. E., van der Meche, F. G., and Hofman, A. (1995). Prevalence of Parkinson's disease in the elderly: The Rotterdam Study. *Neurology* **45**, 2143–2146.

Diamond, S. G., Markham, C. H., Hoehn, M. M., McDowell, F. H., and Muenter, M. D. (1987). Multi-center study of Parkinson mortality with early versus later dopa treatment. *Ann. Neurol.* **22**, 8–12.

Diamond, S. G., Markham, C. H., Hoehn, M. M., McDowell, F. H., and Muenter, M. D. (1990). An examination of male-female differences in progression and mortality of Parkinson's disease. *Neurology* **40**, 763–766.

Dietz, V., Zijlstra, W., Assaiante, Ch., Trippel, M., and Berger, W. (1993). Balance control in Parkinson's disease. *Gait Post.* **1**, 77–84.

Douglas, J., Gelb, E. O., and Gilman, S. (1999). *Arch. Neurol.* **56**, 33–39.

Downton, J. H. (1993). Balance, gait, and falling. *In* "Falls in the Elderly" (J. H. Downton, ed.), pp. 47–63. Arnold, London.

Dubois, B., and Pillon, B. (1999). Dementia in Parkinson's disease. *In* "Mental Dysfunction in Parkinson's Disease II" (E.C. Wolters, P. Scheltens, and H. W. Berendse, eds.), pp. 165–176. Academic Pharmaceutical Productions, Utrecht.

Eidelberg, D., Moeller, J. R., Dhawan, V., Spetsieris, P., Takikawa, S., Ishikawa, T., Chaly, T., Robeson, W., Margouleff, D., Przedborski, S., *et al*. (1994). The metabolic topography of parkinsonism. *J. Cereb. Blood Flow Metab.* **14**, 783–801.

Elble, R. J. (1997). Changes in gait with normal aging. *In* "Gait Disorders of Aging" (J.C. Masdeu, L. Sudarsky, and L. Wolfson, eds.), pp. 93–106. Lippincott-Raven, Philadelphia.

Elble, R. J. (1998). Tremor in ostensibly normal elderly people. *Mov. Disord.* **13**, 457–464.

Elble, R. J., Thomas, S. S., Higgins, C., and Colliver, J. (1991). Stride-dependent changes in gait of older people. *J. Neurol.* **238**, 1–5.

Elble, R. J., Hughes, L., and Higgins, C. (1992). The syndrome of senile gait. *J. Neurol.* **239**, 71–75.

Elble, R. J., Moody, C., Leffler, K., and Sinha, R. (1994). The initiation of normal walking. *Mov. Disord.* **9**, 139–146.

Fearnley, J. M., and Lees, A. J. (1991). Ageing and Parkinson's disease: Substantia nigra regional selectivity. *Brain* **114**, 2283–2301.

Fisher, C. M. (1982). Hydrocephalus as a cause of disturbances of gait in the elderly. *Neurology* **32**, 1358–1363.

Friedman, A. (1994). Old-onset Parkinson's disease compared with young-onset disease: Clinical differences and similarities. *Acta Neurol. Scand.* **89**, 258–261.

Gelb, D. J., Oliver, E., and Gilman, S. (1999). Diagnostic criteria for Parkinson disease. *Arch. Neurol.* (*Chicago*) **56**, 33–39.

Gibb, W. R., and Lees, A. J. (1988a). The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. *J. Neurol., Neurosurg. Psychiatry* **51**, 745–752.

Gibb, W. R., and Lees, A. J. (1988b). A comparison of clinical and pathological features of young- and old-onset Parkinson's disease. *Neurology* **38**, 1402–1406.

Giladi, N., McMahon, D., Przedborski, S., Flaster, E., Guillory, S., Kostic, V., and Fahn, S. (1992). Motor blocks in Parkinson's disease. *Neurology* **42**, 333–339.

Gilmore, G. C., Wenk, H. E., Naylor, L. A., and Stuve, T. A. (1992). Motion perception and aging. *Psychol. Aging* **7**, 654–660.

Gilmore, R. (1984). Movement disorders in the elderly. *Geriatrics* **39**, 65–68, 72–76.

Goetz, C. G., Tanner, C. M., Stebbins, G. T., and Buchman, A. S. (1988). Risk factors for progression in Parkinson's disease. *Neurology* **38**, 1841–1844.

Golbe., L. I. (1993). Progressive supranuclear palsy. *In* "Parkinsonian Syndromes" (M.B. Stern and W.C. Koller, eds.), pp. 227–248. Dekker, New York.

Gorell, J. M., Johnson, C. C., and Rybicki, B. A. (1994). Parkinson's disease and its comorbid disorders: An analysis of Michigan mortality data, 1970 to 1990. *Neurology* **44**, 1865–1868.

Gourie-Devi, M., Gururaj, G., Satishchandra, P., and Subbakrishna, D. K. (1996). Neuro-epidemiological pilot survey of an urban population in a developing country. A study in Bangalore, south India. *Neuroepidemiology* **15**, 313–320.

Graham, J. M., and Sagar, H. J. (1999). A data-driven approach to the study of heterogeneity in idiopathic Parkinson's disease: Identification of three distinct subtypes. *Mov. Disord.* **14**, 10–20.

Hely, M. A., Morris, J. G., Traficante, R., Reid, W. G., Sullivan, D. J., and Williamson, P. M. (1999). The sydney multicentre study of Parkinson's disease: Progression and mortality at 10 years. *J. Neurol. Neurosurg. Psychiatry* **67**, 300–307.

Hoehn, M. M., and Yahr, M. D. (1967). Parkinsonism: Onset, progression and mortality. *Neurology* **17**, 427–442.

Hughes, A. J., Daniel, S. E., and Lees, A. J. (1993). The clinical features of Parkinson's disease in 100 histologically proven cases. *Adv. Neurol.* **60**, 595–599.

Imms, F. J., and Edholm, O. G. (1981). Studies of gait and mobility in the elderly. *Age Ageing* **10**, 147–156.

Jankovic, J., McDermott, M., Carter, J., Gauthier, S., Goetz, C., Golbe, L., Huber, S., Koller, W., Olanow, C., Shoulson, I., Stern, M., Tanner, C., and Weiner, W. (1990). Variable expression of Parkinson's disease: A base-line analysis of the DATATOP cohort. The Parkinson Study Group. *Neurology* **40**, 1529–1534.

Jansson, B., and Jankovic, J. (1985). Low cancer rates among patients with Parkinson's disease. *Ann. Neurol.* **17**, 505–509.

Kessler, I. I. (1972a). Epidemiologic studies of Parkinson's disease. II. A hospital-based survey. *Am. J. Epidemiol.* **95**, 308–318.

Kessler, I. I. (1972b). Epidemiologic studies of Parkinson's disease. III. A community-based survey. *Am. J. Epidemiol* **96**, 242–254.

Koller, W. C., Wilson, R. S., Glatt, S. L., Huckman, M. S., and Fox, J. R. (1983). Senile gait: Correlation with computed tomographic scans. *Ann. Neurol.* **13**, 343–344.

Kompoliti, K., Goetz, C. G., Boeve, B. F., Maraganore, D. M., Ahlskog, J. E., Marsden, C. D., Bhatia, K. P., Greene, P. E., Przedborski, S., Seal, E. C., Burns, R. S., Hauser, R. A., Gauger, L. L., Factor, S. A., Molho, E. S., and Riley, D. E. (1998). Clinical presentation and pharmacological theraphy in corticobasal degeneration. *Arch. Neurol. (Chicago)* **55**, 957–961.

Konrad, H. R., Girardi, M., and Helfert, R. (1999). Balance and aging. *Laryngoscope* **109**, 1454–1460.

Koponen, H., Stenback, U., Mattila, E., Soininen, H., Reinikainen, K., and Riekkinen, P. J. (1989). Delirium among elderly persons admitted to a psychiatric hospital: Clinical course during the acute stage and one-year follow-up. *Acta Psychiatr. Scand.* **79**, 579–585.

Kostic, V., Przedborski, S., Flaster, E., and Sternic, N. (1991). Early development of levodopa-induced dyskinesias and response fluctuations in young-onset Parkinson's disease. *Neurology* **41**, 202–205.

Kovacs, C. S., Howse, D. C., and Yendt, E. R. (1993). Reversible parkinsonism induced by hypercalcemia and primary hyperparathyroidism. *Arch. Intern. Med.* **153**, 1134–1136.

Kurtzke, J. F., and Goldberg, I. D. (1988). Parkinsonism death rates by race, sex, and geography. *Neurology* **38**, 1558–1561.

Lang, A. E., and Lozano, A. M. (1988). Parkinson's disease. First of two parts. *N. Engl. J. Med.* **339**, 1044–1053.

Leonard, B. E. (1999). Prevalence and psychopathology of depression in Parkinson's disease. *In* "Mental Dysfunction in Parkinson's Disease II" (E.C. wolters, P. Scheltens, and H.W. Berendse, eds.), pp. 248–253. Academic Pharmaceutical Productions, Utrecht.

Litvan, I., Agid, Y., Calne, D., Campbell, G., Dubois, B., Duvoisin, R. C., Goetz, C. G., Golbe, L. I., Grafman, J., Grondon, J. H., Hallett, M., JanKonic, J., Quinn, N. P., Tolosa, E., and Zee, D. S. (1996). Clinical research criteria for the diagnosis of progressive supranuclear palsy (Steele-Richardson-Olszewski syndrome): Report of the NINDS-SPSP international workshop. *Neurology* **47**, 1–9.

Louis, E. D., Marder, K., Côté, L., Pullman, S., Ford, B., Wilder, D., Tang, M. X., Lantigua, R., Gurland, B., and Mayeux, R. (1995). Differences in the prevalence of essential tremor among elderly African Americans, whites, and Hispanics in northern Manhattan, NY. *Arch. Neurol. (Chicago)* **52**, 1201–1205.

Louis, E. D., Marder, K., Côté, L., Tang, M., and Mayeux, R. (1997). Mortality from Parkinson disease. *Arch. Neurol. (Chicago)* **54**, 260–264.

Lundgren-Lindquist, B., Aniansson, A., and Rundgren, A. (1983). Functional studies in 79-year-olds. III. Walking performance and climbing capacity. *Scand. J. Rehabil. Med.* **15**, 125–131.

Magalhaes, M., Wenning, G. K., Daniel, S. E., and Quinn, N. P. (1995). Autonomic dysfunction in pathologically confirmed multiple system atropphy and idiopathic Parkinson's disease—a retrospective comparison. *Acta Neurol. Scand.* **91**, 98–102.

Marder, K., Flood, P., Côté, L., and Mayeux, R. (1990). A pilot study of risk factors for dementia in Parkinson's disease. *Mov. Disord.* **5**, 156–161.

Marder, K., Leung, D., Tang, M., Bell, K., Dooneief, G., Côté, L., Stern, Y., and Mayeux, R. (1991). Are demented patients with Parkinson's disease accurately reflected in prevalence surveys? A survival analysis. *Neurology* **41**, 1240–1243.

Marttila, R. J., and Rinne, U. K. (1976). Epidemiology of Parkinson's disease in Finland. *Acta Neurol. Scand.* **53**, 81–102.

Mayeux, R., Stern, Y., Rosenstein, R., Marder, K., Hauser, A., Côté, L., and Fahn, S. (1988). An estimate of the prevalence of dementia in idiopathic Parkinson's disease. *Arch. Neurol. (Chicago)* **45**, 260–262.

Mayeux, R., Denaro, J., Hemenegildo, N., Marder, K., Tang, M. X., Côté, L. J., and Stern, Y. (1992). A population-based investigation of Parkinson's disease with and without dementia. Relationship to age and gender. *Arch. Neurol. (Chicago)* **49**, 492–497.

Mayeux, R., Marder, K., Côté, L. J., Denaro, J., Hemenegildo, N., Mejia, H., Tang, M. X., Lantigua, R., Wilder, D., Gurland, B., and Hauser, A. (1995). The frequency of idiopathic Parkinson's disease by age, ethnic group, and sex in northern Manhattan, 1988–1993. *Am. J. Epidemiol.* **142**, 820–827; erratum: *Ibid.* **143**, 528 (1996).

McGeer, P. L., McGeer, E. G., and Suzuki, J. S. (1977). Aging and extrapyramidal function. *Arch. Neurol. (Chicago)* **34**, 33–35.

McKeith, I. G. (1999). Dementia with Lewy bodies. *In* ''Mental Dysfunction in Parkinson's Disease II'' (E.C. Wolters, P. Scheltens, and H.W. Berendse, eds.), pp. 194–208. Academic Pharmaceutical Productions, Utrecht.

Meyer, J., and Barrow, D. (1960). Apraxia of gait: A clinico-physiological study. *Brain* **83**, 761–784.

Mielke, R., Kessler, J., Szelies, B., Herholz, K., Wienhard, K., and Heiss, W. D. (1998). Normal and pathological aging—findings of positron-emission-tomography. *J. Neural Transm.* **105**, 821–837.

Mitchell, I. J., Boyce, S., Sambrook, M. A., and Crossman, A. R. (1992). A 2-deoxyglucose study of the effects of dopamine agonists on the parkinsonian primate brain. Implications for the neural mechanisms that mediat dopamine agonist-induced dyskinesia. *Brain* **115**, 809–824.

Moeller, J. R., Ishikawa, T., Dhawan, V., Spetsieris, P., Mandel, F., Alexander, G. E., Grady, C., Pietrini, P., and Eidelberg, D. (1996). The metabolic topography of normal aging. *J. Cereb. Blood Flow Metab.* **16**, 385–398.

Moeller, J. R., Nakamura, T., Mentis, M. J., Dhawan, V., Spetsieris, P., Antonini, A., Missimer, J., Leenders, K. L., and Eidelberg, D. (1999). Reproducibility of regional metabolic covariance patterns: Comparison of four populations. *J. Nucl. Med.* **40**, 1264–1269.

Morens, D. M., Davis, J. W., Grandinetti, A., Ross, G. W., Popper, J. S., and White, L. R. (1996). Epidemiologic observations on Parkinson's disease: Incidence and mortality in a prospective study of middle-aged men. *Neurology* **46**, 1044–1050.

Muthane, U., Yasha, T. C., and Shankar, S. K. (1998). Low numbers and no loss of melanized nigral neurons with increasing age in normal human brains form India. *Ann. Neurol.* **43**, 283–287.

Newman, G., Dovermuehle, R. H., and Busse, E. W. (1960). Alterations in neurologic status with age. *J. Am. Geriatr. Soc.* **8**, 915–917.

Osuntokun, B. O., Adeuja, A. O., Schoenbereg, B. S., Bademosi, O., Noddidge, V. A., Olumide, A. O., Ige, O., Yaria, F., and Bolis, C. L. (1987). Neurological disorders in Nigerian Africans: A community-based study. *Acta Neurol. Scand.* **75**, 13–21.

Pakkenberg, B., Moller, A., Gundersen H. J., Mouritzen Dam, A., and Pakkenberg, H. (1991). The absolute number of nerve cells in substantia nigra in normal subjects and in patients with Parkinson's disease estimated with an unbiased stereological method. *J. Neurol. Neurosurg. Psychiatry* **54**, 30–33.

Palombo, E., Porrino, L. J., Bankiewicz, K. S., Crane, A. M., Sokoloff, L., and Kopin, I. J. (1990). Local cerebral glucose utilization in monkeys with hemiparkinsonism induced by intracarotid infusion of the neurotoxin MPTP. *J. Neurosci.* **10**, 860–869.

Pearce, R. K., Hawkes, C. H., and Daniel, S. E. (1995). The anterior olfactory nucleus in Parkinson's disease. *Mov. Disord.* **10**, 283–287.

Potagas, C., Dellatolas, G., Ziegler, M., Leveteau, J., Bathien, N., MacLeod, P., and Rondot, P. (1998). Clinical assessment of olfactory dysfunction in Parkinson's disease. *Mov. Disord.* **13**, 394–399.

Prakash, C., and Stern, G. (1973). Neurological signs in the elderly. *Age Ageing* **2**, 24–27.

Quinn, N. (1989). Multiple system atrophy—the nature of the beast. *J. Neurol. Neurosurg. Psychiatry, Suppl*. pp. 78–89.

Quinn, N., (1995). Parkinsonism—recognition and differential diagnosis. *Br. Med. J.* **310**, 447–452.

Quinn, N., Critchley, P., and Marsden, C. D. (1987). Young onset Parkinson's disease. *Mov. Disord.* **2**, 73–91.

Rajput, A. H., and Rozdilsky, B. (1976). Dysautonomia in Parkinsonism: A clinicopathological study. *J. Neurol. Neurosurg. Psychiatry* **39**, 1092–1100.

Rautakorpi, I., Takala, J., Marttila, R. J., Sievers, K., and Rinne, U. K. (1982). Essential tremor in a Finnish population. *Acta Neurol. Scand.* **66**, 58–67.

Razdan, S., Kaul, R. L., Motta, A., Kaul, S., and Bhatt, R. K. (1994). Prevalence and pattern of major neurological disorders in rural Kashmir (India) in 1986. *Neuroepidemiology* **13**, 113–119.

Richter, E. (1980). Quantitative study of human Scarpa's ganglion and vestibular sensory epithelia. *Acta Oto-Laryngol.* **90**, 199–208.

Rogers, J. D., Brogan, D., and Mirra, S. S. (1985). The nucleus basalis of Meynert in neurological disease: A quantitative morphological study. *Ann. Neurol.* **17**, 163–170.

Schapira, A. H. (1999). Science, medicine, and the future: Parkinson's disease. *Br. Med. J.* **318**, 311–314.

Schoenberg, B. S., Anderson, D. W., and Haerer, A. F. (1985). Prevalence of Parkinson's disease in the biracial population of Copiah County, Mississippi. *Neurology* **35**, 841–845.

Schoenberg, B. S., Osuntokun, B. O., Adeuja, A. O., Bademosi, O., Nottidge, V., Anderson, D. W., and Haerer, A. F. (1988). Comparison of the prevalence of Parkinson's disease in black populations in the rural United States and in rural Nigeria: Door-to-door community studies. *Neurology* **38**, 645–646.

Schultz, A. B., Alexander, N. B., and Ashton Miller, J. A. (1992). Biomechanical analyses of rising from a chair. *J. Biomech.* **25**, 1383–1391.

Skinner, H. B., Barrack, R. L., and Cook, S. D. (1984). Age-related decline in proprioception. *Clin. Orthop. Relat. Res.* 208–211.

Stacy, M., and Jankovic, J. (1992). Differential diagnosis of Parkinson's disease and the parkinsonism plus syndromes. *Neurol. Clin.* **10**, 341–359.

Stern, M., and Koller, W. C. (1993). Parkinson's disease. *In* ''Parkinsonian Syndromes'' (M. B. Stern and W. C. Koller, eds.), pp. 3–29. Dekker, New York.

Stern, M., Dulaney, E., Gruber, S. B., Golbe, L., Bergen, M., Hurtig, H., Gollomp, S., and Stolley, P. (1991). The epidemiology of Parkinson's disease. A case-control study of young-onset and old-onset patients. *Arch. Neurol. (Chicago)* **48**, 903–907.

Sudarsky, L. (1997). Clinical approach to gait disorders of aging: An overview. *In* ''Gait Disorders of Aging'' (J. C. Masdeu, L. Sudarsky, and L. Wolfson, eds.), pp. 147–158. Lippincott-Raven, Philadelphia.

Sudarsky, L., and Ronthal, M. (1983). Gait disorders among elderly patients. A survey study of 50 patients. *Arch. Neurol. (Chicago)* **40**, 740–743.

Sudarsky, L., and Simon, S. (1987). Gait disorder in late-life hydrocephalus. *Arch. Neurol. (Chicago)* **44**, 263–267.

Tanner, C. M., and Ben Shlomo, Y. (1999). Epidemiology of Parkinson's disease. *Adv. Neurol.* **80**, 153–159.

Tanner, C. M., and Goldman, S. M. (1996). Epidemiology of Parkinson's disease. *Neurol. Clin.* **14**, 317–335.

Teasdale, N., Stelmach, G. E., Breunig, A., and Meeuwsen, H. J. (1991). Age differences in visual sensory integration. *Exp. Brain Res.* **85**, 691–696.

Tinetti, M. E., Williams, T. F., and Mayewski, R. (1986). Fall risk index for elderly patients based on number of chronic disabilities. *Am. J. Med.* **80**, 429–434.

Tolosa, E., and Balaguer, E. (1989). Nonparkinsonian movement disorders in the elderly. *In* "Parkinsonism and Aging" (D. B. Calne, D. Crippa, M. Trabucchi, G. Comi, and R. Horowski, eds.), pp. 199–210 Raven Press, New York.

Wenning, G. K., Ben Shlomo, Y., Magalhaes, M., Daniel, S. E., and Quinn, N. P. (1994). Clinical features and natural history of multiple system atrophy. An analysis of 100 cases. *Brain* **117**, 835–845.

Wermuth, L., Stenager, E. N., Stenager, E., and Boldsen, J. (1995). Mortality in patients with Parkinson's disease. *Acta Neurol. Scand.* **92**, 55–58.

Jeffrey N. Joyce

48

The Basal Ganglia Dopaminergic Systems in Normal Aging and Parkinson's Disease

It has been suggested repeatedly that age-related declines in substantia nigra cell number and striatal dopamine, which have been reported in humans with normal aging, may contribute to Parkinson's disease, although the mechanisms by which this occurs have yet to be identified. Many of the neurodegenerative changes in Parkinson's disease are, however, unlikely to be components of aging per se. In fact, some of the changes may be neuroprotective. For example, the age-related loss of dopamine transporter expression in the substantia nigra may reduce the risk of cell death resulting from toxins that enter dopamine neurons via the transporter. In addition, many compensatory changes in the dopamine system appear unique to Parkinson's disease. The elevation of D_2 receptors and decline of D_3 receptors play unique roles in the clinical manifestations of Parkinson's disease and are not observed in aging. In the larger picture, however, some of the age-related declines in behavior resemble those in Parkinson's disease, occur in other parkinsonian disorders, and may reflect exaggerated forms of aging. For example, the parkinsonian features observed in a consequential proportion of Alzheimer's disease cases may be caused by significant changes in the biochemistry of the presynaptic dopaminergic system and loss of postsynaptic D_2 receptors that are also observed with aging in animals and humans. As the beneficial response to L-dopa is typically absent in Alzheimer's disease cases exhibiting parkinsonism, it would suggest the possibility that changes with aging may contribute to a deteriorated response to L-dopa in these and other parkinsonian disorders.

I. Overview

Parkinson's disease is a neurodegenerative disorder with an insidious onset and a prolonged course over many years. The primary cause of the symptoms of this illness is the neuronal death of dopamine-producing neurons of the substantia nigra (SN) and the resultant depletion of dopamine in the striatum (Hornykiewicz, 1998). The therapeutic intervention for Parkinson's disease is based on the assumption that activation of postsynaptically located dopamine receptors will provide some return of balance to the system. The preferred mode of treatment of the symptoms of this illness is with levo-dopa (L-dopa) which is taken up by surviving dopamine neurons and converted to dopamine, which, in turn, can be stored and released. However, there remain long-term complications of L-dopa therapy, particularly motor fluctuations and dyskinesias, but also significant loss of antiparkinsonian effectiveness in many patients and inability to reverse dementia and depression (Fabbrini *et al*., 1988; Mouradian *et al*., 1988). Most symptoms of Parkinson's disease and experimentally induced parkinsonism are thought to occur as a result of loss of the nigrostriatal dopaminergic innervation. However, it is now evident that more than one dopamine system is affected in Parkinson's disease and that different clinical symptoms are linked to the differential degeneration of these systems. Lesions to the nigrostriatal pathway and its cells of origin in the ventral SN are most closely associated with rigidity and hypokinesia (Rinne, 1991; Vingerhoets *et al*., 1997; Jellinger, 1999). Dementia, which is prevalent in Parkinson's disease (Mayeux *et al*., 1992), is correlated with exaggerated cell loss in the medial nuclei of the SN (Jellinger, 1991; Jellinger and Paulus, 1992; Narabayashi, 1995) or origin of the mesolimbic dopaminergic system (Fig 48.1). Dementia is highly correlated with greater depressive symptomatology, greater motor deficits, and poorer response to antiparkinsonian medication, suggesting common mechanisms or alterations in the dopaminergic system (Mayeux *et al*., 1988; Ebmeier *et al*., 1990; Jellinger and Paulus, 1992; Aarsland *et al*., 1996; Tandberg *et al*., 1996, 1997; Hobson and Meara, 1999).

While the causes of this deterioration in Parkinson's disease are unknown, it has been suggested repeatedly (Carlsson and Winblad, 1976; Calne and Langston, 1983; Cohen, 1983;

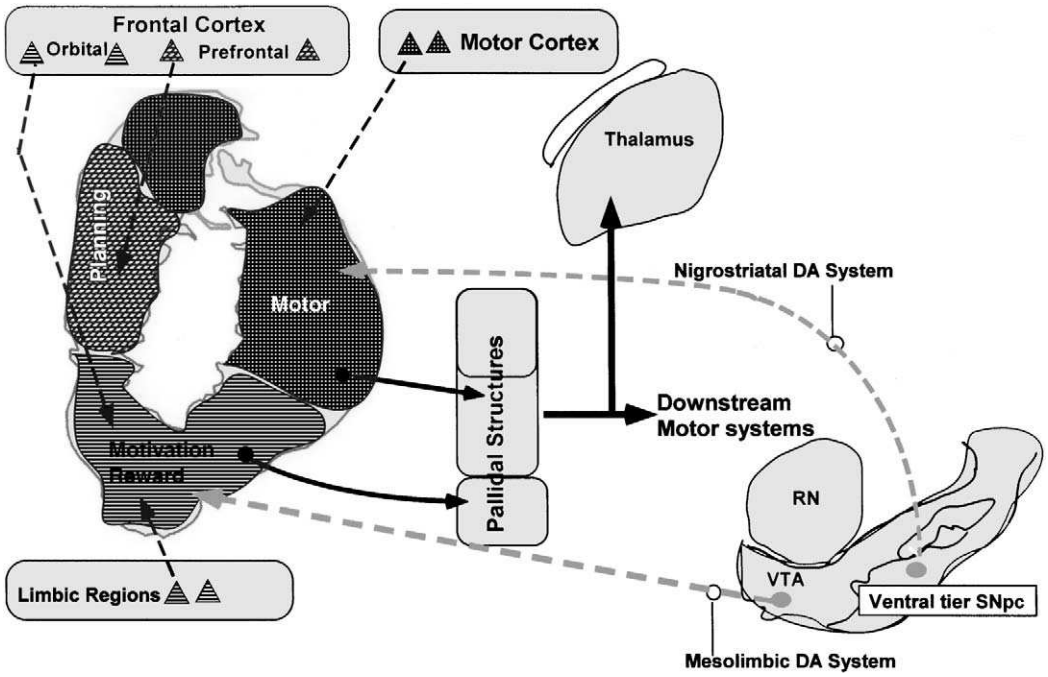

FIG. 48.1. Striatal territories, their cortical projections and innervation by divisions of the dopamine system. The dorsal striatum receives input from motor cortex, associated premotor cortices, and somatosensory cortex, is involved in motor and sensorimotor function and integration, and is innervated by the nigrostriatal dopaminergic system. The ventral striatum receives afferents from the orbital and medial prefrontal cortex and limbic and paralimbic cortices and integrates these signals under the modulatory influence of the mesolimbic dopaminergic system. The ventral striatum and mesolimbic dopaminergic system are involved in goal-directed behavior and changes in affective state.

Mann and Yates, 1983; Calne *et al*., 1986; Calne and Calne, 1988) that age-related declines in SN cell number and striatal dopamine, which have been reported in humans with normal aging (Carlsson and Winblad, 1976; McGeer *et al*., 1977; Fearnley and Lees, 1991), may contribute to Parkinson's disease, although the mechanisms by which this occurs has yet to be determined. Many of the neurodegenerative changes in Parkinson's disease (reduced dopamine terminals; SN cell loss) are, however, unlikely to be components of aging per se (McGeer *et al*., 1977; Gibb and Lees, 1987; German *et al*., 1989; Scherman *et al*., 1989; Fearnley and Lees, 1991). Compensatory changes that occur in Parkinson's disease, such as regulation of dopamine receptor number, also appear quite different from that occurring in aging. However, many aspects of aging, which are common across mammalian species, may contribute to the deterioration in Parkinson's disease. Additionally, parkinsonism may arise from more than one process that is associated with aging. For example, parkinsonism in Alzheimer's disease (AD) can be due to related Parkinson's disease pathology (Leverenz and Sumi, 1986; Ditter and Mirra, 1987; Mölsä *et al*., 1987; Liu *et al*., 1997) or to combination of factors conributing to dysfunction of the dopamine system (Murray *et al*., 1995; Joyce *et al*., 1997, 1998). Hence, an understanding of the relationship between changes in dopaminergic systems with aging and that in various parkinsonian disorders will aid in our understanding of the processes involved in the development of parkinsonian disorders.

II. Organization of the Presynaptic Dopaminergic System and Striatal Territories

It is well accepted that the functions of the striatum are related to its connections with other parts of the brain, particularly with the cortical regions that project to it. Different regions of the cortex provide nonoverlapping projections to the striatum segregated into a number of territories (Alexander *et al*., 1990; Parent and Hazrati, 1995). These territories, in turn, have principal dopamine projections. Two major divisions of the dopaminergic systems exist within the basal ganglia, the nigrostriatal and mesolimbic systems (Fig. 48.1). In the primate the source of the nigrostriatal and mesolimbic dopamine projections reflect a dorsal/ventral differentiation in the SN (Waters *et al*., 1988; Haber and Groenewegen, 1989; Gibb, 1992). Thus, based on the tracer studies, the mesolimbic dopaminergic system of the primate would include the A10 region (ventral tegmental area, parabrachial pigmented, paranigral nuclei) (Jimenez-Castellanos and Graybiel, 1987; Lynd-Balta and Haber, 1994a,b), whereas the origination of the ''nigrostriatal'' dopaminergic system would be more restricted to the ventral tier of the substantia nigra pars compacta SNpc (Szabo, 1980; Tanaka *et al*., 1982; Lynd-Balta and Haber, 1994b). The motor striatum (the dorsal part of the rostral putamen and most of the caudal putamen) receives dopaminergic innervation through the nigrostriatal pathway and topographic projections from the motor cortex, associated premotor cor-

tices (e.g., supplementary motor area, arcuate premotor area) and somatosensory cortex. Motor, premotor, and supplementary motor cortices, and the associated dorsolateral striatum are involved in motor and sensorimotor function and integration. The mesolimbic dopaminergic system innervates the ventral striatum (nucleus accumbens and ventral putamen), or limbic striatum, which is known to receive afferents from the orbital and medial prefrontal cortex, limbic and paralimbic cortices, thalamus, and amygdala (Haber and McFarland, 1999), and integrate these signals under the modulatory influence of the mesolimbic dopaminergic system (Mogenson *et al*., 1988; Lynd-Balta and Haber, 1994b). Association cortical inputs from the dorsolateral prefrontal, temporal, parietal, and cingulate cortices innervate large areas of the caudate nucleus and rostral putamen which receive dopaminergic innervation from both nigrostriatal and mesolimbic dopaminergic pathways. The dorsolateral prefrontal cortex and associated striatal territory is involved in procedural learning and strategic planning (Goldman-Rakic and Selemon, 1986). The limbic striatum has clearly been demonstrated to be involved in goal-directed behavior, locomotor activity, behavioral sensitization, and changes in affective state. The primate striatum (Haber and McFarland, 1999) and human striatum (Reiner *et al*., 1999) exhibit similar neurochemical organization of the striatum and efferent structures, suggesting that there is a similar organization into different territories. This would suggest that in the human striatum dopamine can act to modulate a wide variety of behavioral functions related to motor, cognition, motivation, and affect.

III. Aging and the Presynaptic Dopaminergic System

A. Parkinson's Disease: The Presynaptic Dopaminergic System

The most common methods for determining the extent of damage to the nigrostriatal and mesolimbic dopaminergic systems in Parkinson's disease is through measurement of cell loss in the midbrain dopaminergic neurons (German *et al*., 1989; Fearnley and Lees, 1991; Gibb, 1992), measurement of dopamine transporter levels in the striatum (Murray *et al*., 1995; Miller *et al*., 1997), or measurement of tyrosine hydroxylase (TH) labeling of dopamine terminals in the striatum (Ryoo *et al*., 1998). Parkinson's disease is correlated with specific patterns of cell loss in the substantia nigra, dopamine loss in the striatum, and loss of dopamine transporter binding sites. Cells in the ventrolateral SN, which give rise to the innervation to the dorsal and caudal putamen (Szabo, 1980; Tanaka *et al*., 1982; Lynd-Balta and Haber, 1994a), show the greatest cell loss in Parkinson's disease (German *et al*., 1989; Fearnley and Lees, 1991; Gibb, 1992) and that is correlated with loss of dopamine (Kish *et al*., 1992) and loss of dopamine transporter protein levels in the dorsal and caudal putamen (Chinaglia *et al*., 1992; Murray *et al*., 1995; Miller *et al*., 1997). This has been confirmed with *in vivo* imaging of the dopamine transporter in living Parkinson's disease cases. *In vivo* imaging of dopamine transporter by positron emission tomography (PET) ([^{11}C]WIN 35,428) and single-photon emission computed tomography (SPECT) ([^{123}I]β-CIT) has shown that there is generally a good correlation between symptom severity on the Hoehn and Yahr Staging Scale and Unified Parkinson's Disease Rating Scale (UPDRS) and degree of loss of binding to dopamine transporter (Frost *et al*., 1993; Seibyl *et al*., 1995; Asenbaum *et al*., 1997; Brucke *et al*., 1997; Müller *et al*., 1998; Tissingh *et al*., 1998; Rinne *et al*., 1999). [^{123}I]β-CIT binding shows a significant decrease in comparison to age-expected values ranging from 28 to 36% in Hoehn and Yahr stage 1 to 71 to 76% in Hoehn and Yahr stage V (Seibyl *et al*., 1995; Müller *et al*., 1998; Tissingh *et al*., 1998). Furthermore, the loss of dopamine transporter binding in the caudal putamen is greater than that in the caudate at all stages and those with unilateral symptoms show the greatest losses on the side contralateral to the affected limbs. Thus, the postmortem and *in vivo* findings are in agreement, indicating that, at least initially, the nigrostriatal dopaminergic system is more adversely affected in Parkinson's disease than the mesolimbic dopaminergic system.

Whether aging, per se, can contribute to the cell loss and reduction of dopamine transporter in the Parkinson's disease striatum is unclear. In studies of age-related changes in of the dopaminergic system in human, analyses of Western populations (largely Caucasian) have identified a 20 to 48% decline in melanized neurons between the ages of 20 and 70 years, with a greater emphasis on the smaller decline (McGeer *et al*., 1977; Thiessen *et al*., 1990; Fearnley and Lees, 1991; Tooyama *et al*., 1994). However, postmortem studies of human brain have demonstrated only a small age-related loss of striatal TH after early adolescence (McGeer *et al*., 1977; Wolf *et al*., 1991) and of [^{3}H]α-dihydrotetrabenazine binding to vesicular transporters located on dopamine terminals (Scherman *et al*., 1989). *In vivo* imaging of [^{11}C]dihydrotetrabenazine binding to the vesicular transporter are in agreement with the postmortem findings indicating an age-related loss only in the sixth decade of about 35% as compared to that seen in young adults (Frey *et al*., 1996). This would suggest that dopamine cell and dopamine terminal loss is not that consequential with advancing age in human brain.

Interestingly an analysis of SN in brain material derived from cases obtained in India (Muthane *et al*., 1998) did not observe a decline of melanized nigral neurons with age and, in that study, a much lower density of melanized neurons was observed in the SN as compared to that reported by Fearnley and Lees (1991) for Caucasians living in the United Kingdom. This suggests that a low number of nigral neurons, per se, does not lead to the symptoms of Parkinson's disease as the incidence of Parkinson's disease is lower in that population (Razdan *et al*., 1994) than in the United States or Europe (Pedro-Cuesta and Stawiarz, 1991; de Rijk *et al*., 1997). Fearnley and Lees (1991) also reported that the regions of the SN most susceptible to cell death in Parkinson's disease are the least affected with age. Similarly, age-related decline of dopamine in the striatum does not show regional features similar to that of Parkinson's disease (Kish *et al*., 1992). Thus, age-related SN cell loss per se is unlikely to be contributing to Parkinson's disease. However, as there is an age-related loss of striatal dopamine of a much greater magnitude than the cell loss accounts for (Adolfsson *et al*., 1979; Kish *et al*., 1992), it would suggest that there is decreased synthetic activity of nigral dopaminergic neurons with advanced age in humans that is functionally important.

TABLE 48.1 Presynaptic Dopamine System in Aging and Parkinsonian Disorders

	Striatal DAT	Striatal TH	Striatal DA	SN DAT	SN cell No.
Aging	45% Loss in putamen	↓ 20% In caudate and putamen	↓ 60% In caudate and putamen	↓ 70% In ventrolateral tier	↓ 20 To 30% in all regions
Parkinson's disease[a]	85% Loss in putamen	60% Loss in putamen	90% Loss in putamen	↓ 60% In ventrolateral tier	↓ 60% In ventrolateral tier
Alzheimer's with parkinsonism[a]	80% Loss in putamen	Normal to reduced	↓ 50% In caudate and putamen?	↓ 85% In ventrolateral tier	↓ 26% In ventrolateral tier

[a]All values are in relation to age-matched controls.
DA, dopamine; DAT, dopamine transporter; SN, substantia nigra; TH, tyrosine hydroxylase.

B. Aging and Dopamine Transporter Function

Studies in animals first suggested that decreased function of the presynaptic dopaminergic system could contribute to changes with age (Table 48.1). The overwhelming data indicate that loss of dopaminergic neurons of the SN and striatal dopamine with age is small or nonsignificant in most animal species (Sabel and Stein, 1981; Strong *et al*., 1982; Flood and Coleman, 1988; Marshall and Rosenstein, 1990; Fernandez-Ruiz *et al*., 1992; Friedemann and Gerhardt, 1992; Emerich *et al*., 1993; Irwin *et al*., 1994; Burwell *et al*., 1995). However, synthetic capability may be more affected. For example, Marshall and Rosenstein (1990) demonstrated that DOPA accumulation after NSD-1015 administration was reduced by 40% in aged male F344 rats. In addition, many investigators have found age-related loss in the capacity of dopamine uptake and/or dopamine transporter number in striatum of aged animals (Marshall and Rosenstein, 1990; Shimizu and Prasad, 1991; Friedemann and Gerhardt, 1992; Irwin *et al*., 1994; Gordon *et al*., 1995; Hebert and Gerhardt., 1998, 1999; Hebert *et al*., 1999) and loss of dopamine transporter mRNA in SN (Himi *et al*., 1995). These results suggest that dopamine release and uptake is likely compromised in aged rats (Friedemann and Gerhardt, 1992; Emerich *et al*., 1993; Gordon *et al*., 1995; Hebert and Gerhardt, 1998).

Functionally, this appears important as the age-related reduction in time-related performance and movement velocity are not related to loss of striatal dopamine levels/ nigral cell number (Emerich *et al*., 1993; Zoli *et al*., 1993; Burwell *et al*., 1995) in animal models of aging. These behavioral deficits appear to be more clearly related to reduced capacity of dopaminergic neurons to exhibit evoked release of dopamine (Friedemann and Gerhardt, 1992; Gordon *et al*., 1995; Hebert and Gerhardt, 1998), dopamine transporter levels (Shimizu and Prasad, 1991; Gordon *et al*., 1995; Hebert *et al*., 1999) or dopamine transporter function (Shimizu and Prasad, 1991; Hebert and Gerhardt, 1999), and reduced postsynaptic dopamine D_2 receptor number (De Blasi and Mennini, 1982; Joyce *et al*., 1986a; Lai *et al*., 1987; Han *et al*., 1989; Morelli *et al*., 1990). Thus, aging is not a pre-Parkinson's-disease state but rather may contribute to altered dopamine homeostasis. This has clear implications for other Parkinsonian conditions which may occur as a result of altered dopamine homeostasis and not from loss of dopamine innervation (see section III.C.).

Similar to that in animals age-related losses of striatal dopamine transporter sites and dopamine transporter mRNA in the SN have been identified in human brain (Zelnik *et al*., 1986; de Keyser *et al*., 1990; Bannon *et al*., 1992; van Dyck *et al*., 1995; Bannon and Whitty, 1997; Volkow *et al*., 1998; Ma *et al*., 1999). This suggests some age-related changes in the presynaptic components of the dopamine system are common across species. While PET imaging studies in human brain have suggested that there is a linear loss of striatal dopamine transporter with age (van Dyck *et al*., 1995; Volkow *et al*., 1998), the postmortem studies of dopamine transporter expression in the midbrain dopaminergic neurons indicate that the effect is much more marked after the fourth decade, perhaps after the age of 55 (Bannon *et al*., 1992; Bannon and Whitty, 1997; Ma *et al*., 1999). It is also evident from the postmortem studies that reduction in dopamine transporter expression is not due to some overall biochemical failure of these neurons as mRNA expression for TH is intact. The data, in fact, suggest that these neurons are selectively downregulating expression of dopamine transporter. In addition, there are significant differences in levels of expression of dopamine transporter mRNA among populations of the dopaminergic neurons that give rise to the nigrostriatal, as opposed to that seen in the mesolimbic dopaminergic system, with much higher expression in the ventral tier dopaminergic neurons innervating the dorsal striatum (Hurd *et al*., 1994; Haber *et al*., 1995). Importantly, the age-related loss of dopamine transporter is greatest in the population of cells of the ventral SN that has the highest expression of dopamine transporter in younger adults (Bannon and Whitty, 1997; Ma *et al*., 1999). It would be predicted, therefore, that the nigrostriatal dopaminergic system innervating the territories of the striatum underlying motor function would be functionally more affected than that of the mesolimbic dopamine system. This is meaningful for two reasons. First, the decline in dopamine transporter expression may actually protect those neurons from cell death in Parkinson's disease (Uhl, 1998). Second, there may be similar but far more exaggerated modification in dopamine transporter expression in the nigrostriatal dopamine system in other parkinsonian disorders, reflecting changes in dopamine utilization (Murray *et al*., 1995; Joyce *et al*., 1997).

Based on converging evidence, the formation and/or accumulation of toxic dopamine transporter substrates may be the primary cause of SN degeneration in Parkinson's disease (Hornykiewicz, 1998; Uhl, 1998). A model compound for substantiating this hypothesis is 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is known to produce parkinsonism in humans and in subhuman species via irreversible

damage to the dopaminergic neurons of the SN (Burns *et al*., 1983; Langston *et al*., 1983). The mechanism of the neurotoxic action of MPTP has been postulated to involve three essential steps: (1) crossing by MPTP of the blood–brain barrier, (2) its oxidation to MPP+ (1-methyl-4-phenylpyridinium) occurring largely in glial cells through catalyzation by MAO-B (Ransom *et al*., 1987), and (3) accumulation of MPP+, the toxic agent, in dopaminergic neurons by the high-affinity uptake system for dopamine (dopamine transporter) located on the plasmalemma of these neurons (Pifl *et al*., 1996; Gainetdinov *et al*., 1997). It has been well established that accumulation of MPP+ within dopaminergic neurons via the dopamine transporter is an absolute requirement for its neurotoxicity *in vivo* (Gainetdinov *et al*., 1997; Bézard *et al*., 1999). Inside the neuron, MPP+ is thought to act as a mitochondrial toxin, slowly sapping the neuron of its energy-producing potential and by production of reactive oxygen species (Cassarino and Bennett, 1999). The fact that the reduction of dopamine transporter expression in nigral neurons occurs to the greatest degree in the region most susceptible to cell death in Parkinson's disease suggest that these neurons may become less vulnerable to neurotoxins utilizing dopamine transporter (Bannon and Whitty, 1997; Ma *et al*., 1999). However, several papers have reported increased sensitivity in older C57 mice to neurotoxic effects of MPTP, as measured by initial losses of striatal dopamine levels or striatal dopamine transporter binding (Sershen *et al*., 1985; Ricaurte *et al*., 1987a; Date *et al*., 1990), recovery of striatal dopamine levels or TH-IR fibers with time post-MPTP (Ricaurte *et al*., 1987b; Nishi *et al*., 1989), or extent of cell loss in the SN and ventral tegmental area (VTA) (Gupta *et al*., 1986; Date *et al*.,1990). Importantly, as demonstrated by Irwin and associates (Irwin *et al*., 1992) truly aged mice do show less susceptibility to MPTP. The problem results from inappropriate age comparisons, mature (e.g., 8–12 months) and aged (over 20 months) mice are both likely to show enhanced sensitivity as compared to young (less than 3 months) mice because of development-related changes in the dopaminergic system and/or activity of MAO-B. Irwin and associates demonstrated that 10-, 16-, and 24-month-old mice are more sensitive to MPTP-induced loss of striatal dopamine than 2-month-old mice but 24-month-old mice were actually less sensitive than 10- or 16-month-old mice. Furthermore, the increased sensitivity in 10-, 16-, and 24-month-old mice was mirrored by enhanced activity of striatal MAO-B, but this was slightly reduced in advanced age (see also Rao *et al*., 1995). In contrast, there were no age-related differences in the capability of intraventricular MPP+, the active metabolite of MPTP, to produce striatal dopamine depletion. Hence, the age-related reduction of dopamine transporter in nigral neurons might be protective against the neurotoxicity of compounds that utilize DAT to accumulate inside dopaminergic neurons. Vice versa, it may be that cell death in Parkinson's disease is caused by a failure to downregulate the dopamine transporter (Uhl, 1998).

C. Parkinsonism with Alzheimer's Disease

Parkinsonian features can result from one or more alterations in the nigrostriatal dopaminergic system. Up to 40% of AD patients develop features of Parkinson's disease but because the symptoms of rigidity and bradykinesia predominate, tremor is infrequent and response to L-dopa is minimal it can be clinically differentiated from Parkinson's disease (Chui *et al*., 1985; Mayeux *et al*., 1985; Morris *et al*., 1989). Some studies have identified pathologic alterations similar to those observed in Parkinson's disease, but they occur in only a small percentage of the AD cases with parkinsonian features (Leverenz and Sumi, 1986; Ditter and Mirra, 1987; Mölsä *et al*., 1987; Murray *et al*., 1995; Liu *et al*., 1997). Other evidence suggests a more complex disturbance in the biochemistry of the nigrostriatal dopaminergic system in these patients. PET studies with [^{18}F]fluorodopa uptake show no evidence for marked loss of dopamine terminals in AD cases with parkinsonian signs (Tyrrell *et al*., 1990). Our studies of the neurons of origin of the nigrostriatal dopaminergic system in AD cases with parkinsonian features (AD/Park) indicate that they are not extensively depleted and are capable of synthesizing TH, but are markedly affected in the synthesis of dopamine transporter normally expressed at terminal sites in the striatum (Murray *et al*., 1995; Joyce, *et al*., 1997). Numbers of dopamine transporter binding sites in the striatum are almost as low as that in Parkinson's disease and is correlated with strongly suppressed dopamine transporter mRNA expression in the SN (Fig 48.2). Nonetheless, TH expression in the dopamine terminals and SN appears normal. How this occurs remains unclear but processes associated with AD pathology may lead to accumulation of dopamine transporter protein in the cell body and to downregulation of the dopamine transporter mRNA. Liu and associates (1997) have shown that the numbers of neurofibrillary tangles and neuropil threads are higher in the SNpc of AD/Park than in AD, suggesting that it may be a contributor to cell dysfunction. The presence of neurofibrillary tangles in the dopaminergic neurons might ''trap'' cytoskeletal and noncytoskeletal proteins (e.g., dopamine transporter) that are normally orthogradely transported to neuronal processes (Nakashima and Ikuta, 1985; Tabaton *et al*., 1985; German *et al*., 1987; Mann *et al*., 1987). As in Parkinson's disease with Lewy bodies, these NFTs could induce parallel disturbances in cellular function and processing of proteins so as lead to a feedback regulation of the levels of dopamine transporter mRNA. Thus, presynaptic components of the nigrostriatal dopaminergic system in patients with AD/Park are severely affected but the underlying pathology is distinct from that of Parkinson's disease.

We have hypothesized that these alterations in the presynaptic dopaminergic system in AD/Park directly contribute to the parkinsonian features of AD/Park. We believe that the significantly reduced levels of dopamine transporter would result in very low levels of releasable dopamine and a high dependence on exaggerated synthesis of dopamine to maintain extracellular levels of dopamine. A review of results from the genetic deletion of the dopamine transporter gene in mice demonstrates that lack of dopamine transporter leads to a marked increase in extracellular dopamine but profound changes in pre- and postsynaptic parameters of dopamine homeostasis (Jones *et al*., 1998; Gainetdinov *et al*., 1999). The lack of dopamine transporter leads to 300-fold increase in amount of time needed for dopamine to be cleared *and* a marked ($\sim$75%) reduction in dopamine released in response to electrical stimulation and high K^+ stimulation ($\sim$90%). In fact, the total striatal tissue dopamine levels, reflecting intraneuronal vesicular storage

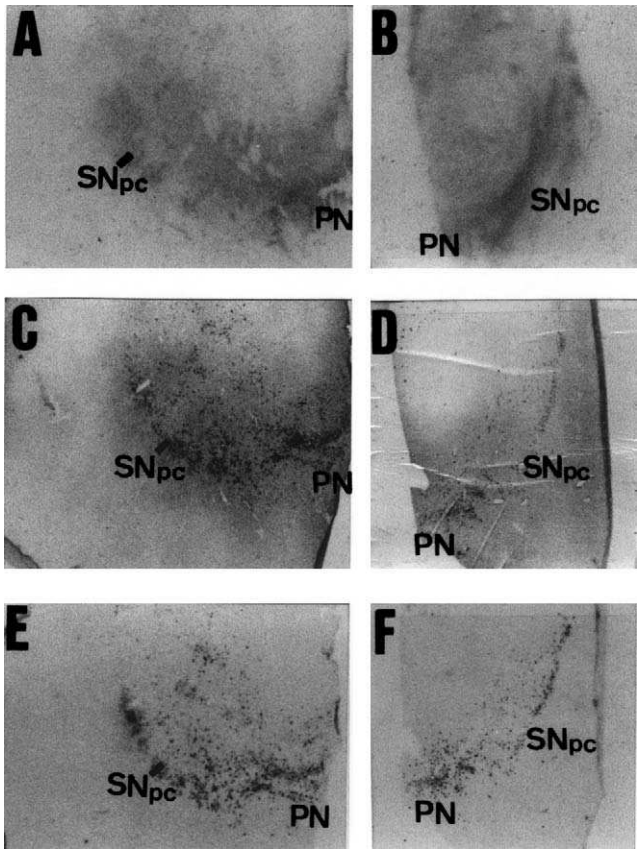

FIG. 48.2. Reduced expression of dopamine transporter mRNA but not TH or TH mRNA in the SN of AD/Park case as compared to age-matched control. Photomicrographs of the original autoradiographs demonstrating the distribution of (A, B) TH protein by immunoautoradiography, (C, D) dopamine transporter mRNA, and (E, F) TH mRNA in the midbrain of a control case (A, C, E) and an AD/Park case (B, D, F) in nearly adjacent sections. Note that the levels of TH-IR and TH mRNA are nearly similar in these cases but DAT mRNA levels are markedly decreased in the AD/Park case (modified from Joyce *et al*., 1997).

pool of dopamine, are drastically reduced (to ~5% of wild type) but dopamine synthesis is elevated by over 200%. In addition, functional studies of D_2 autoreceptor function indicate a marked desensitization to regulation of neuronal firing rate, nerve terminal dopamine release, and synthesis. Thus the maintenance of extracellular dopamine is highly dependent on the ongoing synthesis of dopamine. If dopamine synthesis could not be maintained with increasing age (Marshall and Rosenstein, 1990; Himi *et al*., 1995; Hebert and Gerhardt, 1998), then extracellular dopamine would be significantly decreased along with the loss of releasable dopamine. Thus, a hypoactive dopamine state would exist. At a critical level of hypoactivity a deterioration in function resulting in impairments of time-related performance, movement velocity, and other behavioral changes related to parkinsonism would occur (Marshall and Berrios, 1979; Gallagher and Burwell, 1989; Friedemann and Gerhardt, 1992; Emerich *et al*., 1993; Irwin *et al*., 1994; Burwell *et al*., 1995). We believe that similar conditions prevail in AD/Park and with advancing age a reduction in the capacity to synthesize dopamine by nigral neurons does lead to diminished extracellular dopamine levels. Decreases in postsynaptic D_2 receptor number (Joyce *et al*., 1998), which may result from the sustained extracellular dopamine over a prolonged period of time, would also contribute to diminished response to dopamine (see section V.D). Interestingly, as the mesolimbic dopamine system exhibits much lower native expression of dopamine transporter and dopamine transporter expression is modified to a much lesser degree (e.g., Bannon and Whitty, 1997; Ma *et al*., 1999) but exhibit similarly high levels of TH, age-related changes in dopamine transporter function would be less evident in this dopaminergic system. Thus, an exaggerated hypodopaminergic function would occur in the terminal sites of the nigrostriatal dopaminergic system in AD/Park resulting in parkinsonism that is relatively resistant to relief by L-dopa.

IV. Striatal Circuits and Dopamine Receptors

Five distinct subtypes of G-protein coupled dopamine receptors mediate the actions of dopamine, three of which, D_2, D_3, and D_4, belong to the D_2 subfamily (Civelli *et al*., 1993). These receptor subtypes are differentially expressed in regions of the human brain and, therefore, might mediate different actions of dopamine (Joyce and Meador-Woodruff, 1997). It has been widely studied how dopamine regulates output pathways of the striatum via interaction with different dopamine receptors that are, in turn, differentially expressed in these output pathways (see below). However, to date, all effective dopamine agonists stimulate the D_2 receptor, and changes in expression of this receptor have been hypothesized to underlie the deteriorated response to antiparkinsonian agents in Parkinson's disease. Furthermore, research has almost solely focused on how loss of dopamine regulates expression of D_1 and D_2 receptor mRNAs in the output pathways of the striatum, with little information on other receptor subtypes.

A. D_1 and D_2 Receptors

The functional territories of the striatum have different classes of neurons that give rise to segregated efferents. For the motor striatum (dorsal putamen in human) the striatal-pallidal-thalamo circuitry has been well established. In rat, the caudate-putamen gives rise to two distinct efferent pathways. "Direct" pathway neurons express Substance P (preprotachykinin, mRNA) and project to the entopeduncular nucleus (analogous to the internal segment of the globus pallidus in primates) and substantia nigra pars reticulata (SNr). "Indirect" pathway neurons express enkephalin (preproenkephalin, mRNA) and project to the globus pallidus (external segment in primates) and subthalamic nucleus. The influence of the direct and indirect pathways are considered to be opposing and are thought to function in focusing movements, to increase or decrease the probability of movement, and to scale movement (Levy *et al*., 1997). The neurotransmitter dopamine tonically modulates these two pathways via inhibition of the indirect pathway and excitation of the direct pathway (Fig. 48.3A, see color insert). In the rat neostriatum, "direct," or striatonigral, and "indirect," or striatopallidal, output neu-

rons preferentially express D_1 and D_2 receptors, respectively, with little overlap (Gerfen *et al*., 1990; Curran and Watson, 1995; Le Moine and Bloch, 1995). Loss of dopamine leads to changes in expression levels for D_1 and D_2 receptors, and changes in dopamine-mediated activity of the direct and indirect pathways (Fig. 48.3B, see color insert). It is often assumed that in the primate neostriatum the D_1 and D_2 receptors are similarly segregated. Ontogenetic studies have shown that in the neonatal human neostriatum the D_1 receptor is expressed in neurons expressing substance P but not enkephalin (Brana *et al*., 1996) and the D_2 receptor expression tends to overlap with that of enkephalin. Their findings also demonstrated that, in contrast to what was expected from similar studies in rodents, D_2 receptor mRNA and enkephalin mRNA do not display identical, overlapping expression patterns in striatal neurons during human ontogeny (Brana *et al*., 1997). However, to the best of our knowledge, no comprehensive double-labeling study has been done in the adult primate brain to establish the cellular expression of dopamine receptor mRNAs.

As initially postulated by Heimer and Wilson (1975) it has now been convincingly demonstrated that there are parallel efferent circuits originating from the dorsal and ventral striatum with analogous ''direct'' and ''indirect'' pathways. In the rat, the nucleus accumbens projects primarily to the ventral pallidum and the core of the nucleus accumbens projects to the lateral ventral pallidum which send projections to the subthalamic nucleus and SNr. The shell of the nucleus accumbens project to the medial ventral pallidum, which provides efferent to the mediodorsal thalamus. As cells in the shell coexpress D_1 mRNA and Substance P and the cells in the core preferentially express D_2 mRNA and enkephalin (Curran and Watson, 1995; Lu *et al*., 1998), they are presumed to modulate direct and indirect ventral striato-pallidol-thalamic pathways.

B. D_3 Receptor

The dopamine D_3 receptor has high homology with the D_2 receptor and shares many pharmacological features of the latter including high affinity for D_2 receptor antagonists clinically used in treatment of schizophrenia and D_2 agonists for treatment of Parkinson's disease. In the rodent, the D_3 receptor is generally much less abundant in the brain than the D_2 receptor but the difference is particularly striking in the caudate-putamen. D_3 binding sites and mRNA are densest in the nucleus accumbens the limbic region of the striatum, whereas in the caudate-putamen D_3 binding sites are low and mRNA is barely detectable (Bouthenet *et al*., 1991). The accumbens shell, which is the prime source of the ''direct'' efferents to the medial ventral pallidum contains considerably higher concentrations of D_3 binding sites and D_3 mRNA than the core, which is the opposite for D_2 mRNA and binding sites. Studies have shown that in the rat nucleus accumbens the D_3 receptor mRNA is expressed at higher concentrations in neurons expressing the D_1 dopamine receptor, Substance P, prodynorphin, and/or neurotensin, with little overlap with D_2 mRNA (Curran and Watson, 1995; Ridray *et al*., 1998). However, one comprehensive double-*in situ* hybridization histochemistry study has shown that in the rat nucleus accumbens the D_3 receptor is coexpressed with both D_1 and D_2 receptor in Substance P and enkephalin-expressing neurons, respectively (Le Moine and Bloch, 1996). Nonetheless, the data are strongest that in the rat D_3 receptor is preferentially localized in the direct output neurons to the medial ventral pallidum expressing the D_1 receptor (Curran and Watson, 1995).

This limited distribution of the D_3 receptor suggested that its functions are related to the mesolimbic rather than the nigrostriatal dopamine system (Sokoloff *et al*., 1990). In the human, the highest expression of D_3 receptor is in the ventral striatum (Fig. 48.4, top), but is also enriched in the association areas of the caudate nucleus and putamen (Murray *et al*., 1994; Meador-Woodruff *et al*., 1996; Morissette *et al*., 1998; Gurevich and Joyce, 1999). This indicates a more important role of the D_3 receptor in mediating multiple actions of dopamine. The expected segregation of the D_3 receptor to ventral striatal output neurons that coexpress the D_1 receptor mRNA and preproenkephalin mRNA is less clear for the nonhuman primate and human than the rat. In the monkey very few neurons of the ventral pallidum send projections directly to the thalamus, which indicates that the primate ventral pallidum has characteristics similar to the elements of globus pallidus

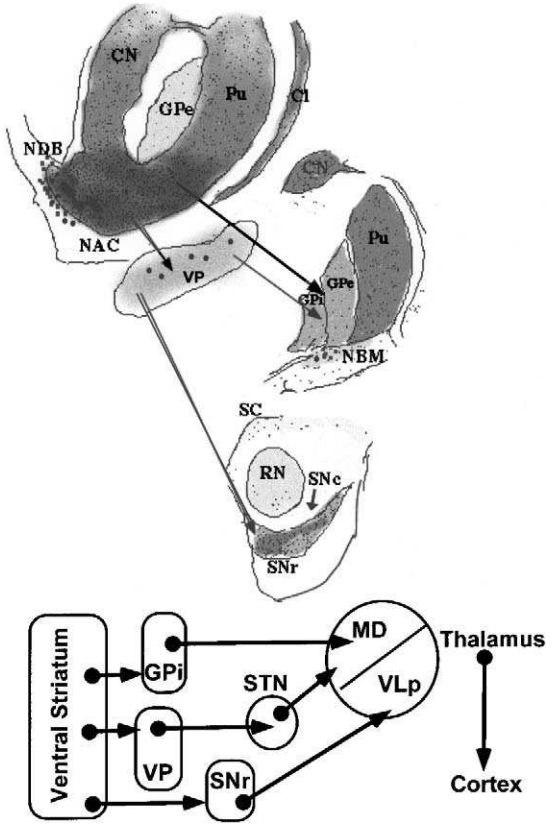

FIG. 48.4. The ventral striatum, D_3 receptors, and the associated circuitry. There are parallel efferent circuits originating from the dorsal and ventral striatum with analogous ''direct'' and ''indirect'' pathways. In the primate, projections from the ventral striatum project to the ventral pallidus which, similar to the GPe, provides projections to the subthalamic nucleus and then the thalamus. Projections from the ventral striatum to the globus pallidus and SNr are, in contrast, directly projecting to the thalamus. The D_3 receptor (gray shading) and D_3 mRNA positive neurons (dots and circles) exhibit higher expression in the ventral striatum than the dorsal striatum and in the regions that receive the projections of the ventral striatum (adapted from Gurevich and Joyce, 1999).

external (GPe) (Haber *et al*., 1993). In addition to the ventral pallidum, the nucleus accumbens also projects to the medial portion of the globus pallidus internal (GPi) and medial portion of the SNr, which do project to thalamus (Fig. 48.4, bottom). This suggests that some neurons of the primate (including the human) nucleus accumbens projecting to the ventral pallidus express enkephalin and that these might coexpress the D_3 receptor and the D_2 receptor. Our studies of the D_3 receptor distribution in the human suggested an overlap in the D_3 and D_2 receptor expression, as D_3 mRNA is expressed in at least 30% of the neurons of the ventral striatum and D_2 mRNA in over 75% of the neurons (Gurevich and Joyce, 1999). Thus, there may be a coexpression of D_3 and D_2 mRNA in a subset of neurons that project to the ventral pallidus as well as in D_1/Substance P neurons. The D_3 receptor-expressing neurons in the human ventral striatum (Fig. 48.4) may give rise not only to the ''direct'' pathway from the ventral striatum to the medial globus pallidus (which innervates anteroventral and mediodorsal nuclei of the thalamus), but also to ''indirect'' projections to the ventral pallidus (which innervates the subthalamic nucleus, substantia nigra pars reticulata and hypothalamus). It seems likely that the D_1/D_3 receptor coexpression and D_2/D_3 receptor coexpression occurs in different projection (neuropeptide expressing) neurons of the ventral striatum. We have hypothesized that in human ventral striatum neurons coexpressing the D_2 and D_3 receptors project to the ventral pallidus (expressing enkephalin) and those coexpressing the D_3 and D_1 receptors ventral pallidus (expressing Substance P) project to the globus pallidus. No studies examining expression of D_1/D_3 or D_2/D_3 receptors with neuropeptide mRNAs have been completed in the human. Expression levels of dopamine receptor mRNAs in these neurons relative to loss of dopamine in Parkinson's disease is also unknown.

V. Dopamine Receptor Contributions to Parkinsonism

A. Dopamine Receptor Changes with Aging

Studies of changes in dopamine receptors in animals have found reductions with age, most consistently for that of the D_2 receptor. In animals the reduction in D_2 receptor number (Severson and Finch, 1980; De Blasi and Mennini, 1982; Joyce *et al*., 1986a; Norman *et al*., 1987; Han *et al*., 1989; Morelli *et al*., 1990) is due to both reduced mRNA levels and synthesis (Norman *et al*., 1987; Han *et al*., 1989; Morelli *et al*., 1990; Della Vedova *et al*., 1992; Weiss *et al*., 1992; Dobie *et al*., 1993; Merchant *et al*., 1993; Mesco *et al*., 1993; Zhang *et al*., 1995). Interestingly, the evident regionally selective effects of the reduction in D_2 receptor number (Joyce *et al*., 1986a; Morelli *et al*., 1990) is not correlated with regionally similar changes in D_2 mRNA levels or enkephalin neurons (colocalized D_2 mRNA) (see Dobie *et al*., 1993; Zoli *et al*., 1993; Zhang *et al*., 1995). This suggests that posttranslational modifications and/or loss of D_2 mRNA-positive neurons may also occur with advanced age (Han *et al*., 1989; Sakata *et al*., 1992; Zhang *et al*., 1995). Reduced D_1 receptor number has also been found in aged rats (Battaglia *et al*., 1988; Han *et al*., 1989; Morelli *et al*., 1990), but other reports are negative (Burwell *et al*., 1995). One study reported reduced D_1 mRNA containing neurons but not mRNA in aging rats (Zhang and Roth, 1997). However, another study has determined that DARP-32 containing neurons (colocalized D_1 mRNA) are not reduced in aged rats but DARP-32 immunoreactive processes were reduced (Zoli *et al*., 1993). Thus, mechanisms underlying the possible reduction in D_1 receptor number are still not conclusive (Table 48.2).

Most studies of the reduction in dopamine receptors with age assumed a linear process and/or utilize a minimal number of time points to establish the age-related decline. Closer examination of scatter plots of data from several studies of striatal tissue, however, suggests a triphasic course of decline (Severson and Finch, 1980; Levin *et al*., 1981; Randall *et al*., 1985; Lai *et al*., 1987). Similarly, in humans it has been assumed that there is a linear process of the age-related decline (Morelli *et al*., 1990; Volkow *et al*., 1998; Wang *et al*., 1998) and this is often utilized to correct for comparisons between neurodegenerative processes and age-matched controls for PET and SPECT imaging. In fact, studies utilizing many age points have identified a nonlinear loss in human striatum (Seeman *et al*., 1987a; Rinne *et al*., 1990a; Antonini *et al*., 1993). This is important, because the loss of D_2 receptors is paralleled by the loss of presynaptically located dopamine transporter number in human (Volkow *et al*., 1998) and animal studies (Marshall and Rosenstein, 1990; Shimizu and Prasad, 1991; Friedemann and Gerhardt, 1992; Irwin *et al*., 1994; Gordon *et al*., 1995; Hebert and Gerhardt, 1998, 1999; Hebert *et al*., 1999). It is now evident that the age-related loss of dopamine transporter in humans is probably not linear with the major

TABLE 48.2 Dopamine Receptors in Aging and Parkinsonian Disorders

	D_2 receptor	D_2 mRNA	D_1 receptor	D_1 mRNA	D_3 receptor
Rat aging	↓ 50–70% In caudate-putamen	↓ 25% In caudate-putamen	↓ 40%? In caudate-putamen	No change	↑ 26% In nuc acb
Human aging	↓ 40–50% In caudate and putamen	Not analyzed	↓ 40% In caudate and putamen	Not analyzed	No change or increase
Parkinson's disease[a]	↑ 26% In putamen	Not analyzed	↑ 26% With dyskinesias	Not analyzed	↓ 20–40% in nuc acb
Alzheimer's with parkinsonism[a]	↓ 40 And 25% in caudate and putamen	Not analyzed	No change	Not analyzed	No change

[a]All values are in relation to age-matched controls. nuc acb, nucleus accumbens.

reduction after age 55 (Bannon *et al*., 1992; Bannon and Whitty, 1997; Ma *et al*., 1999). Although the mechanisms responsible for the decrease in the expression of these proteins with age are not known, the correlation between transporters and receptors, even after accounting for age effects, suggest that their expression may reflect, in part, functional demands on dopaminergic pathways. In fact, they may be linked as is has been shown in dopamine transporter knock-out mice that there is a reduction in D_2 receptor mRNA (Gainetdinov *et al*., 1999). If loss of dopamine transporter leads, initially, to increased dopamine then, as suggested by Shinkai *et al*. (1997), this increased dopamine could lead to death of D_2 receptor synthesizing neurons. While the mechanisms for this are not known, it could possibly contribute to the decline in D_2 receptor number with age (Han *et al*., 1989; Sakata *et al*., 1992; Zhang *et al*., 1995).

This age-related loss of D_2 receptors is functionally relevant as there is a specific correlation between loss of D_2 receptors and reaction time performance and sensorimotor responsiveness in aged rats (MacRae *et al*., 1988) and these impairments are reversed by dopamine uptake inhibitors or the mixed dopamine agonist apomorphine (Marshall and Berrios, 1979; Marshall and Altar, 1986; Hebert and Gerhardt, 1998). The reduced locomotor response to dopaminergic drugs, as well as the loss of spontaneous locomotor and sensorimotor responsiveness (Friedemann and Gerhardt, 1992; Burwell and Gallagher, 1993; Emerich *et al*., 1993; Dorce and Palermo-Neto, 1994; Irwin *et al*., 1994; Hebert and Gerhardt, 1998) in aged animals appears to be most closely related to the loss of D_2 receptors from motor division of the striatum (Joyce *et al*., 1986a; Morelli *et al*., 1990; Dobie *et al*., 1993), but other functional effects have not been explored in detail. One study has not found a reduction in D_2-like receptors, in which age-related behavioral decline was found (Burwell *et al*., 1995). However, that study utilized [^{125}I]epidepride which binds to both D_2 and D_3 receptors (Murray *et al*., 1994). In contrast, selective labeling of the D_3 receptor showed that D_3 receptor is elevated in striatum and nucleus accumbens of aged rats (Wallace and Booze, 1996) and mRNA levels, unlike for D_2, are unchanged in striatum or slightly elevated in nucleus accumbens of aged rats (Valerio *et al*., 1994).

This, coupled with research on the behavioral response to dopamine agonists, suggests that there are functionally important regional and/or receptor specific changes with aging. Whereas there is decreased locomotor response to mixed dopamine agonists (apomorphine) or dopamine-releasing agent amphetamine, there is increased stereotypic response to these drugs with advanced age (Ushijima *et al*., 1987; Stoessl *et al*., 1989; Dorce and Palermo-Neto, 1994; Crawford and Levine, 1997). One study utilizing a more selective D_2/D_3 agonist (quinpirole) also showed that there was a shift in the behavioral response in aged rats to less locomotor activity and rearing to more intense stereotypies (Crawford and Levine, 1997). While this appears contradictory to the reports of the decreased in D_2 receptor number with age, one explanation for this is that the intense stereotypies induced by mixed dopamine agonists may be more dependent on costimulation of D_2 and D_1 receptors in young rats but less so in aged rats. This may occur through changes in the ratio of D_2 to D_1 receptors if the loss of D_2 receptors is greater than D_1 receptors. However, some data suggest that a loss of D_1 receptors occur with age (Battaglia *et al*., 1988; Han *et al*., 1989; Morelli *et al*., 1990), but others are negative (Burwell *et al*., 1995). An alternative explanation is that loss of D_2 receptor and elevation of D_3 receptor with age alters their relative balance. As these two receptors appear to be restricted to different populations of neurons, it may well be that age-related changes in some behavioral responses to dopamine agonists may reflect changes in the responses of D_2 receptor and of D_3 receptor-linked striatal efferents.

B. D_2 and D_3 Receptors and Parkinson's Disease

1. D_2 Receptors Are Functionally Relevant in Parkinson's Disease

Dopaminergic receptor stimulation that results in relief of parkinsonian motor signs is most consistently observed with drugs that interact with D_2-like receptors (Nomoto *et al*., 1985; Loschmann *et al*., 1992; Vermeulen *et al*., 1999). Evidence for efficacy of D_1 agonist stimulation in experimental nonhuman primate models of parkinsonism have been more difficult to interpret as they produce dyskinesias and their antiparkinsonian relief develops rapid tolerance (Vermeulen *et al*., 1999). Recent introduction of new D_1 agonists may challenge this but these agonists are only partially selective for the D_1 receptor having only 14-fold selectivity versus D_2 receptors (Shiosaki *et al*., 1996). Therefore, it is likely that their antiparkinsonian activity occurs through stimulation of D_2-like or D_2 and D_1 receptors. Hence, there is good reason to focus our efforts on understanding where D_2 agonists act to ameliorate Parkinson's disease symptoms. The D_2 receptor family includes three subtypes, D_2, D_3, and D_4 (Joyce and Meador-Woodruff, 1997), which exhibit distinct pharmacological properties and are concentrated in the brain in different regions. Within the basal ganglia D_2 and D_3 and only extremely low levels of the D_4 receptor are expressed (Joyce and Meador-Woodruff, 1997; but see Khan *et al*., 1998), indicating that stimulation of D_2 and D_3 receptors prominently contribute to the antiparkinsonian effects of most compounds used. It has been presumed that it is activity at D_2 receptors that is most relevant to the antiparkinsonian effects of dopaminergic agents (but see section V.B.2). This was because the other subtype, the D_3 receptor, was originally shown to be more selectively localized to the limbic regions of brain in rats and at low levels of expression, whereas D_2 receptors are at high levels throughout the striatum (see section IV.B). In addition, upregulation of the D_2 receptor by dopamine deafferentation and downregulation by dopaminergic stimulation is consistent with a prominent role of the D_2 receptor in this regard (Reches *et al*., 1982, 1984; Alexander *et al*., 1991; Goulet *et al*., 1997; Elsworth *et al*., 1998).

The elevation of striatal D_2 receptor number found in experimental animal models of parkinsonism have also been observed in Parkinson's disease but the relationship to duration of the illness and therapeutic intervention is less clear. We (Joyce, 1993; Ryoo *et al*., 1998) and others (Bokobza *et al*., 1984; Seeman *et al*., 1987b; Piggott *et al*., 1999a) have observed 25–35% increases of D_2 receptors in postmortem studies of Parkinson's disease. *In vivo* studies in Parkinson's

disease with PET ([^{11}C]*N*-methyl-spiperone, [^{11}C]raclopride) and SPECT ([^{123}I]-IBZM) have been utilized to explore the relationship between disease duration and/or chronic drug therapy and D_2 receptor number. Consistent with the postmortem studies, elevation of D_2-like receptor binding of tracer injections of these radioligands have been identified in the striatum of early Parkinson's disease and those without L-dopa treatment (Guttman *et al*., 1986; Rinne *et al*., 1990b,c; Knable *et al*., 1995; Turjanski *et al*., 1997; Ichise *et al*., 1999). Typically the hemisphere contralateral to the parkinsonian symptoms shows the most significant elevation (Rinne *et al*., 1990b,c; Knable *et al*., 1995; Ichise *et al*., 1999) and, with appropriate imaging, the putamen is seen to be most affected (Antonini *et al*., 1997a; Turjanski *et al*., 1997). A reduction in D_2-like receptor binding of these radioligands has been observed with increased disease duration (Hagglund *et al*., 1987; Antonini *et al*., 1995, 1997a) and/or complicated response to L-dopa (Guttman *et al*., 1986; Brooks *et al*., 1992; Pizzolato *et al*., 1995) and has led to the speculation that the loss of D_2 receptors contributes to a deteriorated response to L-dopa in Parkinson's disease (Pizzolato *et al*., 1995). Postmortem studies by ourselves and other groups indicate that with appropriate discrimination between D_2 receptor subtypes that D_2 receptors are *not* reduced at any stage in Parkinson's disease, but the D_3 receptor is reduced (Rinne *et al*., 1991; Ryoo *et al*., 1998; Piggott *et al*., 1999a). This cannot be adequately explored with *in vivo* imaging because: (1) there are not suitable imaging agents that discriminate between D_2 receptor subtypes; and (2) the accurate diagnosis of Parkinson's disease is made with no more than 80% confidence with experienced clinicians (Daniel and Lees, 1993).

Several PET and SPECT imaging studies have suggested that chronic treatment with L-dopa in Parkinson's disease cases leads to downregulation of D_2 receptors, and, thus, to a deteriorated response to treatment (Brucke *et al*., 1991; Brooks *et al*., 1992; Guttman *et al*., 1997; Turjanski *et al*., 1997). However, the results of those studies are questionable since the studies did not compare groups that were similar with respect to duration of illness *and* the presence or absence of drug treatment. Furthermore, the specific examination of short-term treatment of 3 to 4 months with L-dopa or a D_2 agonist could not be observed to downregulate [^{11}C]raclopride binding to D_2 receptors in Parkinson's disease (Antonini *et al*., 1994). Additionally, the results of PET and SPECT studies may be misleading as this requires the accurate diagnosis of Parkinson's disease, which can be wrong in at least 20% of clinically diagnosed parkinsonian disorders (Daniel and Lees, 1993). In fact, some groups have suggested that those parkinsonian cases with lower D_2 receptor number determined by PET/SPECT imaging are typically never responsive to L-dopa or dopamine agonists and are not true Parkinson's disease (Schelosky *et al*., 1993; Antonini *et al*., 1997b; J. C. Schwarz *et al*., 1998). While we (Joyce, 1993) and others (Bokobza *et al*., 1984; Seeman *et al*., 1987b) have observed increases in [^{3}H]spiroperidol binding to D_2 receptors in Parkinson's disease, we did not find an elevation in their number in a recent study using the radioligand [^{125}I]epidepride to label D_2-like receptors (Joyce *et al*., 1998). [^{125}I]epidepride nonselectively labels D_3 and D_2 receptors with equally high affinity and has very low affinity for the D_4 receptor (Murray *et al*., 1994), whereas [^{3}H]spiroperidol has low affinity for the D_3 receptor (Sokoloff *et al*., 1990). In contrast, our results with selective labeling of D_2 and D_3 receptors suggest that D_2 receptors remain elevated in Parkinson's disease even with long disease duration (Ryoo *et al*., 1998; also see Piggott *et al*., 1999a) but a loss of D_3 receptors in the late stages may be correlated with a loss of a therapeutic response to L-dopa or specific clinical symptoms (see below). This issue is important since SPECT imaging agents ([^{123}I]IBZM) and at least one of the PET imaging agents ([^{11}C]raclopride) have equal affinity for D_2 and D_3 receptors. Hence, results of the PET/SPECT imaging studies of depamine receptors and Parkinson's disease may be confounded by the use of nonselective radioligands.

2. Changes in Expression of D_3 Receptors in Parkinson's Disease

Studies in the rat indicate D_3 receptors may be regulated by loss of dopamine afferents differently from the D_2 receptor and produce a reduction in receptor number and mRNA expression (Lévesque *et al*., 1995). Initial reports in MPTP-treated monkey model of parkinsonism and in Parkinson's disease did not identify changes in D_3 receptor number with loss of dopamine innervation (Hurley *et al*., 1996a,b). We examined D_2 and D_3 receptor changes associated with the onset and recovery of parkinsonian signs in MPTP-treated cats (Schneider *et al*., 1986; Schneider and Rothblat, 1991). We established that increases in D_2 receptor number or mRNA expression did not correspond with the onset of parkinsonian signs but peaked after the parkinsonism was established. Loss of D_3 receptors, on the other hand, corresponded to injury to the mesostriatal dopaminergic system and was related to the initial expression of parkinsonian signs (Fig. 48.5). MPTP-treated cats, unlike other animals, exhibit spontaneous recovery from the parkinsonism. Relief from parkinsonism was associated with elevation of D_3 receptor number but permanent reduction of parkinsonian signs was related to normalization of both D_2 and D_3 receptor number. Temporally, the normalization of D_3 receptor number also corresponded to increased extracellular dopamine in the ventral striatum, possible via sprouting dopamine fibers in the area. We hypothesize that in parkinsonism, partial recovery and full recovery may involve regulation of different dopamine receptor subtypes and possible alterations in coexpression of dopamine receptors at least in a subset of striatal neurons. Subsequent studies have partially confirmed those findings, identifying a reduction of D_3 receptor number in MPTP-treated monkeys and in Parkinson's disease when parkinsonian signs are apparent (Morissette *et al*., 1998; Ryoo *et al*., 1998; Piggott *et al*., 1999a).

How does this relate to our findings in Parkinson's disease? We had explored changes in D_2 and D_3 receptor while simultaneously measuring the extent of dopamine fiber loss in striatum of autopsied cases with relatively short or long histories of Parkinson's disease (Ryoo *et al*., 1998). Dopaminergic innervation to the caudal putamen was heavily reduced and to a lesser extent in the rostral putamen in Parkinson's disease but the mesolimbic dopaminergic system was less affected. D_3 receptors were profoundly reduced in Parkinson's disease by 40 to 45%, particularly in the ventral striatum and was due to a change in B_{max} values. In contrast, D_2 receptors were elevated

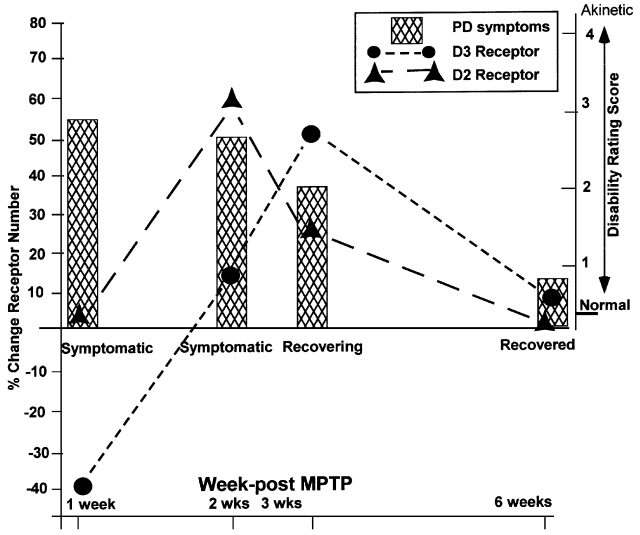

FIG. 48.5 Alterations in striatal D_2 and D_3 receptor number in MPTP-treated cats in relation to symptom severity. MPTP-treated cats initially show severe akinesia related to the loss of dopamine innervation but over 6 weeks exhibit behavioral recovery. The initial akinesia and recovery better correlated with changes in D_3 receptor number than D_2 receptor number. Akinesia rating scale (right axis) is shown in the hatched bars and the percentage change in striatal D_2 and D_3 receptor number (left axis) by the dotted lines.

in Parkinson's disease in the dorsal putamen by 15%, consistent with our previous results (Joyce, 1993). Interestingly, the reduction in D_3 receptor number occurred in Parkinson's disease cases with a duration of greater than 9 years, typically when the clinical benefits of L-dopa were reduced and behavioral symptoms such as depression were present. D_2 receptors were elevated in Parkinson's disease in all cases, regardless of disease duration. As the reduction of D_3 receptor number was small (-17%) in the overall Parkinson's disease sample of Piggott and associates (1999a) and was not found in that of Hurley *et al*., (1996b), it may suggest that clinically defined subgroups are more affected. To provide preliminary information on relationship between clinical subgroups of Parkinson's disease and changes in D_3 receptor number we processed tissue from an additional 13 Parkinson's disease cases that met clinical and neuropathological criteria for Parkinson's disease and 6 controls. Clinical information regarding presence or absence of dementia and whether the Parkinson's disease cases had become relatively resistant to the beneficial response of L-dopa was acquired (poor vs good responder). Tissue from the striatum was processed for D_3 receptor autoradiography, TH immunoautoradiography (TH-IR), and dopamine transporter autoradiography (Fig. 48.6, see color insert).

Dopamine transporter binding in all Parkinson's disease cases was lower than measures of TH-IR, which reflects, in part, the lowered dopamine transporter mRNA but elevated TH mRNA in remaining nigral neurons (Joyce *et al*., 1997). Nonetheless, the dorsal putamen was reduced by 95% for dopamine transporter and 50% for TH, indicating substantial loss of dopaminergic innervation (Fig. 48.6). Overall for the Parkinson's disease group the nucleus accumbens was less affected exhibiting a reduction of 65% for dopamine transporter and 30% for TH-IR. When differentiated by Parkinson's disease only versus Parkinson's disease with dementia (with or without AD) there was a greater loss of dopaminergic innervation in the nucleus accumbens for the Parkinson's disease with dementia as determined with TH-IR, but less of a difference measured with dopamine transporter binding. Measurement of D_3 receptor number showed only a small decline for the Parkinson's disease group as a whole but significant differences emerged (even with the small *n*) for the subgroups. Having Parkinson's disease with an additional diagnosis of dementia (with or without AD) or had become a poor responder to antiparkinsonian medication was correlated with lower D_3 receptor number (-28%). Piggot and associates (1999a) and our own results have shown that D_3 receptor number is elevated in the ventral striatum of AD, so the reduction in the Parkinson's disease with dementia group is unlikely to be due to AD pathology per se. It may be related to the greater loss of dopaminergic innervation in the nucleus accumbens. The Parkinson's disease cases reported as poor responders to Parkinson's disease medication all had originally responded well to Sinemet, were not identified at neuropathology as having progressive supranuclear palsy or other Parkinson-like diagnosis with known poor response to antiparkinsonian medication and typically also did not respond to direct dopamine agonists. Thus, we believe that reduced D_3 receptor number is correlated with certain subgroups of Parkinson's disease and may also be related to a further diminishment in the mesolimbic dopamine system.

While the mesolimbic dopamine system is relatively spared in Parkinson's disease (Murray *et al*., 1995; Dymecki *et al*., 1996; Miller *et al*., 1997, 1999), cell loss within the VTA and loss of dopamine afferents in the nucleus accumbens may increase with duration of illness and/or be associated with specific symptoms (Zweig *et al*., 1989; Jellinger, 1991; Chinaglia *et al*., 1992; Narabayashi, 1995; McRitchie *et al*., 1997). It has been proposed that rigidity, tremor, and secondary akinesia (that related to rigidity) start first with degeneration of the ventral tier of the SNc followed by spread of the pathology to the dorsal tier and medial nuclei (source of the mesolimbic dopamine system), which may produce primary akinesia (bradykinesia and psychomotor retardation), dementia, and depression. This spreading of pathology from one functional system to another might be one of the key factors responsible for the progressive worsening of the disease and increase in nonresponders with disease duration (Jellinger, 1991; Narabayashi, 1995). Studies in experimental models of Parkinson's have found that mesolimbic dopamine loss are related to deficits in initiation of movement and specific aspects of goal-directed behavior (Carey, 1983; Wolterink *et al*., 1990; Salamone *et al*., 1997; Meeker *et al*., 1998), as well as some aspects of cognitive function (Salamone, 1992; McCullough *et al*., 1993; Ploeger *et al*., 1994). While the focus has been on D_2 receptor effects of antiparkinsonian agents, D_3-preferring agonists can be effective in ameliorating motor symptoms in Parkinson's disease patients (Hubble *et al*., 1995; Molho *et al*., 1995; Shannon *et al*., 1997) and MPTP-treated monkeys (Blanchet *et al*.. 1997). In monkeys treated with MPTP to produce severe akinesia, the D_3-preferring agonist PD 128,907 was found to be equally potent as apomorphine in reversing the hypoactivity, and this effect was blocked by the D_3 antagonist U-99194A

(Blanchet *et al*., 1997). Similarly, L-dopa reversal of MPTP-induced hypoactivity in monkeys is dose-dependently inhibited by the D_3 antagonist nafadotride (Hadjtahar *et al*., 1999). Hence, regulation of the D_3 receptor may be important in understanding the response of Parkinson's disease cases to antiparkinsonian medication. We hypothesize that permanent loss of postsynaptically located D_3 receptors in some Parkinson's disease cases, perhaps associated with additional damage to the mesolimbic dopaminergic system, contributes significantly to specific impairments in Parkinson's disease. It is not evident that these changes are caused by age-related alterations in the dopaminergic system. There is no evidence that D_3 receptors decrease with age after 55 years of age (Piggott *et al*., 1999b) but there is some evidence for a greater impact on cell loss in the A10 region and dorsal tier of the SN with advanced age (Fearnley and Lees, 1991; Piggott *et al*., 1999b). A systematic analysis of the relationship between D_3 receptor, clinical state of Parkinson's disease, loss of mesolimbic dopaminergic system, and response to drug treatment would greatly add to our understanding of this illness and new therapeutic interventions.

C. D_1 Receptors and Parkinson's Disease

1. Therapeutic Intervention in Parkinson's Disease and the D_1 Receptor

It is generally thought that dopamine receptor agonists mainly exert their action in Parkinson's disease through stimulation of D_2-like receptors (Langtry and Clissold, 1990; Kopin, 1993), whereas L-dopa through conversion to dopamine acts to stimulate multiple receptors. For several reasons it was considered essential that stimulation of D_1 receptors should provide beneficial actions in Parkinson's disease. As outlined in section IV, the output neurons to the globus pallidus forming the "direct route" (Fig. 48.3), contain D_1 receptors, whereas the cells of origin of the indirect route express D_2 receptors. Dopamine is able to modulate the balance of activity of the direct and indirect pathways by simultaneously facilitating the activity of the direct pathway (stimulation of D_1 receptors) and inhibiting the activity of the indirect pathway (stimulation of D_2 receptors). In addition, D_2 receptor agonist induction of motor activity in rats is abolished by depletion of dopamine or administration of a D_1 receptor antagonist (Molloy *et al*., 1986; Arnt *et al*., 1987; Clark and White, 1987). Behavioral responses to the D_2 agonist could be restored by administration of a D_1 agonist. Moreover, a D_1 agonist given alone to normosensitive rats does not produce stereotypies but does so when coadministered with a D_2 agonist. Rats and mice depleted of dopamine respond to a D_1 agonist with a locomotor response and show additive responses with a D_2 agonist (Arnt *et al*., 1987; Clark and White, 1987). Therefore, it can be suggested that the restoration of a complete repertoire of motor activity requires the stimulation of both D_1 and D_2 receptors in the parkinsonian brain (Clark and White, 1987).

Evidence for efficacy of D_1 agonist stimulation in experimental nonhuman primate models of parkinsonism has been more difficult to interpret (Vermeulen *et al*., 1999). In the MPTP-lesioned monkey model of Parkinson's disease, the partial but selective D_1 agonist SKF-38393 failed to stimulate motor behavior (Nomoto *et al*., 1985; Boyce *et al*., 1990). Newly developed agents (e.g., A-86929) that are full agonists at the D_1 receptor do stimulate motor behavior of MPTP-lesioned monkeys, act synergistically with D_2 receptor agonists, and have efficacy in Parkinson's disease (Grondin *et al*., 1997; Asin *et al*., 1997; Rascol *et al*., 1999). Nonetheless, several of these compounds lose their efficacy in stimulating motor behavior upon sustained administration and induce dyskinetic behavior. Additionally, these agonists are only partially selective for D_1 receptors having only 14-fold selectivity versus D_2 receptors (Shiosaki *et al*., 1996). Therefore, it is likely that their antiparkinsonian activity occurs through stimulation of D_2 or D_2 and D_1 receptors. Their efficacy in Parkinson's disease may be further complicated by the fact that the majority of Parkinson's disease cases are coadministered L-dopa and chronic L-dopa treatment appears to reduce D_1 receptor mRNA (Morissette *et al*., 1996) and produce internalization of the receptor (Muriel *et al*., 1999). While it is not clear what the functional impact of this is, receptor availability for interactions with G-proteins may be compromised. Hence, therapeutic response to D_1 agonists may be unlikely in Parkinson's disease.

2. D_1 Receptors and L-Dopa Induced Dyskinesias

In our recent study of dopamine receptor changes in Parkinson's disease we determined that there was an increase in D_1 receptor number in the putamen (Joyce *et al*., 1998). However, the scatter graph of the data indicated that there were two groups of Parkinson's disease cases, those with high numbers and those with low numbers of D_1 receptors. Previous studies of striatal D_1 receptor expression in Parkinson's disease cases have often shown increases in D_1 receptor number but significant variability is observed suggesting a bimodal population (Joyce, 1993; Rinne *et al*., 1985; Seeman *et al*., 1987b). The increase in D_1 receptors may reflect changes in one subpopulation of the cases as a result of L-dopa treatment (Rinne *et al*., 1985; Seeman *et al*., 1987b). This is supported by results in nonhuman primate models of parkinsonism. The pattern of dopamine fiber loss in Parkinson's disease is closely mimicked by administration of MPTP in monkeys to make them parkinsonian (Burns *et al*., 1983; Joyce *et al*., 1986b). These animals also develop abnormal involuntary movements similar to what happens in patients with advanced Parkinson's disease in response to L-dopa. Stimulation of D_1 receptors plays an important role in the induction of the dyskinesias (Blanchet *et al*., 1996; Grondin *et al*., 1999). Moreover MPTP-treated monkeys show an increase in D_1 receptor number that is enhanced by chronic administration of L-dopa that leads to abnormal involuntary movements (Graham *et al*., 1993; Blanchet *et al*., 1995; Rioux *et al*., 1997). Hence, the increase in D_1 receptors may contribute to the unwanted extrapyramidal side-effects of L-dopa treatment that occurs in some advanced Parkinson's disease cases. This is difficult to reconcile with the fact that L-dopa appears to lead to downregulation of D_1 mRNA and internalization of the D_1 receptor (Morissette *et al*., 1996; Muriel *et al*., 1999). However, it may be that distinct populations of neurons expressing D_1 receptors are differentially affected by dopamine loss and L-dopa administration.

Both D_1 (Gerfen *et al*., 1990; Joyce, 1991) and D_3 receptors (Levesque *et al*., 1995) are reduced following 6-OHDA lesion in rats, which may be due to their extensive colocalization in a subset of striatal neurons expressing Substance P (Curran and Watson, 1995; Le Moine and Bloch, 1996; Ridray *et al*., 1998). The D_3 receptor reduced by dopamine depletion can be induced to increase in the nucleus accumbens and dorsal caudate-putamen (typically exhibiting low expression) by chronic treatment with L-dopa or D_1 agonists (Bordet *et al*., 1997). Since the effect of L-dopa can also be prevented by blockade of the D_1 receptor, this upregulation of the D_3 receptor may occur through stimulation of the D_1 receptor in neurons coexpressing D_1 and D_3 receptors. The elevation of D_3 mRNA levels in 6-OHDA lesioned rats was concomitant with behavioral sensitization to L-dopa that could be blocked by the preferential D_3 receptor antagonist nafadotride. These results have led to the hypothesis that the D_3 receptor plays a direct role in the L-dopa-induced behavioral sensitization (J. Schwartz *et al*., 1998). Morisette and associates (1998) have reached similar conclusions studying MPTP-treated monkeys. The monkeys exhibit substantial declines in D_3 receptor number, which can be reversed with D_1 but not D_2 agonist treatment. As the D_1 agonist treatment resulted in effective alleviation of parkinsonian signs but also induced dyskinesias, D_3:D_1 receptor interactions may be involved in D_1 agonist-induced dyskinesias. As the D_3 receptor is increased with advancing age, it may provide a fundamental underlying change in D_3:D_1 receptor ratio that is exacerbated with dopamine deafferentation and L-dopa treatment. The D_3 receptor elevation in a subpopulation of D_1 neurons seems to play an important role in behavioral sensitization under conditions of excessive dopaminergic stimulation. However, in a specific test of the hypothesis the D_3 receptor antagonist nafadotride failed to reduce L-dopa-induced dyskinesias in MPTP-treated monkeys (Hadjtahar *et al*., 1999). Thus, the role of the D_1 receptor in the therapeutic response or unwanted behavioral effects of L-dopa in Parkinson's disease remain unresolved.

D. Parkinsonism in Alzheimer's Disease and Loss of D_2 Receptors

While the parkinsonian features observed in a consequential proportion of AD cases may be caused by significant changes in the presynaptic dopaminergic system (Tyrrell *et al*., 1990; Murray *et al*., 1995; Joyce *et al*., 1997), other modifications in the dopaminergic system may also contribute to it. This is suggested by studies in other parkinsonian disorders such as progressive supranuclear palsy and multiple system atrophy, where there is both substantial impact on the presynaptic dopaminergic system (Jellinger *et al*., 1980; Kish *et al*., 1985; Goto *et al*., 1989; Brucke *et al*., 1997) and reduced numbers of postsynaptic D_2 receptors (Brooks *et al*., 1992; Antonini *et al*., 1995). Typically these patients do not respond well to L-dopa or direct dopamine agonists (Quinn, 1994). Similar conditions may exist for AD/Park and one study using SPECT identified a reduced number of D_2 receptors in the striatum of AD (Pizzolato *et al*., 1996). The authors hypothesized that a loss of D_2 receptors could contribute to the parkinsonian features that were apparent in a number of the cases. Moreover, the decline in the number of postsynaptic striatal D_2 receptors could be one of the characteristic pathologic changes of AD, which would distinguish it from Parkinson's disease in which the number of D_2 receptors is elevated (Joyce, 1993; Ryoo *et al*., 1998). Several studies of post-mortem brains have also found losses of D_2 and/or D_1 receptors in the striatum of AD cases (Reisine *et al*., 1978; Cross *et al*., 1984; Rinne *et al*., 1986; Cortés *et al*., 1988). However, those studies made no attempt to distinguish between AD with and without Parkinsonian features and the relationship to dopamine receptor changes in the striatum. To that end we characterized the patterns of dopamine receptor changes in cases of AD, Parkinson's disease, and AD/Park compared to neurologically intact controls (Joyce *et al*., 1998). We have observed differences in the two AD groups, with a decreased density of D_2 receptors in AD cases with parkinsonism but not in those without the parkinsonian features. No change in D_1 receptor number was found. We believe that the decline in the number of postsynaptic striatal D_2 receptors could be one of the characteristic pathologic changes of AD/Park that distinguish it from AD without parkinsonian features. Our results also suggest that, along with changes in the ability to process dopamine appropriately (Murray *et al*., 1995; Joyce *et al*., 1997), the loss of D_2 receptors in those AD cases exhibiting parkinsonian symptoms might explain the incomplete response of these patients to L-dopa treatment (Duret *et al*., 1989; Merello *et al*., 1994). Interestingly, the extensive depletion of striatal dopamine transporter sites, reduced dopamine transporter mRNA expression in SN, and lowered D_2 receptor resemble exaggerated phenomenon observed with aging. It would suggest the possibility that changes with aging may contribute to at least some parkinsonian disorders.

VI. Conclusions

It has been often proposed that age-related declines in SN cell number and striatal dopamine which have been reported in humans with normal aging, may contribute to Parkinson's disease. A review of the published papers indicates that the neurodegenerative changes in Parkinson's disease are, however, unlikely to be components of aging per se. The age-related reduction of dopaminergic neurons of the SN and striatal dopamine fibers is relatively restricted and not topographically related to Parkinson's disease. Other changes, in fact, may be neuroprotective. For example, the age-related loss of dopamine transporter expression in the SN may reduce the risk of cell death resulting from toxins that enter dopaminergic neurons via the transporter, and this reduction may fail to take place in Parkinson's disease. The pattern and extent of cell and fiber loss in Parkinson's disease suggests factors unrelated to aging per se contribute to the neuropathology. In addition, many compensatory changes in the dopamine system appear unique to Parkinson's disease. The elevation of D_2 receptors and decline of D_3 receptors play unique roles in the clinical manifestations of Parkinson's disease and are not observed in aging. However, age-related changes in the dopaminergic system may contribute to the deterioration in clinical response to antiparkinsonian drugs in Parkinson's disease and the development of other clinical signs and symptoms. Additional research on the impact of aging on the mesolimbic

dopaminergic system may help in understanding what occurs in late-stage Parkinson's disease. In the larger picture, moreover, some of the age-related declines in behavior resemble that in Parkinson's disease, occur in other parkinsonian disorders, and may reflect exaggerated forms of aging. For example, loss of dopamine transporter expression in the SN, an age-related reduction in dopamine synthesis and loss of postsynaptic D_2 receptors may cause the parkinsonian features observed in a consequential proportion of AD cases. These changes are observed with aging in animals and humans but not to the same degree. Unlike in Parkinson's disease, the beneficial response to L-dopa is typically absent in AD/Park. Hence, a better understanding of age-related modifications in the dopamine system may help us develop novel pharmacological treatments of this and related disorders.

References

Aarsland, D., Tandberg, E., Larsen, J. P., and Cummings, J. L. (1996). Frequency of dementia in Parkinson disease. *Arch. Neurol. (Chicago)*. **53**, 538–542.

Adolfsson, R., Gottfries, C. G., Roos, B. E., and Winblad, B. (1979). Post-mortem distribution of dopamine and homovanillic acid in human brain, variations related to age, and a review of the literature. *J. Neural Transm*. **45**, 81–105.

Alexander, G. E., Crutcher, M. D., and DeLong, M. R. (1990). Basal ganglia-thalamocortical circuits: Parallel substrates for motor, oculomotor, "prefrontal" and "limbic" functions. *Prog. Brain Res*. **85**, 119–146.

Alexander, G. M., Brainard, D. L., Gordon, S. W., Hichens, M., Grothusen, J. R., and Schwartzman, R. J. (1991). Dopamine receptor changes in untreated and (+)-PHNO-treated MPTP parkinsonian primates. *Brain Res*. **547**, 181–189.

Antonini, A., Leenders, K. L., Reist, H., Thomann, R., Beer, H. F., and Locher, J. (1993). Effect of age on D_2 dopamine receptors in normal human brain measured by positron emission tomography and 11C-raclopride. *Arch. Neurol. (Chicago)* **50**, 474–480.

Antonini, A., Schwarz, J., Oertel, W. H., Beer, H. F., Madeja, U. D., and Leenders, K. L. (1994). [^{11}C]raclopride and positron emission tomography in previously untreated patients with Parkinson's disease: Influence of L-dopa and lisuride therapy on striatal dopamine D_2-receptors. *Neurology* **44**, 1325–1329.

Antonini, A., Vontobel, P., Psylla, M., Gunther, I., Maguire, P. R., Missimer, J., and Leenders, K. L. (1995). Complementary positron emission tomographic studies of the striatal dopaminergic system in Parkinson's disease. *Arch. Neurol. (Chicago)* **52**, 1183–1190.

Antonini, A., Schwarz, J., Oertel, W. H., Pogarell, O., and Leenders, K. L. (1997a). Long-term changes of striatal dopamine D_2 receptors in patients with Parkinson's disease: A study with positron emission tomography and [^{11}C]raclopride. *Mov. Disord.* **12**, 33–38.

Antonini, A., Leenders, K. L., Vontobel, P., Maguire, R. P., Missimer, J., Psylla, M., and Gunther, I. (1997b). Complementary PET studies of striatal neuronal function in the differential diagnosis between multiple system atrophy and Parkinson's disease. *Brain* **120**, 2187–2195.

Arnt, J., Hyttel, J., and Perregaard, J. (1987). Dopamine D-1 receptor agonists combined with the selective D-2 agonist quinpirole facilitate the expression of oral stereotyped behaviour in rats. *Eur. J. Pharmacol*. **133**, 137–145.

Asenbaum, S., Brucke, T., Pirker, W., Podreka, I., Angelberger, P., Wenger, S., Wober, C., Muller, C., and Deecke, L. (1997). Imaging of dopamine transporters with iodine-123-beta-CIT and SPECT in Parkinson's disease. *J. Nucl. Med*. **38**, 1–6.

Asin, K. E., Domino, E. F., Nikkel, A., and Shiosaki, K. (1997). The selective dopamine D_1 receptor agonist A-86929 maintains efficacy with repeated treatment in rodent and primate models of Parkinson's disease. *J. Pharmacol. Exp. Ther.* **281**, 454–459.

Bannon, M. J., and Whitty, C. J. (1997). Age-related and regional differences in dopamine transporter mRNA expression in human midbrain. *Neurology* **48**, 969–977.

Bannon, M. J., Poosch, M. S., Xia, Y., Goebel, D. J., Cassin, B., and Kapatos, G. (1992). Dopamine transporter mRNA content in human substantia nigra decreases precipitously with age. *Proc. Natl. Acad. Sci. U.S.A*. **89**, 7095–7099.

Battaglia, G., Norman, A. B., and Creese, I. (1988). Age-related differential recovery rates of rat striatal D-1 dopamine receptors following irreversible inactivation. *Eur. J. Pharmacol*. **145**, 281–290.

Bézard, E., Gross, C. E., Fournier, M. C., Dovero, S., Bloch, B., and Jaber, M. (1999). Absence of MPTP-induced neuronal death in mice lacking the dopamine transporter. *Exp. Neurol*. **155**, 268–273.

Blanchet, P. J., Calon, F., Martel, J. C., Bédard, P. J., Di Paolo, T., Walters, R. R., and Piercey, M. F. (1995). Continuous administration decreases and pulsatile administration increases behavioral sensitivity to a novel dopamine D_2 agonist (U-91356A) in MPTP-exposed monkeys. *J. Pharmacol. Exp. Ther.* **272**, 854–859.

Blanchet, P. J., Grondin, R., and Bédard, P. J. (1996). Dyskinesia and wearing-off following dopamine D_1 agonist treatment in drug-naive 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned primates. *Mov. Disord.* **11**, 91–94.

Blanchet, P. J., Konitsiotis, S., and Chase, T. N. (1997). Motor response to a dopamine D_3 receptor preferring agonist compared to apomorphine in levodopa-primed 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine monkeys. *J. Pharmacol. Exp. Ther.* **283**, 794–799.

Bokobza, B., Ruberg, M., Scatton, B., Javoy-Agid, F., and Agid, Y. (1984). [^{3}H]spiperone binding, dopamine and HVA concentrations in Parkinson's disease and supranuclear palsy. *Eur. J. Pharmacol*. **99**, 167–175.

Bordet, R., Ridray, S., Carboni, S., Diaz, J., Sokoloff, P., and Schwartz, J. C. (1997). Induction of dopamine D_3 receptor expression as a mechanism of behavioral sensitization to levodopa. *Proc. Natl. Acad. Sci. U.S.A*. **94**, 3363–3367.

Bouthenet, M. L., Souil, E., Martres, M. P., Sokoloff, P., Giros, B., and Schwartz, J. C. (1991). Localization of dopamine D_3 receptor mRNA in the rat brain using in situ hybridization histochemistry: Comparison with dopamine D_2 receptor mRNA. *Brain Res*. **564**, 203–219.

Boyce, S., Rupniak, N. M., Steventon, M. J., and Iversen, S. D. (1990). Differential effects of D_1 and D_2 agonists in MPTP-treated primates: Functional implications for Parkinson's disease. *Neurology* **40**, 927–933.

Brana, C., Caille, I., Pellevoisin, C., Charron, G., Aubert, I., Caron, M. G., Carles, D., Vital, C., and Bloch, B. (1996). Ontogeny of the striatal neurons expressing the D_1 dopamine receptor in humans. *J. Comp. Neurol*. **370**, 23–34.

Brana, C., Aubert, I., Charron, G., Pellevoisin, C., and Bloch, B. (1997). Ontogeny of the striatal neurons expressing the D_2 dopamine receptor in humans: An in situ hybridization and receptor-binding study. *Brain Res. Mol. Brain Res*. **48**, 389–400.

Brooks, D. J., Ibanez, V., Sawle, G. V., Playford, E. D., Quinn, N., Mathias, C. J., Lees, A. J., Marsden, C. D., Bannister, R., and Frackowiak, R. S. (1992). Striatal D_2 receptor status in patients with Parkinson's disease, striatonigral degeneration, and progressive supranuclear palsy, measured with ^{11}C-raclopride and positron emission tomography. *Ann. Neurol*. **31**, 184–192.

Brucke, T., Podreka, I., Angelberger, P., Wenger, S., Topitz, A., Kufferle, B., Muller, C., and Deecke, L. (1991). Dopamine D_2 receptor imaging with SPECT: Studies in different neuropsychiatric disorders. *J. Cereb. Blood Flow Metab.* **11**, 220–228.

Brucke, T., Asenbaum, S., Pirker, W., Djamshidian, S., Wenger, S., Wober, C., Muller, C., and Podreka, I. (1997). Measurement of the dopaminergic degeneration in Parkinson's disease with [^{123}I] beta-CIT and

SPECT. Correlation with clinical findings and comparison with multiple system atrophy and progressive supranuclear palsy. *J. Neural. Transm.* **50** (Suppl.), 9–24.

Burns, R. S., Chiueh, C. C., Markey, S. P., Ebert, M. H., Jacobowitz, D. M., and Kopin, I. J. (1983). A primate model of parkinsonism: Selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4546–4550.

Burwell, R. D., and Gallagher, M. (1993). A longitudinal study of reaction time performance in Long-Evans rats. *Neurobiol. Aging* **14**, 57–64.

Burwell, R. D., Lawler, C. P., and Gallagher, M. (1995). Mesostriatal dopamine markers in aged Long-Evans rats with sensorimotor impairment. *Neurobiol. Aging* **16**, 175–186.

Calne, D. B., and Calne, J. S. (1988). Normality and disease. *Can. J. Neurol. Sci.* **15**, 3–4.

Calne, D. B., and Langston, J. W. (1983). Aetiology of Parkinson's disease. *Lancet* **2**, 1457–1459.

Calne, D. B., Eisen, A., McGeer, E., and Spencer, P. (1986). Alzheimer's disease, Parkinson's disease, and motoneurone disease: Abiotrophic interaction between ageing and environment? *Lancet* **2**, 1067–1070.

Carey, R. J. (1983). Differential effects of limbic versus striatal dopamine loss on motoric function. *Behav. Brain Res.* **7**, 283–296.

Carlsson, A., and Winblad, B. (1976). Influence of age and time interval between death and autopsy on dopamine and 3-methoxytyramine levels in human basal ganglia. *J. Neural Transm.* **38**, 271–276.

Cassarino, D. S., and Bennett, J. P., Jr. (1999). An evaluation of the role of mitochondria in neurodegenerative diseases: Mitochondrial mutations and oxidative pathology, protective nuclear responses, and cell death in neurodegeneration. *Brain Res. Rev.* **29**, 1–25.

Chinaglia, G., Alvarez, F. J., Probst, A., and Palacios, J. M. (1992). Mesostriatal and mesolimbic dopamine uptake binding sites are reduced in Parkinson's disease and progressive supranuclear palsy: A quantitative autoradiographic study using [^{3}H]mazindol. *Neuroscience* **49**, 317–327.

Chui, H. C., Teng, E. L., Henderson, V. W., and Moy, A. C. (1985). Clinical subtypes of dementia of the Alzheimer type. *Neurology* **35**, 1544–1550.

Civelli, O., Bunzow, J. R., and Grandy, D. K. (1993). Molecular diversity of the dopamine receptors. *Annu. Rev. Pharmacol. Toxicol.* **33**, 281–307.

Clark, D., and White, F. J. (1987). D_1 dopamine receptor—the search for a function: A critical evaluation of the D_1/D_2 dopamine receptor classification and its functional implications. *Synapse* **1**, 347–388.

Cohen, G. (1983). The pathobiology of Parkinson's disease: Biochemical aspects of dopamine neuron senescence. *J. Neural Transm.* **19** (Suppl.), 89–103.

Cortés, R., Probst, A., and Palacios, J. M. (1988). Decreased densities of dopamine D_1 receptors in the putamen and hippocampus in senile dementia of the Alzheimer type. *Brain Res.* **475**, 164–167.

Crawford, C. A., and Levine, M. S. (1997). Dopaminergic function in the neostriatum and nucleus accumbens of young and aged Fischer 344 rats. *Neurobiol. Aging* **18**, 57–66.

Cross, A. J., Crow, T. J., Ferrier, I. N., Johnson, J. A., and Markakis, D. (1984). Striatal dopamine receptors in Alzheimer-type dementia. *Neurosci. Lett.* **52**, 1–6.

Curran, E. J., and Watson, S. J., Jr. (1995). Dopamine receptor mRNA expression patterns by opioid peptide cells in the nucleus accumbens of the rat: A double in situ hybridization study. *J. Comp Neurol.* **361**, 57–76.

Daniel, S. E., and Lees, A. J. (1993). Parkinson's Disease Society Brain Bank, London: Overview and research. *J. Neural Transm.* **39** (Suppl.), 165–172.

Date, I., Felten, D. L., and Felten, S. Y. (1990). Long-term effect of MPTP in the mouse brain in relation to aging: Neurochemical and immunocytochemical analysis. *Brain Res.* **519**, 266–276.

De Blasi, A., and Mennini, T. (1982). Selective reduction of one class of dopamine receptor binding sites in the corpus striatum of aged rats. *Brain Res.* **242**, 361–364.

de Keyser, J., Ebinger, G., and Vauquelin, G. (1990). Age-related changes in the human nigrostriatal dopaminergic system. *Ann. Neurol.* **27**, 157–161.

Della Vedova, F., Fumagalli, F., Sacchetti, G., Racagni, G., and Brunello, N. (1992). Age-related variations in relative abundance of alternative spliced D_2 receptor mRNAs in brain areas of two rat strains. *Mol. Brain Res.* **12**, 357–359.

de Rijk, M. C., Tzourio, C., Breteler, M. M., Dartigues, J. F., Amaducci, L., Lopez-Pousa, S., Manubens-Bertran, J. M., Alpérovitch, A., and Rocca, W. A. (1997). Prevalence of parkinsonism and Parkinson's disease in Europe: The EUROPARKINSON Collaborative Study. European Community Concerted Action on the Epidemiology of Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* **62**, 10–15.

Ditter, S. M., and Mirra, S. S. (1987). Neuropathologic and clinical features of Parkinson's disease in Alzheimer's disease patients. *Neurology* **37**, 754–760.

Dobie, D. J., Merchant, K. M., Hamblin, M. W., Vatassery, G. T., Dysken, M. W., and Dorsa, D. M. (1993). Blunting of the neurotensin mRNA response to haloperidol in the striatum of aging rats: Possible relationship to decline in dopamine D_2 receptor expression. *Brain Res.* **616**, 105–113.

Dorce, V. A. C., and Palermo-Neto, J. (1994). Behavioral and neurochemical changes induced by aging in dopaminergic systems of male and female rats. *Physiol. Behav.* **56**, 1015–1019.

Duret, M., Goldman, S., Messina, D., and Hildebrand, J. (1989). Effect of L-dopa on dementia-related rigidity. *Acta Neurol. Scand.* **80**, 64–67.

Dymecki, J., Lechowicz, W., Bertrand, E., and Szpak, G. M. (1996). Changes in dopaminergic neurons of the mesocorticolimbic system in Parkinson's disease. *Folia Neuropathol.* **34**, 102–106.

Ebmeier, K. P., Calder, S. A., Crawford, J. R., Stewart, L., Besson, J. A., and Mutch, W. J. (1990). Clinical features predicting dementia in idiopathic Parkinson's disease: A follow-up study. *Neurology* **40**, 1222–1224.

Elsworth, J. D., Brittan, M. S., Taylor, J. R., Sladek, J. R., Jr., Redmond, D. E., Jr., Innis, R. B., Zea-Ponce, Y., and Roth, R. H. (1998). Upregulation of striatal D_2 receptors in the MPTP-treated vervet monkey is reversed by grafts of fetal ventral mesencephalon: An autoradiographic study. *Brain Res.* **795**, 55–62.

Emerich, D. F., McDermott, P., Krueger, P., Banks, M., Zhao, J., Marszalkowski, J., Frydel, B., Winn, S. R., and Sanberg, P. R. (1993). Locomotion of aged rats: Relationship to neurochemical but not morphological changes in nigrostriatal dopaminergic neurons. *Brain Res. Bull.* **32**, 477–486.

Fabbrini, G., Mouradian, M. M., Juncos, J. L., Schlegel, J., Mohr, E., and Chase, T. N. (1988). Motor fluctuations in Parkinson's disease: Central pathophysiological mechanisms. Part I. *Ann. Neurol.* **24**, 366–371.

Fearnley, J. M., and Lees, A. J. (1991). Ageing and Parkinson's disease: Substantia nigra regional selectivity. *Brain* **114**, 2283–2301.

Fernandez-Ruiz, J., De Miguel, R., Hernandez, M. L., Cebeira, M., and Ramos, J. A. (1992). Comparisons between brain dopaminergic neurons of juvenile and aged rats: Sex-related differences. *Mech. Ageing Dev.* **63**, 45–55.

Flood, D. G., and Coleman, P. D. (1988). Neuron numbers and sizes in aging brain: Comparisons of human, monkey, and rodent data. *Neurobiol. Aging* **9**, 453–463.

Frey, K. A., Koeppe, R. A., Kilbourn, M. R., Vander Borght, T. M., Albin, R. L., Gilman, S., and Kuhl, D. E. (1996). Presynaptic monoaminergic vesicles in Parkinson's disease and normal aging. *Ann. Neurol.* **40**, 873–884.

Friedemann, M. N., and Gerhardt, G. A. (1992). Regional effects of aging on dopaminergic function in the Fischer-344 rat. *Neurobiol. Aging* **13**, 325–332.

Frost, J. J., Rosier, A. J., Reich, S. G., Smith, J. S., Ehlers, M. D., Snyder, S. H., Ravert, H. T., and Dannals, R. F. (1993). Positron emission tomographic imaging of the dopamine transporter with 11C-WIN 35,428 reveals marked declines in mild Parkinson's disease. *Ann. Neurol*. **34**, 423–431.

Gainetdinov, R. R., Fumagalli, F., Jones, S. R., and Caron, M. G. (1997). Dopamine transporter is required for in vivo MPTP neurotoxicity: Evidence from mice lacking the transporter. *J. Neurochem*. **69**, 1322–1325.

Gainetdinov, R. R., Jones, S. R., and Caron, M. G. (1999). Functional hyperdopaminergia in dopamine transporter knock-out mice. *Biol. Psychiatry* **46**, 303–311.

Gallagher, M., and Burwell, R. D. (1989). Relationship of age-related decline across several behavioral domains. *Neurobiol. Aging* **10**, 691–708.

Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J., Jr., and Sibley, D. R. (1990). D_1 and D_2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* **250**, 1429–1432.

German, D. C., White, C. L., III, and Sparkman, D. R. (1987). Alzheimer's disease: Neurofibrillary tangles in nuclei that project to the cerebral cortex. *Neuroscience* **21**, 305–312.

German, D. C., Manaye, K., Smith, W. K., Woodward, D. J., and Saper, C. B. (1989). Midbrain dopaminergic cell loss in Parkinson's disease: Computer visualization. *Ann. Neurol*. **26**, 507–514.

Gibb, W. R. (1992). Melanin, tyrosine hydroxylase, calbindin and substance P in the human midbrain and substantia nigra in relation to nigrostriatal projections and differential neuronal susceptibility in Parkinson's disease. *Brain Res*. **581**, 283–291.

Gibb, W. R., and Lees, A. J. (1987). The progression of idiopathic Parkinson's disease is not explained by age-related changes. Clinical and pathological comparisons with post-encephalitic parkinsonian syndrome. *Acta Neuropathol* **73**, 195–201.

Goldman-Rakic, P. S., and Selemon, L. D. (1986). Topography of corticostriatal projections in nonhuman primates and implications for functional parcellation of the neostriatum. *In* "Cerebral Cortex" (E. G. Jones and A. Peters, eds.), pp. 447–466. Plenum, New York.

Gordon, I., Weizman, R., Rosenne, E., and Rehavi, M. (1995). Developmental and age-related alterations in rat brain presynaptic dopaminergic mechanisms. *Dev. Brain Res*. **85**, 225–228.

Goto, S., Hirano, A., and Matsumoto, S. (1989). Subdivisional involvement of nigrostriatal loop in idiopathic Parkinson's disease and striatonigral degeneration. *Ann. Neurol*. **26**, 766–770.

Goulet, M., Morissette, M., Calon, F., Blanchet, P. J., Falardeau, P., Bédard, P. J., and Di Paolo, T. (1997). Continuous or pulsatile chronic D_2 dopamine receptor agonist (U91356A) treatment of drug-naive 4-phenyl-1,2,3,6-tetrahydropyridine monkeys differentially regulates brain D_1 and D_2 receptor expression: In situ hybridization histochemical analysis. *Neuroscience* **79**, 497–507.

Graham, W. C., Sambrook, M. A., and Crossman, A. R. (1993). Differential effect of chronic dopaminergic treatment on dopamine D_1 and D_2 receptors in the monkey brain in MPTP-induced parkinsonism. *Brain Res*. **602**, 290–303.

Grondin, R., Bédard, P. J., Britton, D. R., and Shiosaki, K. (1997). Potential therapeutic use of the selective dopamine D_1 receptor agonist, A-86929: An acute study in parkinsonian levodopa-primed monkeys. *Neurology* **49**, 421–426.

Grondin, R., Doan, V. D., Grégoire, L., and Bédard, P. J. (1999). D_1 receptor blockade improves L-dopa-induced dyskinesia but worsens parkinsonism in MPTP monkeys. *Neurology* **52**, 771–776.

Gupta, M., Gupta, B. K., Thomas, R., Bruemmer, V., Sladek, J. R., Jr., and Felten, D. L. (1986). Aged mice are more sensitive to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine treatment than young adults. *Neurosci. Lett*. **70**, 326–331.

Gurevich, E. V., and Joyce, J. N. (1999). Distribution of dopamine D_3 receptor expressing neurons in the human forebrain: Comparison with D_2 receptor expressing neurons. *Neuropsychopharmacology* **20**, 60–80.

Guttman, M., Seeman, P., Reynolds, G. P., Riederer, P., Jellinger, K., and Tourtellotte, W. W. (1986). Dopamine D_2 receptor density remains constant in treated Parkinson's disease. *Ann. Neurol*. **19**, 487–492.

Guttman, M., Burkholder, J., Kish, S. J., Hussey, D., Wilson, A., DaSilva, J., and Houle, S. (1997). [^{11}C]RTI-32 PET studies of the dopamine transporter in early Dopa-naive Parkinson's disease: Implications for the symptomatic threshold. *Neurology* **48**, 1578–1583.

Haber, S. N., and Groenewegen, H. J. (1989). Interrelationship of the distribution of neuropeptides and tyrosine hydroxylase immunoreactivity in the human substantia nigra. *J. Comp. Neurol*. **290**, 53–68.

Haber, S. N., and McFarland, N. R. (1999). The concept of the ventral striatum in nonhuman primates. *Ann. N. Y. Acad. Sci*. **877**, 33–48.

Haber, S. N., Lynd-Balta, E., and Mitchell, S. J. (1993). The organization of the descending ventral pallidal projections in the monkey. *J. Comp Neurol*. **329**, 111–128.

Haber, S. N., Ryoo, H., Cox, C., and Lu, W. (1995). Subsets of midbrain dopaminergic neurons in monkeys are distinguished by different levels of mRNA for the dopamine transporter: Comparison with the mRNA for the D_2 receptor, tyrosine hydroxylase and calbindin immunoreactivity. *J. Comp Neurol*. **362**, 400–410.

Hadjtahar, A., Grondin, R., Doan, V. D., Grégoire, L., Sokoloff, P., Schwartz, J.-C., and Bédard, P. J. (1999). Behavioral effects of selective dopamine D_3 receptor agonist/antagonist in primate model of Parkinson's disease. *Soc. Neurosci. Abstr.* **25**, 1598.

Hagglund, J., Aquilonius, S. M., Eckernas, S. A., Hartvig, P., Lundquist, H., Gullberg, P., and Langstrom, B. (1987). Dopamine receptor properties in Parkinson's disease and Huntington's chorea evaluated by positron emission tomography using ^{11}C-N-methyl-spiperone. *Acta Neurol. Scand*. **75**, 87–94.

Han, Z., Kuyatt, B. L., Kochman, K. A., DeSouza, E. B., and Roth, G. S. (1989). Effect of aging on concentrations of D_2-receptor-containing neurons in the rat striatum. *Brain Res*. **498**, 299–307.

Hebert, M. A., and Gerhardt, G. A. (1998). Normal and drug-induced locomotor behavior in aging: Comparison to evoked DA release and tissue content in Fischer 344 rats. *Brain Res*. **797**, 42–54.

Hebert, M. A., and Gerhardt, G. A. (1999). Age-related changes in the capacity, rate, and modulation of dopamine uptake within the striatum and nucleus accumbens of Fischer 344 rats: An in vivo electrochemical study. *J. Pharmacol. Exp. Ther.* **288**, 879–887.

Hebert, M. A., Larson, G. A., Zahniser, N. R., and Gerhardt, G. A. (1999). Age-related reductions in [^{3}H]WIN 35,428 binding to the dopamine transporter in nigrostriatal and mesolimbic brain regions of the Fischer 344 rat. *J. Pharmacol. Exp. Ther.* **288**, 1334–1339.

Heimer, L., and Wilson, R. D. (1975). The subcortical projections of the allocortex: Similarities in the neural associations of the hippocampus, the priform cortex, and the neocortex. *In* "Golgi Centennial Symposium" (M. Santini, ed.), pp. 177–193. Raven Press, New York.

Himi, T., Cao, M., and Mori, N. (1995). Reduced expression of the molecular markers of dopaminergic neuronal atrophy in the aging rat brain. *J. Gerontol.* **50**, B193–B200.

Hobson, P., and Meara, J. (1999). The detection of dementia and cognitive impairment in a community population of elderly people with Parkinson's disease by use of the CAMCOG neuropsychological test. *Age Ageing* **28**, 39–43.

Hornykiewicz, O. (1998). Biochemical aspects of Parkinson's disease. *Neurology* **51**, S2–S9.

Hubble, J. P., Koller, W. C., Cutler, N. R., Sramek, J. J., Friedman, J., Goetz, C., Ranhosky, A., Korts, D., and Elvin, A. (1995). Pramipexole in patients with early Parkinson's disease. *Clin. Neuropharmacol*. **18**, 338–347.

Hurd, Y. L., Pristupa, Z. B., Herman, M. M., Niznik, H. B., and Kleinman, J. E. (1994). The dopamine transporter and dopamine D_2 receptor messenger RNAs are differentially expressed in limbic- and motor-related subpopulations of human mesencephalic neurons. *Neuroscience* **63**, 357–362.

Hurley, M. J., Jolkkonen, J., Stubbs, C. M., Jenner, P., and Marsden, C. D. (1996a). Dopamine D_3 receptors in the basal ganglia of the common marmoset and following MPTP and L-dopa treatment. *Brain Res*. **709**, 259–264.

Hurley, M. J., Stubbs, C. M., Jenner, P., and Marsden, C. D. (1996b). D_3 receptor expression within the basal ganglia is not affected by Parkinson's disease. *Neurosci. Lett*. **214**, 75–78.

Ichise, M., Kim, Y. J., Ballinger, J. R., Vines, D., Erami, S. S., Tanaka, F., and Lang, A. E. (1999). SPECT imaging of pre- and postsynaptic dopaminergic alterations in L-dopa-untreated Parkinson's disease. *Neurology* **52**, 1206–1214.

Irwin, I., Finnegan, K. T., DeLanney, L. E., Di Monte, D., and Langston, J. W. (1992). The relationships between aging, monoamine oxidase, striatal dopamine and the effects of MPTP in C57BL/6 mice: A critical reassessment. *Brain Res*. **572**, 224–231.

Irwin, I., DeLanney, L. E., McNeill, T., Chan, P., Forno, L. S., Murphy, G. M., Jr., Di Monte, D. A., Sandy, M. S., and Langston, J. W. (1994). Aging and the nigrostriatal dopamine system: A non-human primate study. *Neurodegeneration* **3**, 251–265.

Jellinger, K., Riederer, P., and Tomonaga, M. (1980). Progressive supranuclear palsy: Clinico-pathological and biochemical studies. *J. Neural Transm.* (Suppl.), 111–128.

Jellinger, K. A. (1991). Pathology of Parkinson's disease. Changes other than the nigrostriatal pathway. *Mol. Chem. Neuropathol*. **14**, 153–197.

Jellinger, K. A. (1999). Post mortem studies in Parkinson's disease—is it possible to detect brain areas for specific symptoms? *J. Neural Transm.* **56** (Suppl.), 1–29.

Jellinger, K. A., and Paulus, W. (1992). Clinico-pathological correlations in Parkinson's disease. *Clin. Neurol. Neurosurg*. **94** (Suppl.), S86–S88.

Jimenez-Castellanos, J., and Graybiel, A. M. (1987). Subdivisions of the dopamine-containing A8-A9-A10 complex identified by their differential mesostriatal innervation of striosomes and extrastriosomal matrix. *Neuroscience* **23**, 223–242.

Jones, S. R., Gainetdinov, R. R., Jaber, M., Giros, B., Wightman, R. M., and Caron, M. G. (1998). Profound neuronal plasticity in response to inactivation of the dopamine transporter. *Proc. Natl. Acad. Sci. U.S.A*. **95**, 4029–4034.

Joyce, J. N. (1991). Differential response of striatal dopamine and muscarinic cholinergic receptor subtypes to the loss of dopamine. II. Effects of 6- hydroxydopamine or colchicine microinjections into the VTA or reserpine treatment. *Exp. Neurol*. **113**, 277–290.

Joyce, J. N. (1993). Differential response of striatal dopamine and muscarinic cholinergic receptor subtypes to the loss of dopamine. III. Results in Parkinson's disease cases. *Brain Res*. **600**, 156–160.

Joyce, J. N., and Meador-Woodruff, J. H. (1997). Linking the family of D_2 receptors to neuronal circuits in human brain: Insights into schizophrenia. *Neuropsychopharmacology* **16**, 375–384.

Joyce, J. N., Loeschen, S. K., Sapp, D. W., and Marshall, J. F. (1986a). Age-related regional loss of caudate-putamen dopamine receptors revealed by quantitative autoradiography. *Brain Res*. **378**, 158–163.

Joyce, J. N., Marshall, J. F., Bankiewicz, K. S., Kopin, I. J., and Jacobowitz, D. M. (1986b). Hemiparkinsonism in a monkey after unilateral internal carotid artery infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is associated with regional ipsilateral changes in striatal dopamine D-2 receptor density. *Brain Res*. **382**, 360–364.

Joyce, J. N., Smutzer, G., Whitty, C. J., Myers, A., and Bannon, M. J. (1997). Differential modification of dopamine transporter and tyrosine hydroxylase mRNAs in midbrain of subjects with Parkinson's, Alzheimer's with parkinsonism, and Alzheimer's disease. *Mov. Disord*. **12**, 885–897.

Joyce, J. N., Murray, A. M., Hurtig, H. I., Gottlieb, G. L., and Trojanowski, J. Q. (1998). Loss of dopamine D_2 receptors in Alzheimer's disease with parkinsonism but not Parkinson's or Alzheimer's disease. *Neuropsychopharmacology* **19**, 472–480.

Khan, Z. U., Gutierrez, A., Martin, R., Penafiel, A., Rivera, A., and De La, C. A. (1998). Differential regional and cellular distribution of dopamine D_2-like receptors: An immunocytochemical study of subtype-specific antibodies in rat and human brain. *J. Comp. Neurol*. **402**, 353–371.

Kish, S. J., Chang, L. J., Mirchandani, L., Shannak, K., and Hornykiewicz, O. (1985). Progressive supranuclear palsy: Relationship between extrapyramidal disturbances, dementia, and brain neurotransmitter markers. *Ann. Neurol*. **18**, 530–536.

Kish, S. J., Shannak, K., Rajput, A., Deck, J. H., and Hornykiewicz, O. (1992). Aging produces a specific pattern of striatal dopamine loss: Implications for the etiology of idiopathic Parkinson's disease. *J. Neurochem*. **58**, 642–648.

Knable, M. B., Jones, D. W., Coppola, R., Hyde, T. M., Lee, K. S., Gorey, J., and Weinberger, D. R. (1995). Lateralized differences in iodine-123-IBZM uptake in the basal ganglia in asymmetric Parkinson's disease. *J. Nucl. Med*. **36**, 1216–1225.

Kopin, I. J. (1993). The pharmacology of Parkinson's disease therapy: An update. *Annu. Rev. Pharmacol. Toxicol*. **33**, 467–495.

Lai, H., Bowden, D. M., and Horita, A. (1987). Age-related decreases in dopamine receptors in the caudate nucleus and putamen of the rhesus monkey (*Macaca mulatta*). *Neurobiol. Aging* **8**, 45–49.

Langston, J. W., Ballard, P., Tetrud, J. W., and Irwin, I. (1983). Chronic parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* **219**, 979–980.

Langtry, H. D., and Clissold, S. P. (1990). Pergolide. A review of its pharmacological properties and therapeutic potential in Parkinson's disease. *Drugs* **39**, 491–506.

Le Moine, C. and Bloch, B. (1995). D_1 and D_2 dopamine receptor gene expression in the rat striatum: Sensitive cRNA probes demonstrate prominent segregation of D_1 and D_2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. *J. Comp. Neurol*. **355**, 418–426.

Le Moine, C., and Bloch, B. (1996). Expression of the D_3 dopamine receptor in peptidergic neurons of the nucleus accumbens: Comparison with the D_1 and D_2 dopamine receptors. *Neuroscience* **73**, 131–143.

Leverenz, J., and Sumi, S. M. (1986). Parkinson's disease in patients with Alzheimer's disease. *Arch. Neurol. (Chicago)* **43**, 662–664.

Lévesque, D., Martres, M. P., Diaz, J., Griffon, N., Lammers, C. H., Sokoloff, P., and Schwartz, J. C. (1995). A paradoxical regulation of the dopamine D_3 receptor expression suggests the involvement of an anterograde factor from dopamine neurons. *Proc. Natl. Acad. Sci. U.S.A*. **92**, 1719–1723.

Levin, P., Janda, J. K., Joseph, J. A., Ingram, D. K., and Roth, G. S. (1981). Dietary restriction retards the age-associated loss of rat striatal dopaminergic receptors. *Science* **214**, 561–562.

Levy, R., Hazrati, L. N., Herrero, M. T., Vila, M., Hassani, O. K., Mouroux, M., Ruberg, M., Asensi, H., Agid, Y., Feger, J., Obeso, J. A., Parent, A., and Hirsch, E. C. (1997). Re-evaluation of the functional anatomy of the basal ganglia in normal and parkinsonian states. *Neuroscience* **76**, 335–343.

Liu, Y., Stern, Y., Chun, M. R., Jacobs, D. M., Yau, P., and Goldman, J. E. (1997). Pathological correlates of extrapyramidal signs in Alzheimer's disease. *Ann. Neurol*. **41**, 368–374.

Loschmann, P. A., Smith, L. A., Lange, K. W., Jahnig, P., Jenner, P., and Marsden, C. D. (1992). Motor activity following the administration of selective D-1 and D-2 dopaminergic drugs to MPTP-treated common marmosets. *Psychopharmacology* **109**, 49–56.

Lu, X. Y., Ghasemzadeh, M. B., and Kalivas, P. W. (1998). Expression of D_1 receptor, D_2 receptor, substance P and enkephalin messenger RNAs in the neurons projecting from the nucleus accumbens. *Neuroscience* **82**, 767–780.

Lynd-Balta, E., and Haber, S. N. (1994a). The organization of midbrain projections to the ventral striatum in the primate. *Neuroscience* **59**, 609–623.

Lynd-Balta, E., and Haber, S. N. (1994). The organization of midbrain projections to the striatum in the primate: Sensorimotor-related striatum versus ventral striatum. *Neuroscience* **59**, 625–640.

Ma, S. Y., Ciliax, B. J., Stebbins, G., Jaffar, S., Joyce, J. N., Cochran, E. J., Kordower, J. H., Mash, D. C., Levey, A. I., and Mufson, E. J. (1999). Dopamine transporter-immunoreactive neurons decrease with age in the human substantia nigra. *J. Comp. Neurol*. **409**, 25–37.

MacRae, P. G., Spirduso, W. W., and Wilcox, R. E. (1988). Reaction time and nigrostriatal dopamine function: The effects of age and practice. *Brain Res*. **451**, 139–146.

Mann, D. M., and Yates, P. O. (1983). Possible role of neuromelanin in the pathogenesis of Parkinson's disease. *Mech. Ageing Dev.* **21**, 193–203.

Mann, D. M., Yates, P. O., and Marcyniuk, B. (1987). Dopaminergic neurotransmitter systems in Alzheimer's disease and in Down's syndrome at middle age. *J. Neurol. Neurosurg. Psychiatry* **50**, 341–344.

Marshall, J. F., and Altar, C. A. (1986). Striatal dopamine uptake and swim performance of the aged rat. *Brain Res*. **379**, 112–117.

Marshall, J. F., and Berrios, N. (1979). Movement disorders of aged rats: Reversal by dopamine receptor stimulation. *Science* **206**, 477–479.

Marshall, J. F., and Rosenstein, A. J. (1990). Age-related decline in rat striatal dopamine metabolism is regionally homogeneous. *Neurobiol. Aging* **11**, 131–137.

Mayeux, R., Stern, Y., and Spanton, S. (1985). Heterogeneity in dementia of the Alzheimer type: Evidence of subgroups. *Neurology* **35**, 453–461.

Mayeux, R., Stern, Y., Rosenstein, R., Marder, K., Hauser, A., Côté, L., and Fahn, S. (1988). An estimate of the prevalence of dementia in idiopathic Parkinson's disease. *Arch. Neurol. (Chicago)* **45**, 260–262.

McCullough, L. D., Sokolowski, J. D., and Salamone, J. D. (1993). A neurochemical and behavioral investigation of the involvement of nucleus accumbens dopamine in instrumental avoidance. *Neuroscience* **52**, 919–925.

McGeer, P. L., McGeer, E. G., and Suzuki, J. S. (1977). Aging and extrapyramidal function. *Arch. Neurol. (Chicago)* **34**, 33–35.

McRitchie, D. A., Cartwright, H. R., and Halliday, G. M. (1997). Specific A10 dopaminergic nuclei in the midbrain degenerate in Parkinson's disease. *Exp. Neurol*. **144**, 202–213.

Meador-Woodruff, J. H., Damask, S. P., Wang, J., Haroutunian, V., Davis, K. L., and Watson, S. J. (1996). Dopamine receptor mRNA expression in human striatum and neocortex. *Neuropsychopharmacology* **15**, 17–29.

Meeker, D., Kim, J. H., and Vezina, P. (1998). Depletion of dopamine in the nucleus accumbens prevents the generation of locomotion by metabotropic glutamate receptor activation. *Brain Res*. **812**, 260–264.

Merchant, K. M., Dobie, D. J., and Dorsa, D. M. (1993). Differential loss of dopamine D_2 receptor mRNA isoforms during aging in Fischer-344 rats. *Neurosci. Lett*. **154**, 163–167.

Merello, M., Sabe, L., Teson, A., Migliorelli, R., Petracchi, M., Leiguarda, R., and Starkstein, S. (1994). Extrapyramidalism in Alzheimer's disease: Prevalence, psychiatric, and neuropsychological correlates. *J. Neurol., Neurosurg. Psychiatry* **57**, 1503–1509.

Mesco, E. R., Carlson, S. G., Joseph, J. A., and Roth, G. S. (1993). Decreased striatal D_2 dopamine receptor mRNA synthesis during aging. *Brain Res. Mol. Brain Res*. **17**, 160–162.

Miller, G. W., Staley, J. K., Heilman, C. J., Perez, J. T., Mash, D. C., Rye, D. B., and Levey, A. I. (1997). Immunochemical analysis of dopamine transporter protein in Parkinson's disease. *Ann. Neurol*. **41**, 530–539.

Miller, G. W., Erickson, J. D., Perez, J. T., Penland, S. N., Mash, D. C., Rye, D. B., and Levey, A. I. (1999). Immunochemical analysis of vesicular monoamine transporter (VMAT2) protein in Parkinson's disease. *Exp. Neurol*. **156**, 138–148.

Mogenson, G. J., Yang, C. R., and Yim, C. Y. (1988). Influence of dopamine on limbic inputs to the nucleus accumbens. *Ann. N. Y. Acad. Sci*. **537**, 86–100.

Molho, E. S., Factor, S. A., Weiner, W. J., Sanchez-Ramos, J. R., Singer, C., Shulman, L., Brown, D., and Sheldon, C. (1995). The use of pramipexole, a novel dopamine (DA) agonist, in advanced Parkinson's disease. *J. Neural Transm.* **45** (Suppl.), 225–230.

Molloy, A. G., O'Boyle, K. M., Pugh, M. T., and Waddington, J. L. (1986). Locomotor behaviors in response to new selective D-1 and D-2 dopamine receptor agonists, and the influence of selective antagonists. *Pharmacol. Biochem. Behav.* **25**, 249–253.

Mölsä, P. K., Sako, E., Paljarvi, L., Rinne, J. O., and Rinne, U. K. (1987). Alzheimer's disease: Neuropathological correlates of cognitive and motor disorders. *Acta Neurol. Scand.* **75**, 376–384.

Morelli, M., Mennini, T., Cagnotto, A., Toffano, G., and Di Chiara, G. (1990). Quantitative autoradiographical analysis of the age-related modulation of central dopamine D_1 and D_2 receptors. *Neuroscience* **36**, 403–410.

Morissette, M., Goulet, M., Calon, F., Falardeau, P., Blanchet, P. J., Bédard, P. J., and Di Paolo, T. (1996). Changes of D_1 and D_2 dopamine receptor mRNA in the brains of monkeys lesioned with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: Correction with chronic administration of L-3,4-dihydroxyphenylalanine. *Mol. Pharmacol*. **50**, 1073–1079.

Morissette, M., Goulet, M., Grondin, R., Blanchet, P., Bédard, P. J., Di Paolo, T., and Levesque, D. (1998). Associative and limbic regions of monkey striatum express high levels of dopamine D_3 receptors: Effects of MPTP and dopamine agonist replacement therapies. *Eur. J. Neurosci*. **10**, 2565–2573.

Morris, J. C., Drazner, M., Fulling, K., Grant, E. A., and Goldring, J. (1989). Clinical and pathological aspects of parkinsonism in Alzheimer's disease. A role for extranigral factors? *Arch. Neurol. (Chicago)* **46**, 651–657.

Mouradian, M. M., Juncos, J. L., Fabbrini, G., Schlegel, J., Bartko, J. J., and Chase, T. N. (1988). Motor fluctuations in Parkinson's disease: Central pathophysiological mechanisms. Part II. *Ann. Neurol*. **24**, 372–378.

Müller, T., Farahati, J., Kuhn, W., Eising, E. G., Przuntek, H., Reiners, C., and Coenen, H. H. (1998). [123I]beta-CIT SPECT visualizes dopamine transporter loss in de novo parkinsonian patients. *Eur. Neurol*. **39**, 44–48.

Muriel, M. P., Bernard, V., Levey, A. I., Laribi, O., Abrous, D. N., Agid, Y., Bloch, B., and Hirsch, E. C. (1999). Levodopa induces a cytoplasmic localization of D_1 dopamine receptors in striatal neurons in Parkinson's disease. *Ann. Neurol*. **46**, 103–111.

Murray, A. M., Ryoo, H. L., Gurevich, E., and Joyce, J. N. (1994). Localization of dopamine D_3 receptors to mesolimbic and D_2 receptors to mesostriatal regions of human forebrain. *Proc. Natl. Acad. Sci. U.S.A*. **91**, 11271–11275.

Murray, A. M., Weihmueller, F. B., Marshall, J. F., Hurtig, H. I., Gottleib, G. L., and Joyce, J. N. (1995). Damage to dopamine systems differs between Parkinson's disease and Alzheimer's disease with parkinsonism. *Ann. Neurol*. **37**, 300–312.

Muthane, U., Yasha, T. C., and Shankar, S. K. (1998). Low numbers and no loss of melanized nigral neurons with increasing age in normal human brains from India. *Ann. Neurol*. **43**, 283–287.

Nakashima, S., and Ikuta, F. (1985). Catecholamine neurons with Alzheimer's neurofibrillary changes and alteration of tyrosine hydroxylase. Immunohistochemical investigation of tyrosine hydroxylase. *Acta Neuropathol*. **66**, 37–41.

Narabayashi, H. (1995). The neural mechanisms and progressive nature of symptoms of Parkinson's disease—based on clinical, neurophysiological and morphological studies. *J. Neural Transm. [Park. Dis. Dement. Sect.]* **10**, 63–75.

Nishi, K., Kondo, T., and Narabayashi, H. (1989). Difference in recovery patterns of striatal dopamine content, tyrosine hydroxylase activity and total biopterin content after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration: A comparison of young and older mice. *Brain Res*. **489**, 157–162.

Nomoto, M., Jenner, P., and Marsden, C. D. (1985). The dopamine D_2 agonist LY 141865, but not the D_1 agonist SKF 38393, reverses parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the common marmoset. *Neurosci. Lett*. **57**, 37–41.

Norman, A. B., Battaglia, G., and Creese, I. (1987). Differential recovery rates of rat D_2-dopamine receptors as a function of aging and chronic

reserpine treatment following irreversible modification: A key to receptor regulatory mechanisms. *J. Neurosci*. **7**, 1484–1491.

Parent, A., and Hazrati, L. N. (1995). Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. *Brain Res. Brain Res. Rev.* **20**, 91–127.

Pedro-Cuesta, J., and Stawiarz, L. (1991). Parkinson's disease incidence: Magnitude, comparability, time trends. *Acta Neurol. Scand.* **84**, 382–388.

Pifl, C., Hornykiewicz, O., Giros, B., and Caron, M. G. (1996). Catecholamine transporters and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity: Studies comparing the cloned human noradrenaline and human dopamine transporter. *J. Pharmacol. Exp. Ther.* **277**, 1437–1443.

Piggott, M. A., Marshall, E. F., Thomas, N., Lloyd, S., Court, J. A., Jaros, E., Burn, D., Johnson, M., Perry, R. H., McKeith, I. G., Ballard, C., and Perry, E. K. (1999a). Striatal dopaminergic markers in dementia with lewy bodies, Alzheimer's and Parkinson's diseases: Rostrocaudal distribution. *Brain* **122**, 1449–1468.

Piggott, M. A., Marshall, E. F., Thomas, N., Lloyd, S., Court, J. A., Jaros, E., Costa, D., Perry, R. H., and Perry, E. K. (1999b). Dopaminergic activities in the human striatum: Rostrocaudal gradients of uptake sites and of D_1 and D_2 but not of D_3 receptor binding or dopamine. *Neuroscience* **90**, 433–445.

Pizzolato, G., Chierichetti, F., Rossato, A., Cagnin, A., Fabbri, M., Dam, M., Ferlin, G., and Battistin, L. (1995). Alterations of striatal dopamine D_2 receptors contribute to deteriorated response to L-dopa in Parkinson's disease: A [123I]-IBZM SPET study. *J. Neural Transm.* **45** (Suppl.), 113–122.

Pizzolato, G., Chierichetti, F., Fabbri, M., Cagnin, A., Dam, M., Ferlin, G., and Battistin, L. (1996). Reduced striatal dopamine receptors in Alzheimer's disease: Single photon emission tomography study with the D_2 tracer [^{123}I]-IBZM. *Neurology* **47**, 1065–1068.

Ploeger, G. E., Spruijt, B. M., and Cools, A. R. (1994). Spatial localization in the Morris water maze in rats: Acquisition is affected by intra-accumbens injections of the dopaminergic antagonist haloperidol. *Behav. Neurosci*. **108**, 927–934.

Quinn, N. (1994). Multiple system atrophy. *In* ''Movement Disorders'' (C. D. Marsden and S. Fahn, eds.), pp. 262–281. Butterworth Heinemann, London.

Randall, P. K., Severson, J. A., Hurd, S. M., and McClure, W. O. (1985). Effect of duration of haloperidol treatment on DA receptor supersensitization in aging C57BL/6J mice. *Brain Res*. **333**, 85–95.

Ransom, B. R., Kunis, D. M., Irwin, I., and Langston, J. W. (1987). Astrocytes convert the parkinsonism inducing neurotoxin, MPTP, to its active metabolite, MPP+. *Neurosci. Lett*. **75**, 323–328.

Rao, K. M., Nagendra, S. N., and Subhash, M. N. (1995). Monoamine oxidase isoenzymes in rat brain: Differential changes during postnatal development but not aging. *Neurobiol. Aging* **16**, 833–836.

Rascol, O., Blin, O., Thalamas, C., Descombes, S., Soubrouillard, C., Azulay, P., Fabre, N., Viallet, F., Lafnitzegger, K., Wright, S., Carter, J. H., and Nutt, J. G. (1999). ABT-431, a D_1 receptor agonist prodrug, has efficacy in Parkinson's disease. *Ann. Neurol*. **45**, 736–741.

Razdan, S., Kaul, R. L., Motta, A., Kaul, S., and Bhatt, R. K. (1994). Prevalence and pattern of major neurological disorders in rural Kashmir (India) in 1986. *Neuroepidemiology* **13**, 113–119.

Reches, A., Wagner, H. R., Jiang, D., Jackson, V., and Fahn, S. (1982). The effect of chronic L-dopa administration on supersensitive pre- and postsynaptic dopaminergic receptors in rat brain. *Life Sci*. **31**, 37–44.

Reches, A., Wagner, H. R., Jackson-Lewis, V., Yablonskaya-Alter, E., and Fahn, S. (1984). Chronic levodopa or pergolide administration induces down-regulation of dopamine receptors in denervated striatum. *Neurology* **34**, 1208–1212.

Reiner, A., Medina, L., and Haber, S. N. (1999). The distribution of dynorphinergic terminals in striatal target regions in comparison to the distribution of substance P-containing and enkephalinergic terminals in monkeys and humans. *Neuroscience* **88**, 775–793.

Reisine, T. D., Yamamura, H. I., Bird, E. D., Spokes, E., and Enna, S. J. (1978). Pre- and postsynaptic neurochemical alterations in Alzheimer's disease. *Brain Res*. **159**, 477–481.

Ricaurte, G. A., Irwin, I., Forno, L. S., DeLanney, L. E., Langston, E., and Langston, J. W. (1987a). Aging and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced degeneration of dopaminergic neurons in the substantia nigra. *Brain Res*. **403**, 43–51.

Ricaurte, G. A., DeLanney, L. E., Irwin, I., and Langston, J. W. (1987b). Older dopaminergic neurons do not recover from the effects of MPTP. *Neuropharmacology* **26**, 97–99.

Ridray, S., Griffon, N., Mignon, V., Souil, E., Carboni, S., Diaz, J., Schwartz, J. C., and Sokoloff, P. (1998). Coexpression of dopamine D_1 and D_3 receptors in islands of Calleja and shell of nucleus accumbens of the rat: Opposite and synergistic functional interactions. *Eur. J. Neurosci*. **10**, 1676–1686.

Rinne, J. O. (1991). Nigral degeneration in Parkinson's disease in relation to clinical features. *Acta Neurol. Scand.* **136** (Suppl.), 87–90.

Rinne, J. O., Rinne, J. K., Laakso, K., Lonnberg, P., and Rinne, U. K. (1985). Dopamine D-1 receptors in the parkinsonian brain. *Brain Res*. **359**, 306–310.

Rinne, J. O., Sako, E., Paljarvi, L., Molsa, P. K., and Rinne, U. K. (1986). Brain dopamine D-2 receptors in senile dementia. *J. Neural Transm*. **65**, 51–62.

Rinne, J. O., Lonnberg, P., and Marjamaki, P. (1990a). Age-dependent decline in human brain dopamine D_1 and D_2 receptors. *Brain Res*. **508**, 349–352.

Rinne, J. O., Laihinen, A., Nagren, K., Bergman, J., Solin, O., Haaparanta, M., Ruotsalainen, U., and Rinne, U. K. (1990b). PET demonstrates different behaviour of striatal dopamine D-1 and D-2 receptors in early Parkinson's disease. *J. Neurosci. Res*. **27**, 494–499.

Rinne, U. K., Laihinen, A., Rinne, J. O., Nagren, K., Bergman, J., and Ruotsalainen, U. (1990c). Positron emission tomography demonstrates dopamine D_2 receptor supersensitivity in the striatum of patients with early Parkinson's disease. *Mov. Disord*. **5**, 55–59.

Rinne, J. O., Laihinen, A., Lonnberg, P., Marjamaki, P., and Rinne, U. K. (1991). A post-mortem study on striatal dopamine receptors in Parkinson's disease. *Brain Res*. **556**, 117–122.

Rinne, J. O., Bergman, J., Ruottinen, H., Haaparanta, M., Eronen, E., Oikonen, V., Sonninen, P., and Solin, O. (1999). Striatal uptake of a novel PET ligand, [^{18}F]beta-CFT, is reduced in early Parkinson's disease. *Synapse* **31**, 119–124.

Rioux, L., Frohna, P. A., Joyce, J. N., and Schneider, J. S. (1997). The effects of chronic levodopa treatment on pre- and postsynaptic markers of dopaminergic function in striatum of parkinsonian monkeys. *Mov. Disord*. **12**, 148–158.

Ryoo, H. L., Pierrotti, D., and Joyce, J. N. (1998). Dopamine D_3 receptor is decreased and D_2 receptor is elevated in the striatum of Parkinson's disease. *Mov. Disord*. **13**, 788–797.

Sabel, B. A., and Stein, D. G. (1981). Extensive loss of subcortical neurons in the aging rat brain. *Exp. Neurol*. **73**, 507–516.

Sakata, M., Farooqui, S. M., and Prasad, C. (1992). Post-transcriptional regulation of loss of rat striatal D_2 dopamine receptor during aging. *Brain Res*. **575**, 309–314.

Salamone, J. D. (1992). Complex motor and sensorimotor functions of striatal and accumbens dopamine: Involvement in instrumental behavior processes. *Psychopharmacology* **107**, 160–174.

Salamone, J. D., Cousins, M. S., and Snyder, B. J. (1997). Behavioral functions of nucleus accumbens dopamine: Empirical and conceptual problems with the anhedonia hypothesis. *Neurosci. Biobehav. Rev.* **21**, 341–359.

Schelosky, L., Hierholzer, J., Wissel, J., Cordes, M., and Poewe, W. (1993). Correlation of clinical response in apomorphine test with D_2-receptor status as demonstrated by ^{123}I IBZM-SPECT. *Mov. Disord*. **8**, 453–458.

Scherman, D., Desnos, C., Darchen, F., Pollak, P., Javoy-Agid, F., and Agid, Y. (1989). Striatal dopamine deficiency in Parkinson's disease: Role of aging. *Ann. Neurol*. **26**, 551–557.

Schneider, J. S., and Rothblat, D. S. (1991). Neurochemical evaluation of the striatum in symptomatic and recovered MPTP-treated cats. *Neuroscience* **44**, 421–429.

Schneider, J. S., Yuwiler, A., and Markham, C. H. (1986). Production of a Parkinson-like syndrome in the cat with N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): Behavior, histology, and biochemistry. *Exp. Neurol*. **91**, 293–307.

Schneider, J. S., Rothblat, D. S., Wade, T., Joyce, J. N., and Ryoo, H. (1999). Differential modulation of dopamine D_3 and D_2 receptor numbr ein symptomatic and recovered parkinsonian cats. *Soc. Neurosci. Abstr.* **25**, 1598–1598.

Schwarz, J., Tatsch, K., Gasser, T., Arnold, G., Pogarell, O., Kunig, G., and Oertel, W. H. (1998). ^{123}I-IBZM binding compared with long-term clinical follow up in patients with de novo parkinsonism. *Mov. Disord*. **13**, 16–19.

Schwartz, J. C., Ridray, S., Bordet, R., Diaz, J., and Sokoloff, P. (1998). D_1/D_3 receptor relationships in brain coexpression, coactivation, and coregulation. *Adv. Pharmacol*. **42**, 408–411.

Seeman, P., Bzowej, N. H., Guan, H. C., Bergeron, C., Becker, L. E., Reynolds, G. P., Bird, E. D., Riederer, P., Jellinger, K., and Watanabe, S. (1987a). Human brain dopamine receptors in children and aging adults. *Synapse* **1**, 399–404.

Seeman, P., Bzowej, N. H., Guan, H. C., Bergeron, C., Reynolds, G. P., Bird, E. D., Riederer, P., Jellinger, K., and Tourtellotte, W. W. (1987b). Human brain D_1 and D_2 dopamine receptors in schizophrenia, Alzheimer's, Parkinson's, and Huntington's diseases. *Neuropsychopharmacology* **1**, 5–15.

Seibyl, J. P., Marek, K. L., Quinlan, D., Sheff, K., Zoghbi, S., Zea-Ponce, Y., Baldwin, R. M., Fussell, B., Smith, E. O., and Charney, D. S. (1995). Decreased single-photon emission computed tomographic [^{123}I]beta-CIT striatal uptake correlates with symptom severity in Parkinson's disease. *Ann. Neurol*. **38**, 589–598.

Sershen, H., Mason, M. F., Hashim, A., and Lajtha, A. (1985). Effect of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on age-related changes in dopamine turnover and transporter function in the mouse striatum. *Eur. J. Pharmacol*. **113**, 135–136.

Severson, J. A., and Finch, C. E. (1980). Reduced dopaminergic binding during aging in the rodent striatum. *Brain Res*. **192**, 147–162.

Shannon, K. M., Bennett, J. P., Jr., and Friedman, J. H. (1997). Efficacy of pramipexole, a novel dopamine agonist, as monotherapy in mild to moderate Parkinson's disease. The Pramipexole Study Group. *Neurology* **49**, 724–728; erratum: *Ibid.* **50**, 838 (1998).

Shimizu, I., and Prasad, C. (1991). Relationship between [^{3}H]mazindol binding to dopamine uptake sites and [^{3}H]dopamine uptake in rat striatum during aging. *J. Neurochem*. **56**, 575–579.

Shinkai, T., Zhang, L., Mathias, S. A., and Roth, G. S. (1997). Dopamine induces apoptosis in cultured rat striatal neurons; possible mechanism of D_2-dopamine receptor neuron loss during aging. *J. Neurosci. Res*. **47**, 393–399.

Shiosaki, K., Jenner, P., Asin, K. E., Britton, D. R., Lin, C. W., Michaelides, M., Smith, L., Bianchi, B., Didomenico, S., Hodges, L., Hong, Y., Mahan, L., Mikusa, J., Miller, T., Nikkel, A., Stashko, M., Witte, D., and Williams, M. (1996). ABT-431: The diacetyl prodrug of A-86929, a potent and selective dopamine D_1 receptor agonist: In vitro characterization and effects in animal models of Parkinson's disease. *J. Pharmacol. Exp. Ther.* **276**, 150–160.

Sokoloff, P., Giros, B., Martres, M. P., Bouthenet, M. L., and Schwartz, J. C. (1990). Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics. *Nature (London)* **347**, 146–151.

Stoessl, A. J., Martin-Iverson, M. T., Barth, T. M., Dourish, C. T., and Iversen, S. D. (1989). Effects of ageing on the behavioural responses to dopamine agonists: Decreased yawning and locomotion, but increased stereotypy. *Brain Res*. **495**, 20–30.

Strong, R., Samorajski, T., and Gottesfeld, Z. (1982). Regional mapping of neostriatal neurotransmitter systems as a function of aging. *J. Neurochem*. **39**, 831–836.

Szabo, J. (1980). Organization of the ascending striatal afferents in monkeys. *J. Comp. Neurol*. **189**, 307–321.

Tabaton, M., Schenone, A., Romagnoli, P., and Mancardi, G. L. (1985). A quantitative and ultrastructural study of substantia nigra and nucleus centralis superior in Alzheimer's disease. *Acta Neuropathol*. **68**, 218–223.

Tanaka, C., Ishikawa, M., and Shimada, S. (1982). Histochemical mapping of catecholaminergic neurons and their ascending fiber pathways in the rhesus monkey brain. *Brain Res. Bull*. **9**, 255–270.

Tandberg, E., Larsen, J. P., Aarsland, D., and Cummings, J. L. (1996). The occurrence of depression in Parkinson's disease. A community-based study. *Arch. Neurol. (Chicago)* **53**, 175–179.

Tandberg, E., Larsen, J. P., Aarsland, D., Laake, K., and Cummings, J. L. (1997). Risk factors for depression in Parkinson disease. *Arch. Neurol. (Chicago)* **54**, 625–630.

Thiessen, B., Rajput, A. H., Laverty, W., and Desai, H. (1990). Age, environments, and the number of substantia nigra neurons. *Adv. Neurol*. **53**, 201–206.

Tissingh, G., Bergmans, P., Booij, J., Winogrodzka, A., van Royen, E. A., Stoof, J. C., and Wolters, E. C. (1998). Drug-naive patients with Parkinson's disease in Hoehn and Yahr stages I and II show a bilateral decrease in striatal dopamine transporters as revealed by [^{123}I]beta-CIT SPECT. *J. Neurol*. **245**, 14–20.

Tooyama, I., McGeer, E. G., Kawamata, T., Kimura, H., and McGeer, P. L. (1994). Retention of basic fibroblast growth factor immunoreactivity in dopaminergic neurons of the substantia nigra during normal aging in humans contrasts with loss in Parkinson's disease. *Brain Res*. **656**, 165–168.

Turjanski, N., Lees, A. J., and Brooks, D. J. (1997). In vivo studies on striatal dopamine D_1 and D_2 site binding in L-dopa-treated Parkinson's disease patients with and without dyskinesias. *Neurology* **49**, 717–723.

Tyrrell, P. J., Sawle, G. V., Ibañez, V., Bloomfield, P. M., Leenders, K. L., Frackowiak, R. S., and Rossor, M. N. (1990). Clinical and positron emission tomographic studies in the 'extrapyramidal syndrome' of dementia of the Alzheimer type. *Arch. Neurol. (Chicago)* **47**, 1318–1323.

Uhl, G. R. (1998). Hypothesis: The role of dopaminergic transporters in selective vulnerability of cells in Parkinson's disease. *Ann. Neurol*. **43**, 555–560.

Ushijima, I., Mizuki, Y., Soeda, K., Kishimoto, O., Hara, T., and Yamada, M. (1987). Effects of age on behavioral responses to dopamine agonists in the rat. *Eur. J. Pharmacol*. **138**, 101–106.

Valerio, A., Belloni, M., Gorno, M. L., Tinti, C., Memo, M., and Spano, P. (1994). Dopamine D_2, D_3, and D_4 receptor mRNA levels in rat brain and pituitary during aging. *Neurobiol. Aging* **15**, 713–719.

van Dyck, C. H., Seibyl, J. P., Malison, R. T., Laruelle, M., Wallace, E., Zoghbi, S. S., Zea-Ponce, Y., Baldwin, R. M., Charney, D. S., and Hoffer, P. B. (1995). Age-related decline in striatal dopamine transporter binding with iodine-123-beta-CITSPECT. *J. Nucl. Med.* **36**, 1175–1181.

Vermeulen, R. J., Drukarch, B., Wolters, E. C., and Stoof, J. C. (1999). Dopamine D_1 receptor agonists. The way forward for the treatment of Parkinson's disease? *CNS Drugs* **11**, 83–91.

Vingerhoets, F. J., Schulzer, M., Calne, D. B., and Snow, B. J. (1997). Which clinical sign of Parkinson's disease best reflects the nigrostriatal lesion? *Ann. Neurol*. **41**, 58–64.

Volkow, N. D., Wang, G. J., Fowler, J. S., Ding, Y. S., Gur, R. C., Gatley, J., Logan, J., Moberg, P. J., Hitzemann, R., Smith, G., and Pappas, N. (1998). Parallel loss of presynaptic and postsynaptic dopamine markers in normal aging. *Ann. Neurol*. **44**, 143–147.

Wallace, D. R., and Booze, R. M. (1996). Dopamine D_3 receptor density elevation in aged Fischer-344 x Brown-Norway (F1) rats. *Eur. J. Pharmacol*. **308**, 283–285.

Wang, Y., Chan, G. L., Holden, J. E., Dobko, T., Mak, E., Schulzer, M., Huser, J. M., Snow, B. J., Ruth, T. J., Calne, D. B., and Stoessl, A. J. (1998). Age-dependent decline of dopamine D_1 receptors in human brain: a PET study. *Synapse* **30**, 56–61.

Waters, C. M., Peck, R., Rossor, M., Reynolds, G. P., and Hunt, S. P. (1988). Immunocytochemical studies on the basal ganglia and substantia nigra in Parkinson's disease and Huntington's chorea. *Neuroscience* **25**, 419–438.

Weiss, B., Chen, J. F., Zhang, S., and Zhou, L. W. (1992). Developmental and age-related changes in the D_2 dopamine receptor mRNA subtypes in rat brain. *Neurochem. Int*. **20** (Suppl.), 49S–58S.

Wolf, M. E., LeWitt, P. A., Bannon, M. J., Dragovic, L. J., and Kapatos, G. (1991). Effect of aging on tyrosine hydroxylase protein content and the relative number of dopamine nerve terminals in human caudate. *J. Neurochem*. **56**, 1191–1200.

Wolterink, G., Van Zanten, E., Kamsteeg, H., Radhakishun, F. S., and van Ree, J. M. (1990). Functional recovery after destruction of dopamine systems in the nucleus accumbens of rats. I. Behavioral and biochemical studies. *Brain Res*. **507**, 92–100.

Zelnik, N., Angel, I., Paul, S. M., and Kleinman, J. E. (1986). Decreased density of human striatal dopamine uptake sites with age. *Eur. J. Pharmacol*. **126**, 175–176.

Zhang, L., and Roth, G. S. (1997). The effect of aging on rat striatal D_1 receptor mRNA-containing neurons. *Neurobiol. Aging* **18**, 251–255.

Zhang, L., Ravipati, A., Joseph, J., and Roth, G. S. (1995). Aging-related changes in rat striatal D_2 receptor mRNA-containing neurons: A quantitative nonradioactive in situ hybridization study. *J. Neurosci*. **15**, 1735–1740.

Zoli, M., Ferraguti, F., Toffano, G., Fuxe, K., and Agnati, L. F. (1993). Neurochemical alterations but not nerve cell loss in aged rat neostriatum. *J. Chem. Neuroanat*. **6**, 131–145.

Zweig, R. M., Jankel, W. R., Hedreen, J. C., Mayeux, R., and Price, D. L. (1989). The pedunculopontine nucleus in Parkinson's disease. *Ann. Neurol*. **26**, 41–46.

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Huntington's Disease

Huntington's disease is one of several devastating neurodegenerative diseases, including amyotrophic lateral sclerosis and Alzheimer's disease (AD), which are characterized by late onset in adult life. Their debilitating symptoms include cognitive dysfunction, behavioral changes and mood disturbances, and movement disorders, and in the cases of amyotrophic lateral sclerosis and Huntington's disease paralysis and death can occur. As the mean age of the population increases, so does the frequency of occurrence of these disorders. Symptoms are associated with region-specific loss of neurons within the central nervous system (CNS), but to date the mechanism of this selective neuronal death remains unknown. Several different etiological processes may play roles, and strong evidence from studies in humans and in animal models suggests the involvement of energy metabolism dysfunction, excitotoxic processes, and oxidative stress. The recent development of transgenic mouse models expressing the human Huntington's disease mutation has provided novel opportunities to determine the chronological order of events underlying the selective neuronal death seen in the disease, which have hitherto been impossible to determine in humans.

I. Introduction

Over a century elapsed between the first description of the Huntington's disease phenotype by George Huntington in 1872 and the discovery of the genetic defect underlying the disease in 1993 (Huntington's Disease Collaborative Research Group, 1993). A physician in Long Island, New York, the familial inheritance of the disease was well known to Huntington from the family histories passed down to him by his grandfather and father. Huntington's disease is now known to be an autosomal dominantly inherited neurodegenerative disorder characterized by the adult onset and progressive development of behavioral abnormalities, cognitive impairment, and involuntary choreiform movements, with a typical duration of 15–20 years. The genetic abnormality in Huntington's disease is a CAG repeat expansion in a gene encoding a 350 kDa protein of unknown function, termed "huntingtin" (Huntington's Disease Collaborative Research Group, 1993). It is thought to be a true dominant disorder since homozygous patients do not seem to differ from heterozygote carriers in either age of onset, duration, or severity of the disease (Durr *et al*., 1999). Despite its identification, the definitive role of mutant huntingtin in neuronal degeneration remains unknown. As discussed below, the insidious progression of motor and behavioral disturbances in Huntington's disease reflects the selective pattern of cell loss in the brain and the specific neurotransmitter pathways affected. The reason for the preferential vulnerability of striatal neurons in Huntington's disease is still enigmatic, and cannot be simply explained in terms of the distribution of abnormal huntingtin since the gene mutation is expressed throughout the body. However, experimental evidence suggests that the pathogenesis of cell death in Huntington's disease is linked to a gain of function of mutant huntingtin. In addition, studies in both human Huntington's disease brain and transgenic mouse model of Huntington's disease have identified widespread neuronal intranuclear and cytoplasmic aggregations of mutant huntingtin that may be linked to the disease process. In the following review we have collated the experimental information available to date to give insight into the link between the Huntington's disease gene mutation and selective neuronal dysfunction and death in this disease.

II. Neuropathological Features and Motor Dysfunction in Huntington's Disease

A. Pathological Changes in Huntington's Disease Brain

The typical neuropathological features of Huntington's disease are progressive caudal to rostral degeneration of the caudate-putamen (Vonsattel and DiFiglia, 1998). Patients are graded at postmortem according to the extent of gross and microscopic measures of neuropathological severity, grades ranging from 0 to 4 with increasing severity and extent of striatal involvement (Vonsattel *et al*., 1985). Briefly, grade 0 brains exhibit 30–40% neuronal loss in the head of the caudate, with no visible signs of reactive gliosis. In contrast, in grade 4 more than 95% of striatal neurons are lost, the striatum is severely atrophic with marked gliosis, and about 50% of end-stage

cases show cell loss in the nucleus accumbens. Most Huntington's disease cases reach grade 3 or 4 by the time of death, by which stage nonstriatal regions are also involved, in particular the globus pallidus, cortex, and, to a lesser extent, thalamus, subthalamic nucleus, substantia nigra, white matter, and cerebellum (Sotrel *et al*., 1991; Braak and Braak, 1992; Vonsattel and DiFiglia, 1998). Fibrillary astrogliosis occurs in the striatum, but has not been reported in other affected areas, and no inflammatory responses are involved (Myers *et al*., 1991). In cases of juvenile onset Huntington's disease cerebellar atrophy is particularly prevalent.

The striatal cells most susceptible to degeneration in Huntington's disease are medium spiny projection neurons (Beal, 1994a). Spiny neurons, which constitute 80% of striatal neurons, are the principal input and output neurons of the striatum. All contain the inhibitory neurotransmitter γ-aminobutyric acid (GABA), while subsets also contain enkephalin (ENK), Substance P (SP), dynorphin, or calbindin. The other major class of striatal neurons are aspiny interneurons. Nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d), neuropeptide Y (NPY), somatostatin, and nitric oxide synthase (NOS) typically colocalize in medium-sized aspiny neurons, and some also contain cholecystokinin or the calcium-binding protein parvalbumin. In Huntington's disease striatum, spiny projection neurons containing SP or ENK degenerate earliest in the course of the disease, whereas aspiny interneurons and the larger cholinergic interneurons are relatively spared (Ferrante *et al*., 1987; Beal *et al*., 1988). There is also some hierarchy in the vulnerability of different spiny neuron subpopulations; ENK-immunoreactive neurons projecting to the external segment of the globus pallidus (GPe) degenerate prior to SP-containing neurons projecting to the internal segment (GPi) (Reiner *et al*., 1988; Richfield *et al*., 1995; Sapp *et al*., 1995). Spiny neurons also undergo morphological changes in the course of the disease process in Huntington's disease, including recurving of the dendrites, altered shape and size of the spines, and increased density of spines.

Surviving neostriatal neurons are generally morphologically normal, although some are reduced in size and contain elevated levels of the oxidative damage marker lipofuscin. A subpopulation of ''neostriatal dark neurons'' has also been described by Vonsattel *et al*. (1985), scattered between the zones of atrophic and healthy cells. Interestingly, markers of apoptotic cell death have been detected in these neostriatal dark neurons (for example, granulation of the cytoplasm and condensation of nuclear chromatin and labeling by TdT-mediated dUTP-biotin nick end-labelling [TUNEL]; Vonsattel and DiFiglia, 1998).

B. Motor Dysfunction

The motor defects typical of Huntington's disease result from the disruption of basal ganglia-thalamocortical pathways which regulate movement control. The neostriatum (caudate nucleus and putamen) receives excitatory glutamatergic inputs from the entire neocortex, the first step in the anatomical loop responsible for the initiation and execution of movement. Processed signals are transmitted via basal ganglia output nuclei (GPi, the substantia nigra pars reticulata, SNr, and ventral pallidum) to the thalamus, which in turn sends excitatory projections to areas of the frontal cortex associated with motor planning and execution (Albin *et al*., 1989; Alexander and Crutcher, 1990; Graybiel *et al*., 1994). The GABAergic basal ganglia output projections to the thalamus maintain a tonic inhibition of their target nuclei, which is modulated by two opposing pathways (direct and indirect) which integrate the input and output compartments within the basal ganglia (Fig. 49.1). It is an imbalance in the relative contributions of these two regulatory pathways that triggers, and dictates the nature of, the motor dysfunction in Huntington's disease. In the direct (monosynaptic) pathway, activation of striatal efferents containing GABA and SP projecting directly to the GPi results in disinhibition of thalamic activity. In contrast, in the indirect (polysynaptic) pathway striatal efferents containing GABA and enkephalin project to the GPe which sends purely GABAergic projections to the subthalamic nucleus. From here, excitatory efferents (probably glutamatergic) project to the basal ganglia output nuclei (SNr and GPi). The GPe projection generally exerts a tonic inhibition on the subthalamic nucleus. Activation of GABA/ENKergic striatal efferents tends to suppress activation of GPe neurons, causing disinhibiton of the subthalamic nucleus and hence an increase in the excitatory innervation of the basal ganglia output nuclei. This leads to an increased inhibitory input to the target thalamic nuclei. Thus, cortical function is differentially modulated depending on which basal ganglia pathway, and therefore which thalamocortical pathway, is activated (Albin *et al*., 1989; Alexander and Crutcher, 1990). In Huntington's disease there is preferential loss of the GABA/ENK-containing neurons comprising the indirect pathway. ''Disinhibition'' of the thalamus results, which is manifest in Huntington's disease patients by the development of involuntary choreic movements. The later onset of a rigid akinetic state in some Huntington's disease patients is thought to result from the additional loss of striatal GABA/SP-containing efferents projecting directly to the GPi (Albin *et al*., 1990).

III. Mutant Huntingtin Protein in Huntington's Disease

The genetic defect in Huntington's disease is an expansion of an unstable CAG repeat encoding polyglutamines (Q_n) at the 5′ end of a gene on chromosome 4, *IT15* (''interesting transcript 15''), now termed *huntingtin (HD*) (Huntington's Disease Collaborative Research Group, 1993). Similar trinucleotide mutations in different genes are responsible for at least seven other neurodegenerative disorders: the spinocerebellar ataxias (SCA1, SCA2, SCA3 [Machado-Joseph disease], SCA6, and SCA7), dentatorubral-pallidoluysian atrophy, and spinal bulbar muscular atrophy (Ross, 1995; Zoghbi, 1997). While the genes affected in these disorders have now been identified, only in spinal bulbar muscular atrophy is the function of the affected protein known (androgen receptor). All the glutamine repeat diseases involve neuronal loss and gliosis. However, while gene expression is widespread throughout the body, cell death occurs in specific regions of the brain and spinal cord. How these genetic defects lead to progressive, selective neurodegeneration, remains elusive. The gene product in Huntington's disease, huntingtin protein, is a 348 kDa protein containing 3144 amino acids. Normal, unaffected indi-

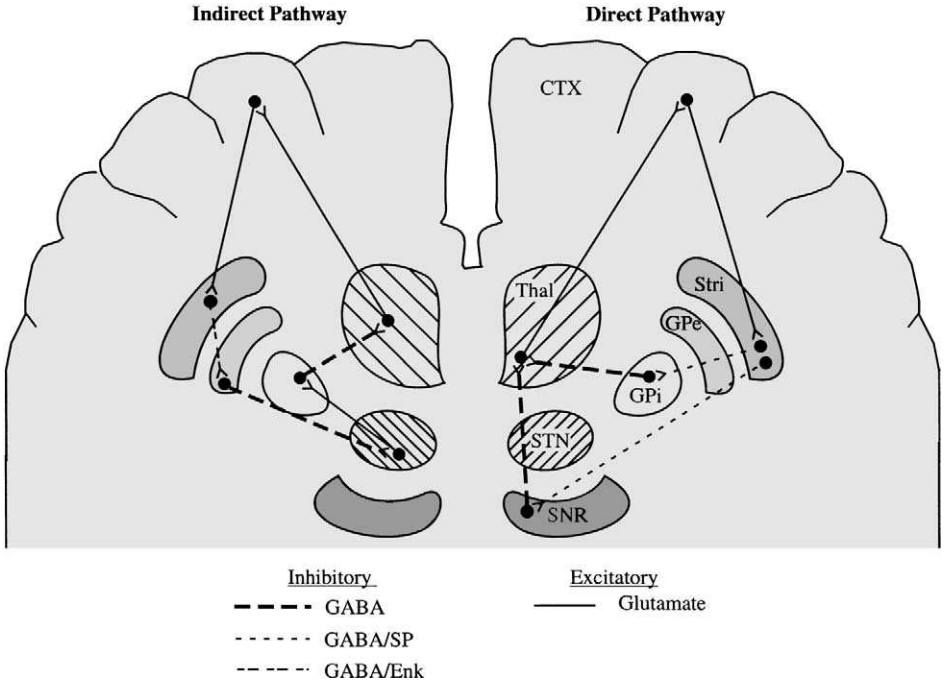

FIG. 49.1. Corticofugal pathways controlling movement. A representative diagram showing the major neurotransmitter systems involved in the direct (right) and indirect (left) basal ganglia output pathways. Activation of the indirect pathway increases the inhibitory input to thalamic nuclei, resulting in reduced excitatory output to the cortex. In contrast, activation of GABA/SP-containing striatal efferents in the direct pathway results in inhibition of the GPi and SNR projection to the thalamus, effectively releasing the thalamus from pallidal inhibition. As a result, the excitatory output to the cortex increases. Choreic movements in Huntington's disease are thought to result from reduced activity of the indirect pathway, due to loss of GABA/Enkephalinergic neurons (Albin *et al*., 1989; Andrews and Brooks, 1998). CTX, cerebral cortex; GPe, globus pallidus, external segment; GPi, globus pallidus, internal segment; Thal, Thalamus; SNR, substantia nigra pars reticulata; STN, subthalamic nucleus; Stri, striatum.

viduals typically have trinucleotide repeats of 11–34 CAGs. Expansion to 34–39 CAG repeats in one or both alleles confers the possibility of developing Huntington's disease, while the disease is inescapable when CAG repeats in either allele exceed 39. The trinucleotide repeat is polymorphic and undergoes alterations during meiosis, generally fluctuating by ± 1–5 repeats per transmission, although larger increases can occur following paternal transmission (Ross, 1995). The physiological functions of both normal and mutant huntingtin have not yet been determined, although several features of the Huntington's disease phenotype are known to be influenced by CAG repeat length, such as age of onset of the disease (Andrew *et al*., 1993; Duyao *et al*., 1993; MacDonald *et al*., 1999) and the extent of DNA fragmentation in Huntington's disease striatal neurons (Butterworth *et al*., 1998). CAG repeat length has also been correlated with neuropathological severity, although this observation is controversial since the grade of disease at time of death is dependent on a number of factors also influenced by repeat length, including age of onset and disease duration (Furtado *et al*., 1996; Sieradzan and Mann, 1998).

Distribution studies give little insight into the involvement of mutant huntingtin in the regional selectivity of cell loss in the disease, since huntingtin protein is ubiquitously expressed throughout the body. The fact that its distribution shows no apparent selectivity for cerebral regions targeted by the disease process suggests that another property of basal ganglia neurons confers vulnerability to degeneration in Huntington's disease (Strong *et al*., 1993; Sharp *et al*., 1995). However, recent immunohistochemical studies suggest that huntingtin may be differentially distributed at the cellular level within the striatum. Ferrante *et al*. (1997) reported that within the striatum huntingtin immunoreactivity is heterogeneous, the patch compartment showing low levels or no immunoreactivity in neurons and neuropil, while levels are relatively higher in the matrix. Double labeling techniques revealed higher levels of huntingtin expression in medium spiny neurons and colocalization with calbindin, in contrast to little or no colocaliztion of huntingtin with NADPH-d or NOS neurons, suggesting that there is some correlation between huntingtin's cellular location and cell vulnerability. A more recent study (Fusco *et al*., 1999) supports Ferrante and colleagues' observations that huntingtin protein load varies within the striatal population of medium-sized neurons and is not consistently found in all medium spiny projection neurons. Taken with their observations that large striatal cholinergic interneurons which are relatively spared in the disease contain high levels of huntingtin, Fusco and colleagues suggest that the huntingtin mutation is not directly toxic to cells.

At the neuronal level, huntingtin protein is widely expressed throughout cells, with a largely cytoplasmic distribution in perikarya, axons, dendrites, and some nerve terminals, and protein fragments have been identified in neuronal nuclei. Subcellular fractionation studies revealed an association of

huntingtin with synaptic vesicles (DiFiglia *et al*., 1995), while another report suggests association with the microtubules (Gutekunst *et al*., 1995), implicating potential roles in intracellular trafficking and synaptic function. N-terminal fragments of huntingtin form ubiquinated protein aggregates in neuronal nuclei (neuronal intranuclear inclusions) and in dystrophic neurites (cytoplasmic inclusions). These protein aggregates have been identified in both Huntington's disease brain and in the brains of transgenic mice expressing a fragment of human mutant huntingtin (Davies *et al*., 1997; DiFiglia *et al*., 1997; Reddy *et al*., 1998; Hodgson *et al*., 1999). The mechanism of inclusion formation has not yet been detrmined, but Perutz and colleagues (1994) suggest that the expanded polyglutamine stretches in mutant huntingtin lend themselves to the formation of β-pleated sheets held together by hydrogen bonds between amide groups. CAG repeat length appears to be critical for aggregate formation (Scherzinger *et al*., 1997). Similar intranuclear inclusions also occur in other CAG repeat disorders including spinocerebellar ataxia type 3 (Paulson *et al*., 1997) and in transgenic mice expressing merely an expanded CAG repeat (Ordway *et al*., 1997), suggesting that nuclear inclusions are a common product of trinucleotide expansions irrespective of the affected gene.

The toxic function associated with mutated huntingtin appears to be due to gain of a novel function, rather than loss of wild-type huntingtin function, since murine *HD* homolog-null mice die *in utero* while heterozygous knock-out mice show little or no pathology (Duyao *et al*., 1995). Further, Huntington's disease knock-out mice are completely rescued by crossing into knock-in Hdh^{Q50} mice with a mutant polyglutamine expansion (48 CAGs), while mice expressing abnormally low levels of murine huntingtin exhibit developmental abnormalities (White *et al*., 1997). Putative mechanisms of huntingtin dysfunction include altered cellular interactions. These include suggestions that expanded glutamine repeats may allow protein–protein interactions or that polyglutamines might be substrates for transglutaminases (Perutz *et al*., 1994; Cooper *et al*., 1997b). The principal candidates for protein interactors are huntingtin-associated protein-1 (HAP-1) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Li *et al*., 1995; Burke *et al*., 1996). Others include calmodulin, caspase-3, α-adaptin, cystathionine β-synthase, and huntingtin interacting protein (HIP-1) (see Aronin *et al*., 1999). Interestingly, in the case of calmodulin the polyglutamine expansion seems to increase huntingtin's affinity (Bao *et al*., 1996). A potential role for HAP-1 in the pathogenesis of Huntington's disease has largely been discounted on the basis of reports that although it is found only in brain it does not show a preferential striatal distribution, and that HAP-1 does not interact with the mutant stretch in huntingtin (Bertaux *et al*., 1998). GAPDH is a critical glycolytic enzyme, which led to intriguing proposals that an interaction with mutant huntingtin might impair metabolic function. Arguing against this idea, we found that the glycolytic function of GAPDH is not altered in postmortem Huntington's disease brain tissue (Browne *et al*., 1997). However, a recent study in fibroblast preparations showed that GAPDH's glycolytic activity is less responsive to metabolic stress in Huntington's disease patients than in fibroblasts from control subjects and unaffected Huntington's disease family members (Cooper *et al*., 1998). GAPDH also has a number of other functions within the cell which might be altered by an interaction with huntingtin. These include a role as a uracil DNA glycosylase in DNA repair, and binding to a number of proteins including DNA, RNA, ATP, actin, tubulin, amyloid precursor protein, and calcyclin. GAPDH expression is very susceptible to metabolic stress, and a glycolytically inactive form of the enzyme found in the nucleus has been implicated in apoptotic mechanisms in neurons and somatic cells (Saunders *et al*., 1997; Sawa *et al*., 1997). The potential for huntingtin to deleteriously affect GAPDH function is supported by a report that both GAPDH and α-ketoglutarate dehydrogenase are inactivated by fusion proteins containing polyglutamine stretches of pathological length, in reactions catalyzed by transglutaminase (Cooper *et al*., 1997a). Tissue transglutaminases (tTGase) are also implicated in the pathogenesis of trinucleotide repeat disorders including Huntington's disease due to another functional role within neurons and astrocytes, catalyzing Ca^{2+}-dependent cross-linking of glutamine residues with lysine and polyamines in other proteins (Aeschlimann and Paulsson, 1994; Kahlem *et al*., 1996; Lorand, 1996; Cooper *et al*., 1997a,b, 1999).

IV. Huntingtin Aggregates: Toxic, Protective, or Inert?

A great deal of debate surrounds the issue of whether neuronal intranuclear inclusion deposition plays a causative role in the pathogenesis of cell death in Huntington's disease, or is merely a secondary event. There is conflicting evidence about the role of neuronal intranuclear inclusions, although at present the weight of opinion seems to favor a lack of involvement, or even a neuroprotective role. The original thesis that nuclear localization of huntingtin aggregates was essential for pathogenic processes came from observations in the R6/2 transgenic mouse line, that neuronal intranuclear inclusion deposition preceded symptoms and pathology in the mouse phenotype (Mangiarini *et al*., 1996; Davies *et al*., 1997). Expanded CAG repeats have also been shown to induce huntingtin aggregation and cell death in transfected cerebellar granule cell cultures (Moulder *et al*., 1998). In addition, transfection of a human mutant huntingtin fragment into *Drosophila* eye cells induced CAG repeat-dependent photoreceptor degeneration and death, putatively via apoptotic mechanisms (Jackson *et al*., 1998).

The case against a pathogenic role of neuronal intranuclear inclusions is steadily growing. In Huntington's disease postmortem brain, neuronal intranuclear inclusion deposition in the cortex and striatum does not mirror the pattern of cell death in the disease (Ferrante *et al*., 1997; Fusco *et al*., 1999). Neuronal intranuclear inclusions and cytoplasmic inclusions are seen in NADPH-d neurons spared in the disease, but are not found in acetylcholinesterase- and choline acetyltransferase-positive interneurons, suggesting that neuronal intranuclear inclusion formation is not critical for cell death mechanisms. Also, in the majority of Huntington's disease patients huntingtin aggregates are most commonly found in neurites, with the exception of 5–10% of patients with juvenile onset of disease in whom neuronal intranuclear inclusions are prevalent in the cortex and striatum (Aronin *et al*., 1999). In addition, Sawa

et al. (1999) demonstrated in cultured Huntington's disease and control lymphoblasts that stress-induced depolarization is much greater in Huntington's disease mitochondria than in mitochondria from controls, in the absence of any neuronal intranuclear inclusion deposition—suggesting that neuronal intranuclear inclusions are not necessary for cellular dysfunction. They went on to demonstrate that this mitochondrial dysfunction is linked to apoptotic cell death. Furthermore, Saudou and colleagues (1998) recently reported cell-selective neurodegeneration resembling apoptotic cell death in cultured striatal (but not hippocampal) neurons transfected with a human mutant huntingtin fragment, independent of the presence of intranuclear inclusions. Observations that suppression of neuronal intranuclear inclusion deposition resulted in increased cell death in this neuronal population led to the proposal that neuronal intranuclear inclusion deposition may reflect a protective mechanism within cells.

Another study in cultured mouse clonal striatal neurons supports the argument that nuclear aggregates are unnecessary for the cell death mechanism to be activated in Huntington's disease. Kim *et al*. (1999) demonstrated that transfecting mouse clonal cells with either full-length or truncated huntingtin containing mutant CAG repeat lengths induced the formation of both nuclear and cytosolic inclusions, whereas huntingtin with wild-type CAG repeats remained within the cytoplasm. Nuclear inclusions consisted largely of N-terminal cleaved fragments of huntingtin, while cytoplasmic inclusions contained both fragments and intact proteins. Apoptotic features were present in both wild-type and mutant huntingtin transfected cells, but were exacerbated in mutant cells. Findings that inhibiting caspase activity with Z-VAD-FMK increased cell survival, but had no effect on either neuronal intranuclear inclusion or cytoplasmic inclusion number, imply that neuronal death is independent of aggregate formation in this model. This hypothesis was supported by observations that another caspase inhibitor, Z-DEVD-FMK, reduced neuronal intranuclear inclusion and cytoplasmic inclusion number but had no effect on cell survival. The authors also demonstrated that only mutant, and not wild-type, huntingtin underwent cleavage to form N-terminal fragments, which suggests that the polyglutamine domain confers some propensity for cleavage by caspases (Kim *et al*., 1999). In another study, nuclear import and export sequences were inserted into huntingtin fragments in 293T cultured cells to alter their normal distribution within the cells (Hackam *et al*., 1999). Results show that toxicity of mutant huntingtin is unaffected by the intracellular location of huntingtin aggregates. Although neither full-length huntingtin or huntingtin fragments have yet been found in mitochondria, an effect on mitochondrial function cannot be ruled out. For instance, huntingtin may play a role in mitochondrial trafficking, or alternatively neuronal intranuclear inclusions may influence nuclear transcription and thus affect the expression of nuclear-encoded proteins including subunits of mitochondrial complex II. The latter is of particular note since complex II activity is impaired in affected brain areas in Huntington's disease.

Transgenic and knock-in mouse models (discussed in section VI) further support the argument that neuronal intranuclear inclusions are not needed for cell toxicity in Huntington's disease models. Although neuronal intranuclear inclusion deposition precedes behavioral symptoms and neurotransmitter changes in R6/2 mice, there is no direct link between the distribution of neuronal intranuclear inclusion deposition and patterns of cell death or dysfunction in several other mutant Huntington's disease mouse models (Reddy *et al*., 1998; Hodgson *et al*., 1999; Levine *et al*., 1999). In addition, R6/2 transgenic mice also develop neuronal inclusions in many postmitotic peripheral tissues from about 6 weeks of age, including both skeletal and cardiac muscle, kidney, liver, pancreas, and adrenal glands (Sathasivam *et al*., 1999). Peripheral aggregate formation seems to coincide with tissue atrophy, but there is no direct evidence of cell death. It is of interest, therefore, that unlike the brain where the bulk of aggregtes are neuritic, inclusions in skeletal muscle cells are found solely within nuclei. There is also increasing evidence from transgenic mouse models that the mechanism of neuronal death in other polyglutamine repeat disorders is independent of nuclear aggregation of the mutant protein. Lin *et al*. (2000) report that polyglutamine expansion in ataxin-1 causes the downregulation of several neuronal genes involved in signal transduction and calcium homeostasis in SCA-1 mice expressing the transgene. More interestingly, these changes precede any pathological changes in the mice by at least 3 weeks (pathology is evident at 6 weeks). Mutant ataxin-1 also forms intranuclear inclusions in cerebellar Purkinje cells of SCA-1 mice, and in humans, by aggregating with proteosomes and ubiquitin (Cummings *et al*., 1998). However, these inclusions do not appear to be pathogenic, since decreasing the frequency of nuclear inclusion formation (by treating with E6-AP ubiquitin ligase) actually exacerbated the rate and extent of pathology in SCA-1 mice (Cummings *et al*., 1999).

V. Putative Mechanisms of Cell Death

The main question still confounding researchers is how the huntingtin mutation results in selective neuronal cell loss in Huntington's disease. The definitive answer is still elusive, but several hypotheses exist.

A. Bioenergetic Defects

One hypothesis is that the gain of function associated with expanded polyglutamine repeats leads either directly or indirectly to a defect in energy metabolism, potentially via secondary excitotoxicity (Albin and Greenamyre, 1992; Beal, 1994b). Reduced ATP production due to impaired energy metabolism can lead to partial cell depolarization by making neurons more vulnerable to endogenous levels of glutamate. The concomitant increase in Ca^{2+} influx into neurons may trigger further free radical production, exacerbating damage to cellular elements. This hypothesis is supported by findings that normally ambient levels of excitatory amino acids become toxic in the presence of oxidative phosphorylation inhibitors, sodium–potassium pump inhibitors, or potassium-induced partial cell membrane depolarization. Further, excitatory amino acid antagonists such as MK-801 can ameliorate cerebral lesions induced by mitochondrial toxins such as 3-nitropropionic acid (3-NP), 3-acetylpyridine (3-AP), aminooxyactic acid (AOAA), 1-methyl-4-phenylpyridinium (MPP^+), and malo-

nate (Storey *et al*., 1992; Beal, 1996; Schulz *et al*., 1996b). The principal indicator of an energetic involvement in the disease process is the observation of insidious weight loss in Huntington's disease patients despite a sustained caloric intake (O'Brien *et al*., 1990). Subsequently, positron emission tomography (PET) and biochemical studies in postmortem brain have shown selective metabolic defects in brain regions targeted by the disease, and mitochondrial abnormalities in Huntington's disease have been identified in ultrastructural studies of cortical biopsies from juvenile and adult-onset Huntington's disease cases (Goebel *et al*., 1978).

PET studies show marked reductions in glucose metabolism in the basal ganglia and cerebral cortex of symptomatic Huntington's disease patients (Kuhl *et al*., 1985; Kuwert *et al*., 1990; Andrews and Brooks, 1998). Caudate hypometabolism in symptomatic patients has been shown to correlate with clinical test scores for bradykinesia, rigidity, dementia, and functional capacity, while the extent of putaminal hypometabolism correlates with chorea and eye-movement dysfunction, and thalamic hypermetabolism correlates with dystonia scales (Young *et al*., 1986; Berent *et al*., 1988; Kuwert *et al*., 1990). Cortical hypometabolism is also seen in patients suffering psychological disturbances and mood changes, before the onset of motor symptoms (Kuwert *et al*., 1990). More convincing evidence of a causative role for energetic defects comes from observations of striatal hypometabolism prior to the bulk of tissue loss, and in asymptomatic subjects at risk of developing the disease (Grafton *et al*, 1992; Kuwert *et al*., 1993; Antonini *et al*., 1996). Approximately 50% of gene-positive mutation carriers exhibit metabolic defects years before the onset of clinical symptoms (Antonini *et al*., 1996). PET techniques have also demonstrated that the first clinical symptoms of the disease correlate with loss of 30–40% of striatal dopamine D1 and D2 receptors, which are localized on the GABAergic medium spiny projection neurons in the striatum (Andrews *et al*., 1997; Hussey *et al*., 1998). More evidence of metabolic dysfunction in Huntington's disease comes from proton nuclear magnetic resonance (^{1}H NMR) imaging studies which show increased lactate production in the basal ganglia and occipital cortex of Huntington's disease patients (Jenkins *et al*., 1993, 1998). Notably, these lactate defects can be ameliorated by treatment with the metabolic cofactor coenzyme Q_{10} (Koroshetz *et al*., 1997).

Biochemical studies in Huntington's disease postmortem tissue have revealed selective dysfunction of components of the oxidative phosphorylation pathway and the tricarboxylic acid (TCA) cycle in brain regions targeted in the disorder. Activities of complexes II-III and IV are markedly reduced in advanced grade Huntington's disease caudate and putamen, while enzyme activities are unaltered in other brain regions (Gu *et al*., 1996; Browne *et al*., 1997). Complex I activity is also reported to be impaired in muscle from Huntington's disease patients, but has not been shown to be affected in brain (Parker *et al*., 1990; Browne *et al*., 1997; Arenas *et al*., 1998). Pyruvate dehydrogenase activity is decreased in Huntington's disease basal ganglia and hippocampus, while polarographic studies show that striatal oxygen consumption in Huntington's disease patients is lower than in age-matched controls (Butterworth *et al*., 1985). The most profound enzyme defect seen in Huntington's disease to date is the dramatic reduction in activity of the TCA cycle enzyme aconitase in affected brain regions and muscle (> 70%; Tabrizi *et al*., 1999). In addition, mitochondrial toxins, which inhibit succinate dehydrogenase in the TCA cycle and complex II (3-nitropropionic acid and malonate), induce selective striatal lesions in rodents and primates which closely resemble those seen in Huntington's disease (Beal *et al*., 1993, 1994; Wullner *et al*., 1994; Schulz *et al*., 1996a,b). Mitochondrial abnormalities and metabolic defects are also features of other trinucleotide repeat disorders, including SCA1, SCA2, and SCA3, leading to the proposal that energetic dysfunction may play a common role in these disorders, and is directly linked to the polyglutamine defect (Matthew *et al*., 1993; Mastrogiacomo and Kish, 1994; Mastrogiacomo *et al*., 1994; Matsuishi *et al*., 1996).

Further indirect evidence that energetic defects contribute to neurodegenerative processes in Huntington's disease is provided by findings that agents which enhance energy production in the brain exert neuroprotective effects. Preliminary studies in rodent mitochondrial toxin models, and NMR measurements of lactate production in man, suggest that coenzyme Q_{10} and creatine are neuroprotective, putatively via enhancing cerebral energy metabolism (Koroshetz *et al*., 1997; Matthews *et al*., 1998). Coenzyme Q_{10} also has potent antioxidant effects. Oral administration of coenzyme Q_{10} improves symptoms in some other mitochondrial-associated disorders including MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes) and Kearns-Sayre syndrome (reducing cerebrospinal fluid, serum lactate and pyruvate levels, and enhancing mitochondrial enzyme activities in platelets) (Bresolin *et al*., 1988; Ihara *et al*., 1989). We recently showed that oral administration of coenzyme Q_{10} ameliorated elevated lactate levels seen in the cortex of Huntington's disease patients, an effect which was reversible on withdrawal of coenzyme Q_{10} (Koroshetz *et al*., 1997). Furthermore, coenyme Q_{10} attenuates neurotoxicity induced by the mitochondrial toxins MPTP and malonate in animal models (Beal *et al*., 1994; Schulz *et al*., 1996b). An alternative strategy is to increase brain energy stores of the high-energy compound phosphocreatine by creatine administration. We recently showed that oral creatine administration in rats attenuates neurotoxicity induced by the succinate dehydrogenase inhibitor 3-NP (Matthews *et al*., 1998). In addition, increases in cerebral lactate levels and decreases in levels of high-energy phosphate compounds seen in the striata of 3-NP treated rats were attenuated by pretreatment with creatine.

B. Oxidative Damage

Oxidative damage can affect cell viability directly, via oxidation of DNA and other neuronal macromolecules, or indirectly, for example by impairing mitochondrial energy metabolism (Schulz *et al*., 1996b; Browne *et al*., 1999a). Evidence for oxidative damage in Huntington's disease is steadily accumulating. Findings include increased incidence of DNA strand breaks, exacerbated lipofuscin accumulation (a marker of lipid peroxidation), elevated DNA oxidative damage products such as 8-hydroxydeoxyguanosine (OH8dG), and increased immunohistochemical staining of oxidative damage products in Huntington's disease striatum and cortex, including staining for 3-nitrotyrosine (a marker for peroxynitrite-

mediated protein nitration), malondialdehyde (marker for oxidative damage to lipids), heme oxygenase (formed during oxidative stress), and OH8dG (Goebel *et al*., 1978; Tellez-Nagel *et al*., 1995; Ferrante *et al*., 1996; Browne *et al*., 1997, 1999a).

A potential mechanism of mitochondrial dysfunction is via increased generation of free radicals and oxidants. Free radicals including superoxide ($O_2^{\cdot -}$) and hydroxyl radicals ($HO^{\cdot}$) are constantly produced as by-products of aerobic metabolism, but production increases under circumstances of electron transport chain inhibition or molecular defects (Schapira *et al*., 1992). These agents can induce oxidative damage to cell macromolecules including DNA, proteins, and lipids by a number of different mechanisms, including DNA strand breaks or formation of DNA adducts (e.g., OH8dG), protein carbonylation, or lipid peroxidation. Potential functional consequences include perturbations in DNA transcription and translation, protein synthesis, enzyme activities, and membrane fluidity. Mitochondria are thought to be particularly vulnerable to oxidative injury since most intracellular free radicals are generated by the mitochondrial electron transport chain. Mitochondrial DNA is extremely susceptible due to its localization in the mitochondrial matrix, lack of protective histones, and limited repair mechanisms (Linnane *et al*., 1989). Thus any increase in free radical production, for example due to impaired activity of a regulatory enzyme such as Cu/Zn SOD or glutathione peroxidase, could reduce the functional capacity of the respiratory transport chain. In addition, the tricarboxylic acid cycle enzyme aconitase, which is severely affected in Huntington's disease, is a prime target for free radical-mediated oxidative damage. The slow, progressive nature of neuronal injury in neurodegenerative disorders may be explained by cycling of free radicals and mitochondrial dysfunction. We have previously found increased levels of oxidative markers in both Huntington's disease and aminotrophic lateral sclerosis. Nuclear DNA OH8dG levels are significantly elevated in Huntington's disease caudate relative to controls (Browne *et al*., 1997, 1999a). Further evidence supporting a role for oxidative damage in Huntington's disease is that the energetic defects seen in Huntington's disease brain are similar to those induced in cell culture by peroxynitrite, which preferentially inhibits complex II-III and (to a lesser extent) complex IV activity in the electron transport chain (Bolanos *et al*., 1995).

VI. State of the Art Approaches: Animal Models Provide Insights into Disease Etiology

A. Mitochondrial Toxin Models

A role for mitochondrial energy metabolism dysfunction in the pathogenesis of neuronal degeneration in Huntington's disease is further supported by observations, in both humans and in experimental animals, that the basal ganglia neurons are particularly vulnerable to mitochondrial toxins. These include the complex II inhibitors 3-NP and malonate, AOAA (complex I), potassium cyanide, and sodium azide (complex IV) (Browne and Beal, 1994). Ingestion of 3-NP, an irreversible inhibitor of succinate dehydrogenase (complex II), produces selective basal ganglia lesions and delayed dystonia in humans (Ludolph *et al*., 1990). Systemic administration of 3-NP to both rats and primates produces age-dependent striatal lesions which are strikingly similar to those seen in Huntington's disease (Brouillet *et al*., 1993, 1995). In primates, chronic 3-NP administration produces selective striatal lesions which spare NADPH-d neurons, and induce proliferative changes in the dendrites of spiny neurons. Animals also show both spontaneous and apomorphine-inducible movement disorders resembling Huntington's disease (Brouillet *et al*., 1995). 3-NP basal ganglia lesions in rats are associated with elevated lactate levels, similar to the increased lactate production seen in Huntington's disease patients (Jenkins *et al*., 1993). 3-NP lesions can be prevented by prior removal of glutamatergic excitatory corticostriatal inputs by decortication, by glutamate release inhibitors, and by glutmate receptor antagonists, suggesting that 3-NP toxicity is mediated by secondary excitotoxic mechanisms (Beal, 1994a; Schulz *et al*., 1996b).

Intrastriatal injection of either malonate or 3-NP in rats is also associated with increased oxidative damage. We found that the rate of hydroxyl free radical production is elevated in the striatum, as detected by microdialysis (Schulz *et al*., 1996a). Increased OH^8dG levels in striatum are also detected following systemic 3-NP administration, and elevated 3-nitrotyrosine concentrations are reported after either systemic 3-NP or intrastriatal malonate injection. Further, the finding that 3-NP-induced lesions and concomitant increases in oxidative damage markers were markedly attenuated in mice overexpressing the superoxide free radical scavenger Cu/Zn superoxide dismutase (SOD1) implies that oxidative free radicals contribute to lesion formation (Beal *et al*., 1995). Furthermore, malonate and 3-NP striatal lesions were attenuated by free radical spin traps and NOS inhibitors. Inhibition of nitric oxide (NO) generation in mice lacking the gene for the neuronal isoform of NOS (nNOS) also resulted in reduced volume of malonate lesions (Schulz *et al*., 1996b). Hence there is substantial evidence that nitric oxide-mediated oxidative damage is involved in cell death processes following energetic disruption in these models.

B. Transgenic Mouse Models of Huntington's Disease

One of the major drawbacks of relying on human tissue for asessment of neurological disease progression is the inability to adequately map early events in the disease etiology. Substantial evidence of a causative role in the disorder would be provided by evidence of occurrence prior to symptoms and pathology in models of Huntington's disease. Over the past few years, the development of methodology to generate transgenic mouse lines expressing the physiological phenotypes associated with human gene mutations has provided much needed *in vivo* models to circumvent many of these issues. A number of different groups have developed several different transgenic and ''knock-in'' mouse models of Huntington's disease, which vary in terms of the transgene incorporation technique employed. As a result, mouse phenotypes vary between lines, the features manifested by the animals depending on the nature of the transgene incorporated (i.e., full-length human mutant huntingtin, or a huntingtin *HD* gene fragment incorporating the mutant region in exon 1, or merely an expansion

inserted into the murine *HD* homolog *Hdh*); CAG repeat length; copy number of the mutant gene incorporated; promoter used, and hence cellular specificity of expression; background strains; and expression levels of the mutant gene. The different mouse lines reported to date are discussed below and listed in Table 49.1, which compares the salient features of each of the models.

1. Transgenic Mice Expressing Full-Length Human Mutant Huntingtin

The *HD89* and YAC72 mice discussed below represent two of the most useful transgenic mouse lines developed to date. Their utility is encumbent on the fact that disease-length huntingtin mutations are expressed in the context of the human

TABLE 49.1 Characteristics of Transgenic and Knock-in Mouse Models Expressing the Huntington Disease Mutation

Mutant mouse lines	Background strain	Promoter	Insert	CAG repeat	Symptom onset (weeks)	NII onset (weeks)	Neuronal loss (at end stage)	Life span (weeks)
HD[a]								
HD16 (Wt)	FVB/N	CMV	Full-length	16+/− (A–E)	None	None	None	Normal
HD48			*HD* cDNA,	48 +/− (B,D)	8	12	Stri, CTX >	29–31
HD89			2–22 copies	48 +/− (C)	25	Stri, CTX,	Thal, Hip	29–31
				89 +/− (A–C)	8	Thal, Hip,	(Not 48C)	29–31
				48B +/+	0–8	CBL		21–23
				89A +/+	0–8			21–23
YAC[b]								
YAC18 (Wt)	FVB/N	Constitutive	Full-length	18	None	None	None	Normal
YAC46			*HD* DNA,	46	42	None	None	N/D
YAC72			1–2 copies	72 (2511 line)	26	None	Stri[j]	N/D
Hdh Knock-in[c]								
Hdh^{Q7} (Wt)	C57B16/J	Murine	Full length	7	None	None	None	Normal
Hdh^{Q50}		endogenous	mouse *Hdh*,	48	None	None	None	Normal
Hdh^{Q92}			CAG insert	90	16	60	None	Normal
Hdh^{Q111}				109		16	None	Normal
CAG Knock-in[d]								
CAG 71	C57B16/J	Constitutive	Mouse *Hdh*	71	12	None	None	N/D
CAG 94			−100 bp,	94	8	None	None	N/D
			CAG insert					
Hdh4/6 Knock-in[e]								
Hdh 6/Q72	RF8	Constitutive	Full length	72	12	None	None	N/D
Hdh 4/Q80	JM-1		mouse *Hdh*,	80	12	None	None	N/D
			CAG insert					
N171[f]								
N171-18Q (Wt)	C57B16/C3	Mouse prion	N-terminal	18	None	None	None	Normal
N171-44Q		protein	truncated	44	None	None	None	Normal
N171-82Q			*HD* cDNA,	82	14	21–26	Some; CTX,	21–26
			171 aa				Stri, Hip, CBL	
L63[g]								
L63-46	SJL/B6	Rat NSE	N-terminal	46	12+	N/D	None	N/D
L63-100			fragment,	100	12	CTX, Stri	Stri	N/D
			epitope tag					
R6[h]								
Hdex6 (Wt)	C57B16/CBA	Constitutive	1.9-kb *HD*	18	None	None	None	Normal
Hdex27 (Wt)			DNA	18	None	None	None	Normal
R6/0			fragment	142	None	None	None	Normal
R6/1				113	16–20	Yes	Atrophy/loss?	
R6/2				144	8	3.5	Atrophy/loss?	17–25
R6/5				128–156 +/+	36	Yes	Atrophy/loss?	
				128–156 +/−	None	None	None	Normal
Tet-Off[i]								
		CamKII	Full length		Tet-on	Yes, reversible	Atrophy/loss?	N/D
			mouse *Hdh*,					
			CAG insert					

Note. The models listed reflect those reported by February 2000. CBL, cerebellum; CTX, neocortex; Hip, hippocampus; N/D, not determined to date; NII, neuronal intranuclear inclusions; Stri, striatum; Tet, tetracyclin; Thal, thalamus; wks, weeks of age; Wt, wild type.
[j]Evident in striatal medium spiny neurons by 52 weeks of age.

full-length gene inserted into the mouse genome, rather than just a gene fragment. In contrast to some other putative Huntington's disease models (such as the *Hdh* knock-in mice and R6/2 mice), animals develop region-specific neuronal degeneration over their life span, which provides a suitable context for measuring the efficacy of potential neuroprotective agents.

a. HD48 *and* HD89 *Mice.* Tagle and colleagues generated mice expressing 16 (wild type), 48, and 89 glutamines from full-length human huntingtin cDNA constructs. Using the human promoter, huntingtin expression is widespread throughout the brain and periphery. In these mice, copy number of the gene incorporated varied from two to six in mutant CAG lines. Both 48 and 89 CAG repeat mice showed motor deficits from an early age, developing foot clasping and stereotypic hyperkinetic activity, followed by hypokinesis and locomotor deterioration. Mice die prematurely (24–32 weeks). By about 24 weeks of age marked neuronal cell loss and astrogliosis is evident in the striata of both *HD48* and *HD89* mice, but few neuronal intranuclear inclusions occur. The lack of a distinct correlation between CAG repeat number and disease progression is thought to be associated with the levels of expression in each of the mouse lines, which vary from approximately endogenous levels in 89/89 mice to fivefold higher in 48/48 mice (Reddy *et al*., 1998).

b. YAC HD Tg *Mice.* Hodgson *et al*. (1999) used yeast artificial chromosome (YAC) technology to generate transgenic mice expressing normal (YAC18: 18 glutamines) and mutant huntingtin (YAC46 and 72: 46 and 72 glutamines, respectively). Mutations are expressed in the context of full-length huntingtin protein. By 12 months of age YAC72 mice have a selective degeneration of medium spiny neurons in the lateral striatum (similar to the selective cell death seen in Huntington's disease patients). Neurodegeneration seems to require nuclear translocation of N-terminal huntingtin fragments, but not neuronal intranuclear inclusion formation. Both YAC46 and YAC72 develop progressive electrophysiological abnormalities at approximately 7 months of age that precede nuclear translocation of huntingtin and cell death. Behavioral changes are manifest in YAC72 mice at 7 months of age.

2. "Knock-in" Mice Expressing Full-Length Huntingtin

"Knock-in" mice arguably represent an excellent model system for investigating the effects of the huntingtin mutation, since the mutation is expressed in the context of the full-length murine huntingtin analog, *Hdh*. Hence, the system uses the endogenous promoter to produce protein at normal murine expression levels, so any differences between animals may be attributed to different polyglutamine repeat lengths. In reality their utility is confounded to some extent by the fact that they do not develop overt, quantifiable, neurodegenerative, or behavioral changes over the animals' life spans. However, as discussed below, there is some evidence of cellular dysfunction associated with the CAG expansion in these models which may be useful as testable parameters for investigating disease mechanisms.

The reason for the lack of neuropathological features of Huntington's disease in these models is thought to result from the combination of low expression levels of the gene and the short life span of mice. In support of this hypothesis, HD48 mice, which do show selective cell death, neuronal intranuclear inclusion, cytoplasmic inclusion formation, and motor disturbances, express up to 5 × wild-type endogenous levels of full-length huntingtin protein (Reddy *et al*., 1998). However, in the knock-in lines only endogenous murine levels of huntingtin are expressed. Also, the CAG repeat number required to confer toxicity in mice seems to be longer than in humans, perhaps reflecting the short life span of the animals.

a. H*dh knock-in* Mice. White *et al*. (1997) developed a mouse model of Huntington's disease by extending the polyglutamine tract of the murine homolog (*Hdh*) of the human huntingtin gene (*HD*). CAG repeats from an *HD* chromosome were inserted into the appropriate position in *Hdh* exon 1, altering the mouse *HD* homolog to encode huntingtin protein with 50, 92, or 111 glutamine residues, instead of the 7 normally found in the mouse protein. The transgene has the endogenous promoter, and mice express wild-type levels of huntingtin protein. Mice homozygous for mutant huntingtin do not exhibit any behavioral symptomatology up to 18 months of age. However, recent observations have shown CAG length-dependent translocation of huntingtin protein from cytosol to the nucleus, and eventual neuronal intranuclear inclusion formation in Hdh^{Q92} and Hdh^{Q111} mice (Wheeler *et al*., 2000). Although there is no evidence of selective cellular pathology in any of these mouse lines yet, we have found that cerebral glucose metabolism and mitochondrial enzyme activities are impaired in Hdh^{Q92} mice at 4 months of age (Browne *et al*., 1999b, and unpublished observations). This corresponds with a time point preceding neuronal intranuclear inclusion formation in these mice, and may be indicative of early bioenergetic changes associated with the huntingtin mutation (Fig. 49.2, see color insert).

b. CAG71 *and* CAG94 *Mice.* Similar to the technique in *Hdh* mice, the endogenous murine *Hdh* gene was modified by replacing a portion of mouse exon 1 and the adjacent intron with a human sequence containing an expanded CAG repeat region (71 or 94) from a juvenile Huntington's disease lymphoblastoid cell line (Levine *et al*., 1999). Although no overt cell loss has been reported to date in this model, there is evidence of neuronal dysfunction in CAG94 knock-ins, which showed an increased sensitivity of cortical and striatal cells to *N*-methyl-D-aspartate (NMDA) receptor activation. In contrast, mice expressing fewer glutamines (94) CAG71 knock-ins were less affected than CAG94 knock-ins, showing NMDA responses similar to littermate controls. No cerebral neuronal intranuclear inclusions have been detected in either mouse line.

c. H*dh*4/Q80 *and* H*dh*6/Q72 *Mice.* Another knock-in Huntington's disease model was generated by Shelbourne and colleagues (1999). They inserted an expanded CAG repeat (72–80) into the murine *Hdh* by homologous recombination and generated two heterozygous knock-in lines, *Hdh4/Q80* and *Hdh6/Q72*. These mice do not develop any neuropathological abnormalities at the cellular level by 17 months of age, but do exhibit a 10–15% reduction in brain size, which is evident by 4 months of age. Neuronal intranuclear inclusions have not been

seen in either line to date, but mice do display an abnormal behavioral phenotype, in the form of chronically increased aggressive behavior toward other mice from about 3 months of age, which is more prevalent in males than in females. There is also evidence of reduced long-term potentiation in hippocampal neurons (Usdin *et al*., 1999), which the authors suggest reflects the cognitive impairments seen at an early stage in Huntington's disease.

3. Transgenic Mice Expressing Human Mutant Huntingtin Fragments

a. N171 HD M*ice*. N171 transgenic mice express a truncated portion of Huntington's disease cDNA, consisting of 171 amino acids from the N-terminal, with an expanded glutamine repeat of 44 or 82 (N171-44Q and N171-82Q). Wild-type N171-18Q express 18 glutamines (Schilling *et al*., 1999). N171-82Q mice display a phenotype of uncoordination, ataxia, and weight loss with onset at approximately 3–4 months, and die prematurely at about 6 months of age. On pathologic examination brains show neuronal intranuclear inclusions when labeled with antibodies to ubiquitin or the N-terminal of huntingtin protein and evidence of neuronal degeneration in the striatum.

b. L63 HD M*ice*. The L63 mouse line express a FLAG–huntingtin cDNA fusion protein consisting of the first 3221 huntingtin bases from the N-terminal, with 46 or 100 glutamines (Laforet *et al*., 1998). Motor defects and approximately 20% loss of striatal neuron occur in most mice with 100 CAGs by 6 months of age. Low levels of neuronal intranuclear inclusions are also seen in striatum by 6 months (Laforet *et al*., 1998; Aronin *et al*., 1999).

c. R6 HD M*ice*. The R6 mouse lines were the first Huntington's disease transgenic mice developed and are therefore the best characterized to date. Importantly, it was the development of this model which led to the identification of neuronal intranuclear inclusion formation in the disease paradigm. The R6 mouse lines are transgenic for a fragment of the human *HD* gene, containing 1 kb of the *HD* promoter region and exon 1 containing the abnormal CAG repeat expansion and 262 bp of intron 1 (Mangiarini *et al*., 1996). The R6/2 line expressing 144 CAGs is the most studied to date, since these animals develop a rapidly progressing neurological phenotype reminiscent of Huntington's disease, before dying prematurely at 17–22 weeks of age. The first motor symptoms occur at about 8 weeks of age, but are preceded by neuronal intranuclear inclusion deposition throughout the brain by 3.5 weeks of age (although it may occur even earlier) (Davies *et al*., 1997). The motor disorder includes abnormal gait, a resting tremor, abrupt shuddering movements, and stereotypic grooming. However, some features of the disease phenotype do not bear much resemblance to the human disease, including the propensity for seizures in these animals, the lack of overt striatal-specific cell death, and the development of diabetes (Mangiarini *et al*., 1996; Davies *et al*., 1997; Hughes *et al*., 1999; Hurlbert *et al*., 1999; Sathasivam *et al*., 1999). Neurotransmitter and receptor abnormalities also occur in R6/2 mice, including dopamine D1 and D2 receptor loss by 8 weeks of age, and alterations in levels of the glutamatergic mGluR1, mGluR2, mGluR3, and mGluR5 metabotropic receptors by 12 weeks of age (Cha *et al*., 1998). Further, *in situ* studies revealed that D1 and mGluR receptor mRNA is abnormal by as early as 4 weeks of age, preceding the onset of symptoms in these animals. However, contrary to the picture in Huntington's disease brain, GABAergic and NMDA receptor binding levels are not altered in these animals, although AMPA and kainate receptors show some downregulation (Cha *et al*., 1998). Similarly, neurotransmitter changes do not bear close resemblance to the pattern seen in Huntington's disease. For instance, Reynolds *et al*. (1999) report that striatal GABA levels are unaltered in symptomatic 12-week-old mice, although a slight decrease was observed in the cerebellum. Further, levels of serotonin and 5-hydroxyindoleacetic acid (5-HIAA) are reduced in all brain regions of R6/2 mice, while noradrenaline is decreased in the hippocampus. In contrast, dopamine levels in the striatum are reduced in aged animals, consistent with changes seen in Huntington's disease brain.

As discussed earlier, metabolic defects prior to disease onset are also typical of Huntington's disease. In the R6/2 mouse line, Tabrizi *et al*. (2000) reported that complex II-III and aconitase activities are impaired in the brains of 12-week-old R6/2 Huntington's disease transgenic mouse brains, but these alterations do not precede the onset of the behavioral phenotype and neuronal intranuclear inclusion deposition (S. E. Browne and M. F. Beal, unpublished observations). However, at 12 weeks of age R6/2 mice show an increased vulnerability to metabolic stress, as demonstrated by increased free radical generation and lesion size in response to a 3-NP toxic insult (Bogdanov *et al*., 1998). Further, we have recently found that treatment of R6/2 mice with creatine, administered in feed from the time of weaning, significantly increases survival and delays brain atrophy, striatal neuron atrophy, and the formation of nuclear inclusions (Ferrante *et al*., 2000). In addition, prevention of huntingtin cleavage by caspase inhibitors has recently been shown to delay phenotype onset and animal death in this mouse model (Ona *et al*., 1999).

4. "Inducible" Transgenic Mice Transiently Expressing Mutant Huntingtin

Perhaps the most exciting development in the past few years is the generation of a reversible Huntington's disease mouse model, which incorporates a "tet off" system to modulate expression of the huntingtin gene mutation (Yamamoto *et al*., 2000). The expression promoter is α-CamKII, which facilitates high levels of expression in the forebrain. Mutant gene expression is under the regulation of a tetracyclin binding sequence, bi-TetO, linked to galactosidase, and then to exon 1 of mutant huntingtin containing an expanded CAG repeat. Tetracyclin binding switches huntingtin transcription off. Yamamoto and colleagues raised mice up to 18 weeks of age in the absence of tetracyclin. Animals developed a severe, progressive motor phenotype, characterized by tremor and foot clasping. Galactosidase staining showed that mutant huntingtin was widely expressed throughout the forebrain in homozygous gene-positive mice, as well as the striatum, hippocampus, hypothalamus, septum, and neocortex. No immunoreactivity was detectable in heterozygote mice. The animals also developed neuronal intra-

nuclear inclusions in all gal-positive regions, but neuronal intranuclear inclusion deposition was limited to brain regions where mutant huntingtin was expressed. Mice went on to develop striatal atrophy, gliosis, and reduced D1 and D2 receptor binding densities in the striatum, indicative of GABAergic cell loss or dysfunction. Most interestingly, all of these parameters of neuronal dysfunction could be reversed, to some extent, by effectively switching expression of the transgene off by treating the animals with the tetracycline analog doxycycline. Animals treated for 4 months showed a marked reduction in the number of neuronal intranuclear inclusions in the striatum and neocortex and partial recovery of striatal atrophy and D1 and D2 binding levels, suggesting that there may be a therapeutic window for disease treatment in postsymptomatic patients. This breakthrough development opens up a plethora of opportunities to observe the consequences of manipulating huntingtin gene expression and to test potential therapeutic strategies.

VII. Conclusions

Although the pathogenic mechanism in Huntington's disease does not seem to be directly linked to the formation of intranuclear huntingtin inclusions, there is an association between the cell death process and CAG repeat length in mutant huntingtin. This is well demonstrated by the observation that incorporation of an expanded CAG repeat stretch (146 CAGs) into a murine gene normally lacking CAG repeats, the hypoxanthine phosphoribosyltransferase gene (*hprt*), resulted in a mouse phenotype reminiscent of other human CAG repeat disorders (JO1 mice, Ordway *et al*., 1997). Mice develop a progressive neurological phenotype consisting of a late-onset resting tremor, ataxia, decreased open field motor activity, propensity to fall from the rotarod, foot clasping, some incidence of seizures, and premature death at approximately 42–53 weeks of age. CAG length-dependence of these traits was verified by the observations that mice expressing 70 CAG repeats in the *hprt* gene did not develop a behavioral phenotype by 35 weeks of age, whereas the first behavioral symptoms are evident by 12 weeks in JO1 mice (Ordway *et al*., 1997). A great deal of conjecture now surrounds the question of whether translocation of huntingtin protein into the nucleus, with or without aggregate formation, is an essential step in the pathogenic process in Huntington's disease. Several groups have suggested that huntingtin translocation into the nucleus precedes cell pathology (Saudou *et al*., 1998; Hackam *et al*., 1999; Hodgson *et al*., 1999; Wheeler *et al*., 1999). Interestingly, in another polyglutamine disease ataxin-1 movement into the nucleus has been shown to be a prerequisite for pathogenesis, and has been associated with gene downregulation in SCA-1 transgenic mice (Lin *et al*., 2000).

Whereas the definitive pathway underlying cell death in Huntington's disease is yet to be determined, the development and characterization of transgenic and knock-in mouse models of the disorder can only help achieve this goal. In the meantime, it is heartening that initial studies of metabolic enhancers and caspase inhibitors are showing some degree of efficacy in delaying disease onset and extending survival in animal models of the disease.

References

Aeschlimann, D., and Paulsson, M. (1994). Transglutaminases: Protein cross-linking enzymes in tissues and body fluids. *Thromb. Haemostasis* **71**, 402–415.

Albin, R. L., and Greenamyre, J. T. (1992). Alternative excitotoxic hypotheses. *Neurology* **42**, 733–738.

Albin, R. L., Young, A. B., and Penney, J. B. (1989). The functional anatomy of basal ganglia disorders. *Trends Neurosci*. **12**, 366–375.

Albin, R. L., Reiner, A., Anderson, K. D., Penney, J. B., and Young, A. B. (1990). Striatal and nigral neuron subpopulations in rigid Huntington's disease: Implications for the functional anatomy of chorea and rigidity-akinesia. *Ann. Neurol*. **27**, 357–365.

Alexander, G. E., and Crutcher, M. D. (1990). Functional architecture of basal ganglia circuits: Neural substrates of parallel processing. *Trends Neurosci*. **13**, 266–271.

Andrew, S. E., Goldberg, Y. P., Kremer, B., Telenius, H., Theilmann, J., Adam, S., Starr, E., Squitieri, F., Lin, B., Kalchman, M. A. *et al*. (1993). The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. *Nat. Genet*. **4**, 398–403.

Andrews, T. C., and Brooks, D. J. (1998). Advances in the understanding of early Huntington's disease using the functional imaging techniques of PET and SPET. *Mol. Med. Today* **4**, 532–539.

Andrews, T. C., Weeks, R. A., and Brooks, D. J. (1997). [^{11}C]Raclopride and [^{11}C]SCH 23390 PET and the unified Huntington's disease rating scale can be used to monitor disease progression in asymptomatic Huntington's disease gene carriers. *Soc. Neurosci. Abstr.* **23**, 1912.

Antonini, A., Leenders, K. L., Spiegel, R., Meier, D., Vontobel, P., Weigell-Weber, M., Sanchez-Pernaute, R., de Yebenez, J. G., Boesiger, P., Weindl, A., and Maguire, R. P. (1996). Striatal glucose metabolism and dopamine D2 receptor binding in asymptomatic gene carriers and patients. *Brain* **119**, 2085–2095.

Arenas, J., Campos, Y., Ribacoba, R., Martin, M. A., Rubio, J. C., Ablanedo, P., and Cabello, A. (1998). Complex I defect in muscle from patients with Huntington's disease. *Ann. Neurol*. **43**, 397–400.

Aronin, N., Kim, M., Laforet, G., and DiFiglia, M. (1999). Are there multiple pathways in the pathogenesis of Huntington's disease? *Philos. Trans. R. Soc. London* **354**, 995–1003.

Bao, J., Sharp, A. H., Wagster, M. V., Becher, M., Schilling, G., Ross, C. A., Dawson, V. L., and Dawson, T. M. (1996). Expansion of polyglutamine repeat in huntingtin leads to abnormal protein interactions involving calmodulin. *Proc. Natl. Acad. Sci. U.S A*. **93**, 5037–5042.

Beal, M. F. (1994a). Huntington's disease, energy, and excitotoxicity. *Neurobiol. Aging*. **15**, 275–276.

Beal, M. F. (1994b). Neurochemistry and toxin models in Huntington's disease. *Curr. Opin. Neurol*. **7**, 542–547.

Beal, M. F. (1996). Mitochondria, free radicals, and neurodegeneration. *Curr. Opin. Neurobiol*. **6**, 661–666.

Beal, M. F., Ellison, D. W., Mazurek, M. F., Swartz, K. J., Malloy, J. R., Bird, E. D., and Martin, J. B. (1988). A detailed examination of substance P in pathologically graded cases of Huntington's disease. *J. Neurol. Sci*. **84**, 51–61.

Beal, M. F., Brouillet, E., Jenkins, B. G., Ferrante, R. J., Kowall, N. W., Miller, J. M., Storey, E., Srivastava, R., Rosen, B. R., and Hyman, B. T. (1993). Neurochemical and histologic characterization of excitotoxic lesions produced by mitochondrial toxin 3-nitropropionic acid. *J. Neurosci*. **13**, 4181–4192.

Beal, M, F., Henshaw, D, R., Jenkins, B. G., Rosen, B. R., and Schulz, J. B. (1994). Coenzyme Q_{10} and nicotinamide block striatal lesions produced by the mitochondrial toxin malonate. *Ann. Neurol*. **36**, 882–888.

Beal, M. F., Ferrante, R. J., Henshaw, R., Matthews, R. T., Chan, P. H., Kowall, N. W., Epstein, C. J., and Schulz, J. B. (1995). 3-Nitropropionic acid neurotoxicity is attenuated in copper/zinc superoxide dismutase transgenic mice. *J. Neurochem*. **65**, 919–922.

Berent, S., Giordani, B., Lehtinen, S., Markel, D., Penney, J. B., Buchtel, H. A., Starosta-Rubinstein, S., Hichwa, R., and Young, A. B. (1988). Positron emission tomographic scan investigations of Huntington's disease: Cerebral metabolic correlates. *Ann. Neurol*. **23**, 541–546.

Bertaux, F., Sharp, A. H., Ross, C. A., Lehrach, H., Bates, G. P., and Wanker, E. (1998). HAP1-huntingtin interactions do not contribute to the molecular pathology in Huntington's disease transgenic mice. *FEBS Lett*. **426**, 229–232.

Bogdanov, M. B., Ferrante, R. J., Kuemmerle, S., Klivenyi, P., and Beal, M. F. (1998). Increased vulnerability to 3-nitropropionic acid in an animal model of Huntington's disease. *J. Neurochem*. **71**, 2642–2644.

Bolanos, J. P., Heales, S. J. R., Land, J. M., and Clark, J. B. (1995). Effect of peroxynitrite on the mitochondrial respiratory chain: Differential susceptibility of neurones and astrocytes in primary culture. *J. Neurochem*. **64**, 1965–1972.

Braak, H., and Braak, E. (1992). Allocortical involvement in Huntington's disease. *Neuropathol. Appl. Neurobiol*. **18**, 539–547.

Bresolin, N., Bet, L., Binda, A., Moggio, M., Comi, G., Nador, F., Ferrante, C., Carenzi, A., and Scarlato, G. (1988). Clinical and biochemical correlations in mitochondrial myopathies treated with coenzyme Q_{10}. *Neurology* **38**, 892–899.

Brouillet, E. P., Jekins, B. G., Hyman, B. T., Ferrante, R. J., Kowall, N. W., Srivastava, R., Samanta-Roy, D., Rosen, B. R., and Beal, M. F. (1993). Age-dependent vulnerability of the striatum to the mitochondrial toxin 3-nitropropionic acid. *J. Neurochem*. **60**, 356–359.

Brouillet, E., Hantraye, P., Ferrante, R. J., Dolan, R., Leroy-Willig, A., Kowall, N. W., and Beal, M. F. (1995). Chronic mitochondrial energy impairment produces selective striatal degeneration and abnormal choreiform movements in primates. *Proc. Natl. Acad. Sci. U.S. A*. **92**, 7105–7109.

Browne, S. E., and Beal, M. F. (1994). Oxidative damage and mitochondrial dysfunction in neurodegenerative diseases. *Biochem. Soc. Trans*. **22**, 1002–1006.

Browne, S. E., Bowling, A. C., MacGarvey, U., Baik, M. J., Berger, S. C., Muqit, M. M. K., Bird, E. D., and Beal, M. F. (1997). Oxidative damage and metabolic dysfunction in Huntington's disease: Selective vulnerability of the basal ganglia. *Ann. Neurol*. **41**, 646–653.

Browne, S. E., Ferrante, R. J., and Beal, M. F. (1999a). Oxidative stress in Huntington's disease. *Brain Pathol*. **9**, 147–163.

Browne, S. E., Wheeler, V., White, J. K., Fuller, S. W., MacDonald, M., and Beal, M. F. (1999b). Dose-dependent alterations in local cerebral glucose use associated with the huntingtin mutation in *Hdh* CAG knock-in transgenic mice. *Soc. Neurosci. Abstr.* **25**, 218.11.

Burke, J. R., Enghild, J. J., Martin, M. E., Jou, Y.-S., Myers, R. M., Roses, A. D., Vance, V. M., and Strittmatter, W. J. (1996). Huntingtin and DRPLA proteins selectively interact with the enzyme GAPDH. *Nat. Med*. **2**, 347–350.

Butterworth, J., Yates, C. M., and Reynolds, G. P. (1985). Distribution of phosphate-activated glutaminase, succinic dehydrogenase, pyruvate dehydrogenase, and γ-glutamyl transpeptidase in post-mortem brain from Huntington's disease and agonal cases. *J. Neurol. Sci*. **67**, 161–171.

Butterworth, N. J., Williams, L., Bullock, J. Y., Love, D. R., Faull, R. L., and Dragunow, M. (1998). Trinucleotide (CAG) repeat length is positively correlated with the degree of DNA fragmentation in Huntington's disease striatum. *Neuroscience* **87**, 49–53.

Cha, J. H., Kosinski, C. M., Kerner, J. A., Alsdorf, S. A., Mangiarini, L., Davies, S. W., Penney, J. B., Bates, G. P., and Young, A. B. (1998). Altered brain neurotransmitter receptors in transgenic mice expressing a portion of an abnormal human Huntington disease gene. *Proc. Natl. Acad. Sci. U. S. A*. **95**, 6480–6485.

Cooper, A. J. L., Sheu, K.-F. R., Burke, J. R., Onodera, O., Strittmatter, W. J., Roses, A. D., and Blass, J. P. (1997a). Transglutaminase-catalyzed inactivation of glyceraldehyde 3-phosphate dehydrogenase and α-ketoglutarate dehydrogenase complex by polyglutamine domains of pathological length. *Proc. Natl. Acad. Sci. U. S. A*. **94**, 12604–12609.

Cooper, A. J., Sheu, K. -F. R., Burke, J.R., Onodera, O., Strittmatter, W. J., Roses, A. D., and Blass, J. P. (1997b). Polyglutamine domains are substrates of tissue transglutaminase: Does transglutaminase play a role in expanded CAG/poly-Q neurodegenerative diseases? *J. Neurochem.* **69**, 431–434.

Cooper, A. J., Sheu, K. F., Burke, J. R., Strittmatter, W. J., and Blass, J. P. (1998). Glyceraldehyde 3-phosphate dehydrogenase abnormality in metabolically stressed Huntington disease fibroblasts. *Dev. Neurosci*. **20**, 462–468.

Cooper, A. J., Sheu, K. F., Burke, J. R., Strittmatter, W. J., Gentile, V., Peluso, G., and Blass, J. P. (1999). Pathogenesis of inclusion bodies in (CAG)n/Qn-expansion diseases with special reference to the role of tissue transglutaminase and to selective vulnerability. *J. Neurochem*. **72**, 889–899.

Cummings, C. J., Mancini, M. A., Antalffy, B., DeFranco, D. B., Orr, H. T., and Zoghbi, H. Y. (1998). Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1. *Nat. Genet*. **19**, 148–154.

Cummings, C. J., Reinstein, E., Sun, Y., Antalffy, B., Jiang, Y., Ciechanover, A., Orr, H. T., Beaudet, A. L., and Zoghbi, H. Y. (1999). Mutation of the E6-AP ubiquitin ligase reduces nuclear inclusion frequency while accelerating polyglutamine-induced pathology in SCA1 mice. *Neuron* **24**, 879–892.

Davies, S. W., Turmaine, M., Cozens, B., DiFiglia, M., Sharp, A., Ross, C. A., Scherzinger, E., Wanker, E. E., Mangiarini, L., and Bates, G. (1997). Formation of neuronal intranuclear inclusions (NII) underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell (Cambridge, Mass.)* **90**, 537–548.

DiFiglia, M., Sapp, E., Chase, K., Schwarz, C., Meloni, A., Young, C., Martin, E., Vonsattel, J. -P., Carraway, R., Reeves, S. A., Boyce, F. M., and Aronin, N. (1995). Huntingtin is a cytoplasmic protein associated with vesicles in human and rat brain neurons. *Neuron* **14**, 1075–1081.

DiFiglia, M., Sapp, E., Chase, K. O., Davies, S. W., Bates, G. P., Vonsattel, J. -P., and Aronin, N. (1997). Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science* **277**, 1990–1993.

Durr, A., Hahn-Barma, V., Brice, A., Pecheux, C., Dode, C., and Feingold, J. (1999). Homozygosity in Huntington's disease. *J. Med. Genet*. **36**, 172–173.

Duyao, M., Ambrose, C., Myers, R., Novelletto, A., Persichetti, F., Frontali, M., Folstein, S., Ross, C., Franz, M., Abbott, M. *et al*. (1993). Trinucleotide repeat length instability and age of onset in Huntington's disease. *Nat. Genet*. **4**, 387–392.

Duyao, M. P., Auerbach, A. B., Persichetti, F., Barnes, G. T., McNeil, S. M., Ge, P., Vonsattel, J.-P., Gusella, J. F., Joyner, A. L., and MacDonald, M. E. (1995). Inactivation of the mouse Huntington's disease gene homolog *Hdh. Science* **269**, 407–410.

Ferrante, R. J., Kowall, N. W., Beal, M. F., Martin, J. B., Bird, E. D., and Richardson, E. P. (1987). Morphologic and histochemical characteristics of a spared subset of striatal neurons in Huntington's disease. *J. Neuropathol. Exp. Neurol*. **46**, 12–27.

Ferrante, R. J., Kowall, N. W., Hersch, S. M., Brown, R. H., and Beal, M. F. (1996). Immunohistochemical localization of markers of oxidative injury in Huntington's disease. *Soc. Neurosci. Abstr.* **22**, 92.5.

Ferrante, R. J., Gutekunst, C. A., Persichetti, F., McNeil, S. M., Kowall, N. W., Gusella, J. F., MacDonald, M. E., Beal, M. F., and Hersch, S. M. (1997). Heterogeneous topographic and cellular distribution of huntingtin expression in the normal human neostriatum. *J. Neurosci*. **17**, 3052–3063.

Ferrante, R. J., Andreassen, O. A., Dedeoglu, A., Kuemmerle, S., Kubilus, J. K., Kaddurah-Daouk, R., Hersch, S. M., and Beal, M. F. (2000). Neuroprotective effects of creatine in a transgenic mouse model of Huntington's disease. *J. Neurosci*. (in press).

Furtado, S., Suchowersky, O., Rewcastle, B., Graham, L., Klimek, M. L., and Garber, A. (1996). Relationship between trinucleotide repeats and neuropathological changes in Huntington's disease. *Ann. Neurol*. **39**, 132–136.

Fusco, F. R., Chen, Q., Lamoreaux, W. J., Figueredo-Cardenas, G., Jiao, Y., Coffman, J. A., Surmeier, D. J., Honig, M. G., Carlock, L. R., and Reiner, A. (1999). Cellular localization of huntingtin in striatal and cortical neurons in rats: Lack of correlation with neuronal vulnerability in Huntington's disease. *J. Neurosci*. **19**, 1189–1202.

Goebel, H. H., Heipertz, R., Scholz, W., Iqbal, K., and Tellez-Nagel, I. (1978). Juvenile Huntington chorea: Clinical, ultrastructural, and biochemical studies. *Neurology* **28**, 23–31.

Grafton, S. T., Mazziotta, J. C., Pahl, J. J., St. George-Hyslop, P., Haines, J. L., Gusella, J., Hoffman, J. M., Baxter, L. R., and Phelps, M. E. (1992). Serial changes of cerebral glucose metabolism and caudate size in persons at risk for Huntington's disease. *Arch. Neurol. (Chicago)* **49**, 1161–1167.

Graybiel, A. M., Aosaki, T., Flaherty, A. W., and Kimura, M. (1994). The basal ganglia and adaptive motor control. *Science* **265**, 1826–1831.

Gu, M., Gash, M. T., Mann, V. M., Javoy-Agid, F., Cooper, J. M., and Schapira, A. H. V. (1996). Mitochondrial defect in Huntington's disease caudate nucleus. *Ann. Neurol*. **39**, 385–389.

Gutekunst, C.-A., Levey, A. I., Heilman, C. J., Whaley, W. L., Yi, H., Nash, N. R., Rees, H. D., Madden, J. J., and Hersch, S. M. (1995). Identification and localization of huntingtin in brain and human lymphoblastoid cell lines with anti-fusion protein antibodies. *Proc. Natl. Acad. Sci. U. S. A*. **92**, 8710–8714.

Hackam, A. S., Singaraja, R., Zhang, T., Gan, L., and Hayden, M. R. (1999). In vitro evidence for both the nucleus and cytoplasm as subcellular sites of pathogenesis in Huntington's disease. *Hum. Mol. Genet*. **8**, 25–33.

Hodgson, J. G., Agopyan, N., Gutekunst, C. -A., Leavitt, B. R., LePiane, F., Singaraja, R., Smith, D. J., Bissada, N., McCutcheon, K., Nasir, J., Jamot, L., Li, X. -J., Rosemond, E., Roder, J. C., Phillips, A. G., Rubin, E. M., Hersch, S. M., and Hayden, M. R. (1999). A YAC mouse model for Huntington's disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration. *Neuron* **23**, 1–20.

Hughes, D. B., Thomas, M., Dedeoglu, A., Browne, S. E., Ferrante, R. J., Andreassen, O. A., and Beal, M. F. (1999). Hyperglycemia in a transgenic animal model of Huntington's disease. *Soc. Neurosci. Abstr.* **25**, 237.14.

Hurlbert, M. S., Zhou, W., Wasmeier, C., Kaddis, F. G., Hutton, J. C., and Freed, C. R. (1999). Mice transgenic for an expanded CAG repeat in the Huntington's disease gene develop diabetes. *Diabetes* **48**, 649–651.

Hussey, D., Stewart, D., Houle, S., and Guttman, M. (1998). [^{11}C]Raclopride striatal binding potential as a measure of Huntington's disease pregression: Implications for prospective neuroprotective studies. *J. Nucl. Med*. **39**, 209.

Huntington's Disease Collaborative Research Group. (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosome. *Cell (Cambridge, Mass*. **72**, 971–983.

Ihara, Y., Namba, R., Kuroda, S., Sato, T., and Shirabe, T. (1989). Mitochondrial encephalomyopathy (MELAS): Pathological study and successful therapy withcoenzyme Q_{10} and idebenone. *J. Neurol. Sci*. **90**, 263–271.

Jackson, G. R., Salecker, I., Schulze, K. L., Dong, X., Yao, X., Arnheim, N., Faber, P., MacDonald, M. E., and Zipursky, S. L. (1998). Age- and polyglutamine length-dependent degeneration of *Drosophila* photoreceptor neurons induced by human huntingtin transgenes. *Soc. Neurosci. Abstr.* **24**, 205.2.

Jenkins, B. G., Koroshetz, W., Beal, M. F., and Rosen, B. (1993). Evidence for an energy metabolism defect in Huntington's disease using localized proton spectroscopy. *Neurology* **43**, 2689–2695.

Jenkins, B. G., Rosas, H. D., Chen, Y. C., Makabe, T., Myers, R., MacDonald, M., Rosen, B. R., Beal, M. F., and Koroshetz, W. J. (1998). ^{1}H NMR spectroscopy studies of Huntington's disease: Correlations with CAG repeat numbers. *Neurology* **50**, 1357–1365.

Kahlem, P., Terré, C., Green, H., and Djian, P. (1996). Peptides containing glutamine repeats as substrates for transglutaminase-catalyzed cross-linking: Relevance to diseases of the nervous system. *Proc. Natl. Acad. Sci. U. S. A*. **93**, 14580–14585.

Kim, M., Lee, H. S., LaForet, G., McIntyre, C., Martin, E. J., Chang, P., Kim, T. W., Williams, M., Reddy, P. H., Tagle, D., Boyce, F. M., Won, L., Heller, A., Aronin, N., and DiFiglia, M. (1999). Mutant huntingtin expression in clonal striatal cells: Dissociation of inclusion formation and neuronal survival by caspase inhibition. *J. Neurosci*. **19**, 964–973.

Koroshetz, W. J., Jenkins, B. G., Rosen, B. R., and Beal, M. F. (1997). Energy metabolism defects in Huntington's disease and possible therapy with coenzyme Q_{10}. *Ann. Neurol*. **41**, 160–165.

Kuhl, D. E., Markham, C. H., Metter, E. J., Riege, W. H., Phelps, M. E., and Mazziotta, J. C. (1985). Local cerebral glucose utilization in symptomatic and presymptomatic Huntington's disease. *Res. Publ. Assoc. Res. Nerv. Ment. Dis*. **63**, 199–209.

Kuwert, T., Lange, H. W., Langer, K.-J., Herzog, H., Aulich, A., and Feinendegen, L. E. (1990). Cortical and subcortical glucose consumption measured by PET in patients with Huntington's disease. *Brain* **113**, 1405–1423.

Kuwert, T., Lange, H. W., Boecker, H., Titz, H., Herzog, H., Aulich, A., Wang, B. C., Nayak, U., and Feinendegen, L. E. (1993). Striatal glucose consumption in chorea-free subjects at risk of Huntington's disease. *J. Neurol*. **241**, 31–36.

Laforet, G. A., Lee, H. S., Cadigan, B., Chang, P., Chase, K. O., Sapp, E., Martin, E. M., McIntyre, C., Williams, M., Reddy, P. H., Tagle, D., Stein, J. S., Boyce, F. M., DiFiglia, M., and Aronin, N. (1998). Development and chracterization of a novel transgenic model of Huntington's disease which recapitulates features of the human illness. *Soc. Neurosci. Abstr.* **24**, 380.8

Levine, M. S., Klapstein, G. J., Koppel, A., Gruen, E., Cepeda, C., Vargas, M. E., Jokel, E. S., Carpenter, E. M., Zanjani, H., Hurst, R. S., Efstratiadis, A., Zeitlin, S., and Chesselet, M. F. (1999). Enhanced sensitivity to N-methyl-D-aspartate receptor activation in transgenic and knockin mouse models of Huntington's disease. *J. Neurosci. Res*. **58**, 515–532.

Li, X.-J., Li, S. -H., Sharp, A. H., Nucifora, F. C., Jr., Schilling, G., Lanahan, A., Worley, P., Snyder, S. H., and Ross, C. A. (1995). Huntingtin-associated protein enriched in brain with implicatins for pathology. *Nature (London)* **378**, 398–402.

Lin, X., Antalffy, B., Kang, D., Orr, H. T., and Zoghbi, H. Y. (2000). Polyglutamine expansion down-regulates specific neuronal genes before pathologic changes in SCA1. *Nat. Neurosci*. **3**, 157–163.

Linnane, A. W., Marzuki, S., Ozawa, T., and Tanaka, M. (1989). Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. *Lancet* **1**, 642–645.

Lorand, L. (1996). Neurodegenerative diseases and transglutaminase. *Proc. Natl. Acad. Sci. U. S. A*. **93**, 14310–14313.

Ludolph, A. C., He, F., Spencer, P. S., Hammerstad, J., and Sabri, M. (1990). 3-nitropropionic acid: Exogenous animal neurotoxin and possible human striatal toxin. *Can. J. Neurol. Sci*. **18**, 492–498.

MacDonald, M. E., Vonsattel, J. -P., Shrinidhi, J., Couropmitree, N. N., Cupples, L. A., Bird, E. D., Gusella, J. F., and Myers, R. H. (1999). Evidence for the GluR6 gene associated with younger onset age of Huntington's disease. *Neurology* **53**, 1330–1332.

Mangiarini, L., Sathasivam, K., Seller, M., Cozens, B., Harper, A., Hetherington, C., Lawton, M., Trottier, Y., Lehrach, H., Davies, S. W., and Bates, G. (1996). Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell (Cambridge, Mass.)* **87**, 493–506.

Mastrogiacomo, F., and Kish, S. J. (1994). Cerebellar_-ketoglutarase dehydrogenase activity is reduced in spinocerebellar ataxia type 1. *Ann. Neurol*. **35**, 624–626.

Mastrogiacomo, F., LaMarche, J., Dozic, S., Lindsay, G., Bettendorff, L., Robitaille, Y., Schut, L., and Kish, S. J. (1994). Immunoreactive levels of α-ketoglutarase dehydrogenase subunits in Friedreich's ataxia and spinocerebellar ataxia type 1. *Neurodegeneration* **5**, 27–33.

Matsuishi, T., Sakai, T., Naito, E., Nagamitsu, S., Kuroda, Y., Iwashita, H., and Kato, H. (1996). Elevated cerebrospinal fluid lactate/pyruvate ratio in Machado-Joseph disease. *Acta Neurol. Scand.* **93**, 72–75.

Matthew, E., Nordahl, T., Schut, L., King, A. C., and Cohen, R. (1993). Metabolic and cognitive changes in hereditary ataxia. *J. Neurol. Sci.* **119**, 134–140.

Matthews, R. T., Yang, L., Jenkins, B. J., Ferrante, R. J., Rosen, B. R., Kaddurah-Daouk, R., and Beal, M. F. (1998). Neuroprotective effects of creatine and cyclocreatine in animal models of Huntington's disease. *J. Neurosci.* **18**, 156–163.

Moulder, K. L., Onodera, O., Burke, J., Strittmatter, W. J., and Johnson, E. M., Jr. (1998). Polyglutamine containing proteins produce length-dependent aggregation and death in cultured cerebellar granule cells. *Soc. Neurosci. Abstr.* **24**, 379.5.

Myers, R. H., Vonsattel, J. P., Paskevich, P. A., Kiely, D. K., Stevens, T. J., Cupples, L. A., Richardson, E. P., Jr., and Bird, E. D. (1991). Decreased neuronal and increased oligodendroglial densities in Huntington's disease caudate nucleus. *J. Neuropathol. Exp. Neurol.* **50**, 729–742.

O'Brien, C. F., Miller, C., Goldblatt, D., Welle, S., Forbes, G., Lipinski, B., Panzik, J., Peck, R., Plumb, S., Oakes, D., Kurlan, R., and Shoulson, I. (1990). Extraneural metabolism in early Huntington's disease. *Ann. Neurol.* **28**, 300–301.

Ona, V. O., Li, M., Vonsattel, J. P., Andrews, L. J., Khan, S. Q., Chung, W. M., Frey, A. S., Menon, A. S., Li, X. J., Stieg, P. E., Yuan, J., Penney, J. B., Young, A. B., Cha, J. H., and Friedlander, R. M. (1999). Inhibition of caspase-1 slows disease progression in a mouse model of Huntington's disease. *Nature (London)* **399**, 263–267.

Ordway, J. M., Tallaksen-Greene, S., Gutekunst, C. A., Bernstein, E. M., Cearley, J. A., Wiener, H. W., Dure, L. S., 4th, Lindsey, R., Hersch, S. M., Jope, R. S., Albin, R. L., and Detloff, P. J. (1997). Ectopically expressed CAG repeats cause intranuclear inclusions and a progressive late onset neurological phenotype in the mouse. *Cell (Cambridge, Mass.)* **91**, 753–763.

Parker, W. D., J. r., Boyson, S. J., Luder, A. S., and Parks, J. K. (1990). Evidence for a defect in NADH: Ubiquinone oxidoreductase (complex I) in Huntington's disease. *Neurology* **40**, 1231–1234.

Paulson, H. L., Perez, M. K., Trottier, Y., Trojanowski, J. Q., Subramony, S. H., Das, S. S., Vig, P., Mandel, J. L., Fischbeck, K. H., and Pittman, R. N. (1997). Intranuclear inclusions of expanded polyglutamine protein in spinocerebellar ataxia type 3. *Neuron* **19**, 333–344.

Perutz, M., Johnson, T., Suzuki, M., and Finch, J. T. (1994). Glutamine repeats as polar zippers: Their possible role in inherited neurodegenerative diseases. *Proc. Natl. Acad. Sci. U. S. A.* **91**, 5355–5358.

Reddy, P. H., Williams, M., Charles, V., Garrett, L., Pike-Buchanan, L., Whetsell, W. O., Jr., Miller, G., and Tagle, D. A. (1998). Behavioural abnormalities and selective neuronal loss in HD transgenic mice expressing mutated full-length HD cDNA. *Nat. Genet.* **20**, 198–202.

Reiner, A., Albin, R. L., Anderson, K. D., D'Amato, C. J., Penney, J. B., and Young, A. B. (1988). Differential loss of striatal projection neurons in Huntington disease. *Proc. Natl. Acad. Sci. U. S. A.* **85**, 5733–5737.

Reynolds, G. P., Dalton, C. F., Tillery, C. L., Mangiarini, L., Davies, S. W., Bates, G. P. (1999). Brain neurotransmitter deficits in mice transgenic for the Huntington's disease mutation. *J. Neurochem.* **72**, 1773–1776.

Richfield, E. K., Maguire-Zeiss, K. A., Cox, C., Gilmore, J., and Voorn, P. (1995). Reduced expression of preproenkephalin in striatal neurons from Huntington's disease patients. *Ann. Neurol.* **37**, 335–343.

Ross, C. A. (1995). When less is more: Pathogenesis in glutamine repeat neurodegenerative diseases. *Neuron* **15**, 493–496.

Sapp, E., Ge, P., Aizawa, H., Bird, E., Penney, J., Young, A. B., Vonsattel, J. P., and DiFiglia, M. (1995). Evidence for a preferential loss of enkephalin immunoreactivity in the external globus pallidus in low grade Huntington's disease using high resolution image analysis. *Neuroscience* **64**, 397–404.

Sathasivam, K., Hobbs, C., Turmaine, M., Mangiarini, L., Mahal, A., Bertaux, F., Wanker, E. E., Doherty, P., Davies, S. W., and Bates, G. P. (1999). Formation of polyglutamine inclusions in non-CNS tissue. *Hum. Mol. Genet.* **8**, 813–822.

Saudou, F., Finkbeiner, S., Devys, D., and Greenberg, M. E. (1998). Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. *Cell (Cambridge, Mass.)* **95**, 55–66.

Saunders, P. A., Chalecka-Franaszek, E., and Chuang, D. M. (1997). Subcellular distribution of glyceraldehyde-3-phosphate dehydrogenase in cerebellar granule cells undergoing cytosine arabinoside-induced apoptosis. *J. Neurochem.* **69**, 1820–1828.

Sawa, A., Khan, A. A., Hester, L. D., and Snyder, S. H. (1997). Glyceraldehyde-3-phosphate dehydrogenase: Nuclear translocation participates in neuronal and nonneuronal cell death. *Proc. Natl. Acad. Sci. U. S. A.* **94**, 11669–11674.

Sawa, A., Wiegand, G. W., Cooper, J., Margolis, R., Sharp, A. H., Lawler, J. F., Greenamyre, J. T., Snyder, S. H., and Ross, C. A. (1999). Increased apoptosis of Huntington disease lymphoblasts associated with repeat length-dependent mitochondrial depolarization. *Nature Med.* **5**, 1194–1198.

Schapira, A. H. V., Mann, V. M., Cooper, J. M., Krige, D., Jenner, P. J., and Marsden, C. D. (1992). *Ann. Neurol.* **32**, S116–S124.

Scherzinger, E., Kurz, R., Turmaine, M., Mangiarini, L., Hollenbach, B., Hasenbank, R., Bates, G. P., Davies, S. W., Lehrach, H., and Wanker, E. E. (1997). Huntingtin-encoded polyglutamine expansions form amyloid-like aggregates in vitro and in vivo. *Cell (Cambridge, Mass.)* **90**, 549–558.

Schilling, G., Bacher, M. W., Sharp, A. H., Jinnah, H. A., Duyao, K., Kotzuk, J. A., Slunt, H. H., Ratovitski, T., Cooper, J. A., Jenkins, N. A. *et al.* (1999). Intranuclear inclusions and neuritic aggregates in transgenic mice expressing a mutant N-terminal fragment of huntingtin. *Hum. Mol. Genet.* **8**, 397–407.

Schulz, J. B., Henshaw, D. R., MacGarvey, U., and Beal. M. F., (1996a). Involvement of oxidative stress in 3-nitropropionic acid neurotoxicity. *Neurochem. Int.* **29**, 167–171.

Schulz, J. B., Matthews, R. T., Henshaw, D. R., and Beal, M. F. (1996b). Neuroprotective strategies for treatment of lesions produced by mitochondrial toxins: Implications for neurodegenerative diseases. *Neurosci.* **71**, 1043–1048.

Sharp, N. H., Loev, S. J., Schilling, G., Li, S.-H., Li, X.-J., Bao, J., Wagster, M. V., Kotzuk, J. A., Steiner, J. P., Lo, A., Hedreen, J., Sisodia, S., Snyder, S. H., Dawson, T. M., Ryugo, D. K., and Ross, C. A. (1995). Widespread expression of the Huntington's disease gene (IT-15) protein product. *Neuron* **14**, 1065–1074.

Shelbourne, P. F., Killeen, N., Hevner, R. F., Johnston, H. M., Tecott, L., Lewandoski, M., Ennis, M., Ramirez, L., Li, Z., Iannicola, C., Littman, D. R., and Myers, R. M. (1999). A Huntington's disease CAG expansion at the murine Hdh locus is unstable and associated with behavioural abnormalities in mice. *Hum. Mol. Genet.* **8**, 763–774.

Sieradzan, K. A., and Mann, D. M. (1998). On the pathological progression of Huntington's disease. *Ann. Neurol.* **44**, 148–149.

Sotrel, A., Paskevich, P. A., Kiely, D. K., Bird, E. D., Willimas, R. S., and Myers, R. H. (1991). Morphometric analysis of the prefrontal cortex in Huntington's disease. *Neurology* **41**, 1117–1123.

Storey, E., Hyman, B. T., Jenkins, B. T., Brouillet, E., Miller, J. M., Rosen, B. R., and Beal, M. F. (1992). MPP^+ produces excitotoxic lesions in rat striatum due to impairment of oxidative metabolism. *J. Neurochem.* **58**, 1975–1978.

Strong, T. V., Tagle, D. A., Valdes, J. M., Elmer, L. W., Boehm, K., Swaroop, M., Kaatz, K. W., Collins, F. S., and Albin, R. L. (1993). Widespread expression of the human and rat Huntington's disease gene in brain and nonneural tissues. *Nat Genet.* **5**, 259–265.

Tabrizi, S. J., Cleeter, M. W., Xuereb, J., Taanman, J. W., Cooper, J. M., and Schapira, A. H. (1999). Biochemical abnormalities and excitotoxicity in Huntington's disease brain. *Ann. Neurol*. **45**, 25–32.

Tabrizi, S. J., Workman, J., Hart, P. E., Mangiarini, L., Mahal, A., Bates, G., Cooper, J. M., and Schapira, A. H. (2000). Mitochondrial dysfunction and free radical damage in the Huntington R6/2 transgenic mouse. *Ann. Neurol*. **47**, 80–86.

Tellez-Nagel, I., Johnson, A. B., and Terry, R. D. (1995). Studies on brain biopsies of patients with Huntington's chorea. *J. Neuropathol. Exp. Neurol*. **33**, 308–332.

Usdin, M. T., Shelbourne, P. F., Myers, R. M., and Madison, D. V. (1999). Impaired synaptic plasticity in mice carrying the Huntington's disease mutation. *Hum. Mol. Genet*. **8**, 839–846.

Vonsattel, J.-P., Myers, R. H., Stevens, T. J., Ferrante, R. J., Bird, E. D., and Richardson, E. P. (1985). Neuropathological classification of Huntington's disease. *J. Neuropathol. Exp. Neurol*. **44**, 559–577.

Vonsattel, J. P. G., and DiFiglia, M. (1998). Huntington disease. *J. Neuropathol. Exp. Neurol*. **57**, 369–384.

Wheeler, V. C., White, J. K., Gutekunst, C. A., Vrbanac, V., Weaver, M., Li, X. J., Li, S. H., Yi, H., Vonsattel, J. P., Gusella, J. F., Hersch, S., Auerbach, W., Joyner, A. L. and MacDonald, M. E. (2000). Long glutamine tracts cause nuclear localization of a novel form of huntingtin in medium spiny striatal neurons in HdhQ92 and HdhQ111 knock-in-mice. *Hum. Mol. Genet.* **9**, 503–513.

White, J. K., Auerbach, W., Duyao, M. P., Vonsattel, J.-P., Gusella, J. F., Joyner, A. L., and MacDonald, M. E. (1997). Huntingtin function is required for mouse brain development and is not impaired by the Huntington's disease CAG expansion mutation. *Nat. Genet*. **17**, 404–410.

Wullner, U., Young, A. B., Penney, J. B., and Beal, M. F. (1994). 3-Nitropropionic acid toxicity in the striatum. *J. Neurochem*. **63**, 1772–1781.

Yamamoto, A., Lucas, J. J., Hen, R. (2000). Reversal of nuropathology and motor dysfunction in a conditional model of Huntington's disease. *Cell* **101**, 57–66.

Young, A. B., Penney, J. B., Starosta-Rubinstein, S., Markel, D. S., Berent, S., Giordani, B., Ehrenkaufer, R., Jewett, D., and Hichwa, R. (1986). PET scan investigations of Huntington's disease: Cerebral metabolic correlates of neurological features and functional decline. *Ann. Neurol*. **20**, 296–303.

Zoghbi, H. Y. (1997). CAG repeats in SCA6. Anticipating new clues. *Neurology* **49**, 1196–1199.

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Biochemical and Anatomical Changes in Basal Ganglia of Aging Animals

Deterioration of motor function is a hallmark of senescence in humans and other mammalian species. The similarity of age-related motor deterioration to the symptoms of Parkinson's disease has focused research efforts upon the basal ganglia and particularly the nigrotriatal dopaminergic system. In this chapter, age-related structural and functional characteristics of basal ganglia of several laboratory animal species were reviewed. Although some studies have demonstrated loss of substantia nigra pars compacta dopaminergic neurons with age, the degree of loss does not appear sufficient to account for the marked motor decrements that occur in senescence. Consequently, studies of age-related changes in nigrostriatal function have increased. Reported changes in the basal ganglia of aged animals include decreased numbers of dopamine receptors and transporters as well as decreased dopamine synthesis, release, and uptake. There is also evidence for changes in the interactions between dopamine and other neurotransmitters in the basal ganglia. These age-related changes in nigrostriatal dopaminergic function may account for the majority of the motor deficits that accompany normal aging.

I. Introduction

Along with memory loss, a cardinal feature of senescence is deteriorating motor function. Indeed, slow movements, tremor, stooped posture, and a shuffling gait—also symptoms of Parkinson's disease—are hallmarks of old age as well as parkinsonism in the elderly (Mortimer and Webster, 1982; Teräväinen and Calne, 1983; Bennett *et al.*, 1996). Similar motoric disturbances have also been reported in a variety of mammalian species (e.g., Irwin *et al.*, 1994; Emborg *et al.*, 1998; Hebert and Gerhardt, 1998). When it was discovered that the brains of individuals who suffered from Parkinson's disease were deficient of the neurotransmitter dopamine (Ehringer and Hornykiewicz, 1960) and that these symptoms were successfully reversed by the dopamine precursor L-dopa (Cotzias *et al.*, 1967, 1969), researchers were provided with a neurochemical mechanism underlying these deficits. Consequently, the basal ganglia became the primary neuroanatomical loci for studies of not only Parkinson's disease but also of nonpathological age-related declines in motor function. Although the majority of previous studies have focused upon age-related structural alterations in the basal ganglia, more recent studies have attempted to characterize changes in neuronal function. These functional decrements arguably account for much of the deterioration of motor capabilities that are observed in senescence.

A. The Basal Ganglia

The basal ganglia are group of interconnected subcortical nuclei that interact with neocortical and limbic regions to produce meaningful, goal-directed behavior (Gray, 1995). They are composed of the striatal and pallidal portions of the basal telencephalon, the substantia nigra, ventral tegmental area, and the subthalamic nucleus (Marin *et al.*, 1998; Wilson, 1998). The striatal and pallidal components of the basal ganglia are functionally segregated into dorsal and ventral portions. The dorsal component is composed of the dorsal striatum, i.e., caudate nucleus and putamen (in primates and carnivores) or caudate-putamen (in other mammals), the internal and external segments of the globus pallidus (in primates), or globus pallidus and entopeduncular nucleus, respectively (in nonprimates). The ventral component is composed of the ventral striatum, i.e., the nucleus accumbens and portions of the olfactory tubercle and the ventral pallidum (Marin *et al.*, 1998).

B. Animal Models of Aging

Understanding the basic mechanisms leading to age-related functional impairment in humans necessitates the use of animal models. As mentioned previously, a variety of mammalian species exhibit age-related motor deficits that parallel those observed in humans. While many laboratory animals species

are used in aging studies, the majority of studies of age-related changes in the nigrostriatal dopaminergic system have been conducted with rodents, specifically Fischer 344 (F344), Wistar, and Sprague–Dawley rats or C57BL mice (Austad, 1997). Recent studies involving nonhuman primates have also uncovered age-related changes in nigrostriatal and mesolimbic dopaminergic systems. These studies have provided new insights into the mechanisms underlying age-related alterations in movement.

One outstanding issue in aging research has been the lack of a standardized scheme of classifying an animal of a particular species as ''aged.'' Ideally such a scheme would be based upon comparative data regarding the species' life span and the extent to which the species' age-related alterations parallel those of aging humans. Considering the absence of such standards, a review of the literature suggests considerable uniformity in what is defined as ''aged'' in aging studies. Although there is some variation between rat strains regarding longevity (Masoro, 1990; Sprott and Austad, 1996), researchers have generally defined rats as aged at $\geq$22 months and C57BL mice are generally classified as aged at $\geq$21 months. Cats are typically labeled aged at $\geq$11 years, rabbits at 5 years, and the aged group in studies using monkeys is typically $\geq$22 years of age. Subjects in human studies in which age-related effects have been reported are generally $\geq$70. In each of these species, marked age-related deficits in movements have been reported. Because, with few exceptions, these are the ages typically employed to classify subjects as ''aged,'' studies that report a lack of age effect in animals younger than the aforementioned values are not included in this review. Age-related changes in humans will be cited throughout the chapter for comparative purposes.

C. Changes to Be Covered

Because of its role in motor behavior, the nigrostriatal dopaminergic system has been the focus of the majority of studies examining age-related decrements in basal ganglia function. For this reason, this chapter will focus predominately upon the ascending nigrostriatal dopaminergic tract. The discussion will include alterations in the structural integrity of dopaminergic neurons and their connections in senescence, as well as the functional capacity of pre- and postsynaptic neuronal mechanisms. The chapter will conclude with a brief review of age-related alterations in other neurotransmitter systems that interact with dopaminergic neurons in the aged basal ganglia. In all cases we have tried to be comprehensive. However, undoubtedly we have failed to include some pertinent data and citations.

II. Morphological Changes

A. Cell Number

It has been a biological doctrine that normal (i.e., nonpathological) aging is accompanied by widespread neuronal loss. This commonly accepted belief was based largely upon early studies in which extensive cell death was inferred from measurements of decreased neuronal densities in brain regions known to be affected in Alzheimer's disease—specifically, regions of the neocortex and hippocampus (Brody, 1955; Dayan, 1970; Colon, 1972). Likewise, the prevalence of parkinsonian-like symptoms that often accompany aging has led to the suggestion that normal aging occupies a ''preparkinsonian'' position on a continuum which includes Parkinson's disease as a manifestation of accelerated aging (Barbeau, 1973; McGeer *et al.*, 1977; Beck, 1978; Mann and Yates, 1982; Mortimer and Webster, 1982). This hypothesis seems to be supported by reports of age-associated decreases in the number of human substantia nigra pars compacta neurons on the order of 30 to 50% between 20 and 90 years of age (McGeer *et al.*, 1977; Mann and Yates, 1983; Fearnley and Lees, 1991; however, see references cited in McNeill and Koek, 1990, for evidence to the contrary). Likewise, a 50% decrease in the number of substantia nigra neurons of aged (25- to 27-year-old) Rhesus monkeys has also been reported (Emborg *et al.*, 1998). However, dopaminergic cell loss in the aged squirrel monkey and rodent substantia nigra pars compacta has not been observed (Flood and Coleman, 1988; McNeill and Koek, 1990; Emerich *et al.*, 1993; Irwin *et al.*, 1994).

Several caveats regarding the interpretation of age-related dopaminergic cell death as it relates to functional deficits should be considered. While there have been reports of decreased numbers of dopaminergic neurons in aged primates, there are quantitative as well as qualitative differences between the physiological substrates of normal age-related motor deficits and the pathophysiological substrates of Parkinson's disease (e.g., Fearnley and Lees, 1991; Kish *et al.*, 1992; Hubble, 1998). These differences have become recognized as a result of critical analyses of previous data, improvements in histopathological methodology, and analysis of the distinct etiologies of the motor deficits that accompany each of these conditions. Other considerations relate to the methods used to determine age-related loss of dopaminergic neurons. Recent studies using unbiased stereological cell-counting methods in regions other than the basal ganglia have cast doubt on the long-held belief that neuronal death is an inevitable consequence of aging (West and Gundersen, 1990; West, 1993; West *et al.*, 1994; see also Morrison and Hof, 1997). Stereological methods have also challenged previous findings regarding the inevitability of nigral cell loss with age (Strothjohann *et al.*, 1993; Irwin *et al.*, 1994; Pakkenberg *et al.*, 1995). Nevertheless, a recent study using stereological methods to determine age-related nigral cell loss reported a 50% reduction of tyrosine hydroxylase (TH)-immunoreactive neurons in the substantia nigra of aged monkeys (Emborg *et al.*, 1998). Interestingly, these investigators also determined that their quantification of dopaminergic cell number did not correlate with age-related functional changes in the aged monkeys. Caution should be exercised when interpreting studies in which the number of dopamine-containing neurons was determined based on the immunoreactivity of cells to TH, however, as decreases in TH immunoreactivity may overestimate dopaminergic neuronal loss as a result of functional deficiencies in residual cells and staining methods (McGeer *et al.*, 1977; Emborg *et al.*, 1998).

Notwithstanding these methodological concerns, the extent of age-related dopaminergic cell loss reported in these studies was generally less than 50%. Although age-related motor deficits may coincide with modest dopaminergic neuronal loss,

reported decreases in the number of dopaminergic neurons barely approach the magnitude of loss that would be necessary to decrease striatal dopaminergic levels by ≥80%, the amount required for gross functional impairment in humans with Parkinson's disease or in animal models of Parkinson's disease (Hornykiewicz, 1963; Stricker and Zigmond, 1976; Lloyd, 1977). Indeed, if Parkinson's disease cell-loss criteria were employed, extrapolation of the regression lines reported in studies of age-related dopaminergic cell loss would not place individuals at risk of gross motor decline until they were 100 years old (McGeer *et al.*, 1977; Mortimer and Webster, 1982; Kish *et al.*, 1992). Clearly, dopaminergic cell loss is an insufficient explanation for gross, age-related declines in motor function.

B. Pathological Accumulations

The escalating presence of lipofuscin in nerve cells is considered to be a sign of neuronal aging, as the amount of the pigment has been shown to gradually increase with age in a variety of tissues in several species (Sohal and Wolfe, 1986; Peters *et al.*, 1991). Age-related accumulation of lipofuscin in dopaminergic neurons of the substantia nigra pars compacta have been reported in C57BL/6J and C57BL/6NNia mice (McNeill *et al.*, 1984; Ingram *et al.*, 1993). While the observations of McNeill and colleagues (1984) were not quantitative, they reported increases in lipofuscin beginning at 10 months of age, with a preponderance of accumulation at 30 months. Ingram and colleagues (1993) quantified the increase, reporting that lipofuscin accumulation was approximately 20% higher in 18-month-old as compared to 3-month-old mice. Although the presence of lipofuscin at low levels does not necessarily support pathology, with increasing accumulation it may be associated with progressively lower levels of ribonucleic acid in the cytoplasm (Peters *et al.* 1991). This may indicate an increasing decrement of the cell's exocytotic capacity (Sohal and Wolfe, 1986) or an alteration in the cell's metabolic or neuronal activity (McNeill *et al.*, 1984).

While the pathological significance of lipofuscin is debatable, Lewy bodies, which are a histological signature of Parkinson's disease (Kopin, 1993), are readily associated with basal ganglia pathology. Lewy bodies have been observed in the substantia nigra of a small percentage of nonparkinsonian human controls over the age of 50 (e.g., 6% reported by Fearnley and Lees, 1991). Although Fearnley and Lees (1991) reported increases in the prevalence of incidental Lewy bodies with age, they argued that the presence of Lewy bodies are not an aspect of normal aging, but are indicative of presymptomatic Parkinson's disease. This suggestion is supported by a lack of reports of Lewy bodies in aged animals.

C. Connections

In studies that have reported age-related changes in morphological features of neurons in the basal ganglia, most have focused on the dendrites of striatal neurons. Levine (1988) reported decreases of 40–49% in spinal densities of medium spiny GABAergic neurons in the caudate nucleus of old (15–18 years) cats compared to mature (1–3 years) cats. Furthermore, total dendritic length, average dendrite length, average branch length, and the radius of the dendritic field of these principal neurons were also decreased by between 30 and 40% in the caudate nucleus of the aged cats. These decreases began in 13-year-old cats and may have been the basis for altered striatal electrophysiological responses to cortical and nigral stimulation.

In a study utilizing C57BL/6N mice, McNeill and colleagues (1990) found that medium spiny neurons in the caudal striata of aged mice exhibited significant dendritic elongation (138%) between 25 and 30 months. This elongation was partially attributed to compensatory responses to degeneration of neighboring neurons. The caudal striatal medium spiny neurons of young (3 month), motor-unimpaired aged (30 months), and motor-impaired aged (30 month) mice were compared. Although each group had total dendritic lengths that were comparable, when frequency distributions based upon ranges of dendritic lengths (0–600, 601–1200, 1201–1800, >1800 μm) were compared, the neurons of the motor-impaired aged mice differed from those of the other two groups. Specifically, the number of neurons containing "compact dendritic arbors" (i.e., neurons with total dendritic lengths of <600 μm) in the motor-impaired aged mice was 244% higher than the young mice and 684% higher than the motor-unimpaired aged mice (recall that the dendrites were elongated in the nonimpaired aged group, accounting for a lower frequency of neurons with compact dendritic arbors). The authors also reported such qualitative observations as an increased prevalence of cells exhibiting small shrunken dendrites, and atypical dendrites with terminal swelling (instead of the growth cones observed in the nonimpaired aged mice) indicative of cellular degeneration in the motor-impaired aged mice.

Although the majority of studies examining age-related morphological changes in the basal ganglia have been limited to the striatum, a few have examined dopaminergic neurons in the substantia nigra. Emborg and colleagues (1998) recently reported qualitative observations of degeneration (i.e., decreased neuropil, abnormally shaped perikarya, stunted neurites) of nigrostriatal dopaminergic neurons in aged (25–27 years) Rhesus monkeys. The altered morphological structure of nigral dopaminergic neurons is consistent with similar findings in the substantia nigra pars compacta of aged humans (Cruz-Sánchez *et al.*, 1995).

D. Receptors and Transporters

Numerous studies have reported decreases in dopamine receptor densities—especially the D_2-type—with age in mammals. In humans (>70 years) and Rhesus monkeys (22 years), age-related decreases of 30–42% of D_2 binding sites in caudate nucleus have been reported (Severson *et al.*, 1982; Wong *et al.*, 1984; Lai *et al.*, 1987; Morgan *et al.*, 1987). Age-related decreases in D_1 and D_2 receptor concentrations have been reported to range from 30–60% in rats and mice (>22 months) (Severson and Randall, 1985; Giorgi *et al.*, 1987; Lai *et al.*, 1987; Morgan and Finch, 1988; Han *et al.*, 1989). Decreases in dopamine receptor densities have been attributed to decreased production of the receptor protein, as both steady-state levels and synthesis of the D_2 receptor mRNA have been reported to be diminished by 50% in the striata of aged (>22 month) Wistar rats (Mesco *et al.*, 1991, 1993).

Several groups have reported reductions in dopamine-stimulated striatal adenylate cyclase in aging. Govoni and colleagues (1977) reported that the activity of adenylate cyclase in 20- to 24-month-old Sprague–Dawley rats was 64% lower than that of 2- to 3-month-old rats following dopamine stimulation. Likewise, Makman and colleagues (1979, 1980) reported that dopamine-stimulated adenylate cyclase activity was 50% lower in rabbits $\geq$5 years old than it was in rabbits $<$1 year old. These reported alterations may be the result of age-related decreases in D_1 dopamine receptor densities (Giorgi *et al.*, 1987) since this receptor has been demonstrated to be positively coupled with adenylate cyclase (Kebabian and Calne, 1979). Age-related changes in cAMP and adenylate cyclase activity appear to be regionally specific, as changes have been reported in the striatum but not within the nucleus accumbens (Sugawa and May, 1993).

Age-related decreases in transmembrane proteins are not limited to dopamine receptors. Positron emission tomography studies in humans have revealed that age-related losses in D_2 dopamine receptors were correlated with losses in dopamine transporters (Volkow *et al.*, 1998). Dopamine transporters provide the primary mechanism for terminating synaptic dopaminergic signals (Giros *et al.*, 1996) and are therfore essential in regulating dopaminergic neurotransmission. Age-related decreases of approximately 70% in the number of dopamine transporters between 19 and 90 years have been reported in humans (Allard and Marcusson, 1989; De Keyser *et al.*, 1990). Significant age-related decreases in dopamine transporters have also been reported in rats and monkeys, although the relative decrease is less than the decrease observed in humans. Emborg and colleagues (1998) reported decreases in dopamine transporter number of 33% and dopamine transporter density of 24% in the substantia nigra of 25- to 27-year-old Rhesus monkeys. Hebert and colleagues (1999) reported decreases in dopamine transporters of $>$50% in striatum and $>$74% in substantia nigra of aged 24-month-old F344 rats. Age-related decreases in dopamine transporter mRNA in 24-month-old rats ($\sim$22%; Himi *et al.*, 1995) and 65- to 72-year-old humans (up to 75%; Bannon and Whitty, 1997) have also been reported. Since age-related decreases in dopamine transporter mRNA exceed the magnitude of dopaminergic neuron loss, the observed reductions in dopamine transporters have been attributed primarily to decreased dopamine transporter mRNA rather than decreased numbers of dopaminergic terminals (De Keyser *et al.*, 1990).

III. Functional Changes

Many factors intrinsic to principal dopaminergic neuronal elements are essential for optimal functioning. In the previous section, evidence regarding age-associated structural changes in the basal ganglia was reviewed. While the notion of widespread neuronal death in the aging basal ganglia is not supported by the literature, there is evidence for such morphological alterations in dopaminergic neurons as decreased dendritic complexity and density of receptor and uptake sites in the striatum. Reports of these alterations are corroborated by the functional changes that are addressed below. These changes include such presynaptic processes as neurotransmitter synthesis, storage, metabolism, release, and reuptake/uptake, as well as such postsynaptic processes as receptor functioning, metabolism, and interactions with other neuromodulators. Compromises in these processes potentially account for the majority of age-related changes in the basal ganglia.

A. Presynaptic Changes

1. Dopamine Synthesis

The synthesis of dopamine has been reported to be decreased by 26–28% in the striatum of aged ($\geq$25 months) rats (Ponzio *et al.*, 1978; Watanabe, 1987; Marshall and Rosenstein, 1990). This decrease in dopamine synthesis may be related to reported age-related decreases of approximately 40% in the level of TH mRNA in substantia nigra of aged (24 months) rats (Himi *et al.*, 1995), and consequential decreased TH activity in striatum (93% decrease reported by Algeri *et al.*, 1977; 32% decrease reported by Ponzio *et al.*, 1982) and substantia nigra (31% decrease reported by Ponzio *et al.*, 1982) of aged (18–30 months) rats. Likewise, decreases of 24–28% in levels of dihydroxy-L-phenylalanine (DOPA) accumulation following the administration of the aromatic acid decarboxylase inhibitor NSD-1015 have been reported in aged (24–27 months) rats (Watanabe, 1987; Marshall and Rosenstein, 1990; Venero *et al.*, 1991). Some researchers, however, have reported no changes in striatal dopamine synthesis in aged rats as assessed by levels of dopamine and its metabolites in postmortem tissues (Demarest *et al.*, 1980; Friedemann and Gerhardt, 1992; Hebert and Gerhardt, 1998). However, these tissue measures may reflect synthesis capacity and not necessarily synthesis rates of dopamine in aged rats.

2. Dopamine Storage

Studies examining age-related differences in dopamine storage (or content) have also produced conflicting results. For example, decreases of 20–60% in dopamine whole tissue content have been reported in the striata of rodents (24- to 29-month-old rats; 24- to 28-month-old mice) and in the caudate nucleus and putamen of humans ($>$72.5 years) (Finch, 1973; Carlsson and Winblad, 1976; Joseph *et al.*, 1978; Demarest *et al.*, 1980; Osterburg *et al.*, 1981; Kish *et al.*, 1992; Yurek *et al.*, 1998). Likewise, a 34% decrease in striatal dopamine levels was also reported in aged (18 years) Rhesus monkeys (Goldman-Rakic and Brown, 1981). Furthermore, Irwin and colleagues (1994) reported a 30% decrease in dopamine levels in putamen, but not caudate nucleus in aged (20 years) squirrel monkeys. However, no significant differences in dopamine levels between young and aged animals and humans were found in other studies (Adolfsson *et al.*, 1979; Rose *et al.*, 1986; Friedemann and Gerhardt, 1992; Hebert and Gerhardt, 1998). Although there is a lack of consensus regarding age-related alterations in dopamine content, the point may be functionally moot. These reported decreases in dopamine levels are not considered large enough to produce Parkinson's disease-like effects in animals and humans (Hornykiewicz, 1963; Stricker and Zigmond, 1976; Lloyd, 1977). Furthermore, it has been demonstrated that the amount of

dopamine stored within neurons does not necessarily relate to the release capacity of dopaminergic neurons (Rose *et al.*, 1986; Friedemann and Gerhardt, 1992; Dobrev *et al.*, 1995; Hebert and Gerhardt, 1998).

3. Dopamine Release

Numerous studies have reported decreases in stimulus-evoked dopamine release in the aged striatum (e.g., Rose *et al.*, 1986; Gordon *et al.*, 1995; Kametani *et al.*, 1995; Friedemann and Gerhardt, 1996; Hebert and Gerhardt, 1998; Yurek *et al.*, 1998). Diminished potassium-evoked dopamine release of 22–50% has been demonstrated in the striatum of aged (12–24 months) rats (Gregerson and Selmanoff, 1990; Dobrev *et al.*, 1995; however, see Kametani *et al.*, 1995, and Gerhardt and Maloney, 1999, for exceptions) and monkeys (Gerhardt *et al.*, 1995). Likewise, age-related decreases of 30–60% in amphetamine-evoked dopamine overflow has been reported in the rat (Dluzen *et al.*, 1991; Kametani *et al.*, 1995; Yurek *et al.*, 1998; Gerhardt and Maloney, 1999). Since the neuronal release of dopamine has been demonstrated to be mediated through either calcium-dependent or calcium-independent mechanisms, both of these mechanisms may be susceptible to age-related alterations. While decreased calcium regulation in aged animals has been reported (Reimann *et al.*, 1993), other mechanisms thought to be responsible for diminished dopamine release include alterations in the efficacy of neuromodulators and other neurotransmitters that modulate dopamine release (Buck *et al.*, 1981; Chesselet, 1984; Joseph and Roth, 1988a,b; Friedemann and Gerhardt, 1996) and decreases in dopamine synthesis (Nakano and Mizuno, 1996).

Decreased dopamine release may be responsible for decreased locomotor activity observed in aging. For example, although some studies have failed to reveal age-related decreases in spontaneous locomotor activity in rats, when rats are habituated to the activity monitor, age-related decreases are robust and stable over time (Hebert and Gerhardt, 1998). The finding that potassium-stimulated dopamine release was lower in aged rats than in young rats, along with the demonstration that nomifensine, a dopamine-uptake inhibitor, increased locomotor activity in aged rats (Hebert and Gerhardt, 1998), suggests that prolonging the postsynaptic dopaminergic signal may reverse the age-related decline in spontaneous locomotor activity.

4. Autoreceptor Feedback

As previously mentioned, age-related decreases in the number of D_2 dopamine receptors have been reported for a number of species. The fact that presynaptic dopaminergic autoreceptors are classified as the D_2 type (Morelli *et al.*, 1988) leads to the question of whether dopaminergic autoreceptors are likewise affected by age. In a behavioral assay that is thought to reflect dopaminergic autoreceptor function in rats, Stoessl and colleagues (1989) reported that old (23–26 months) Sprague–Dawley rats exhibit diminished yawning following low-dose apomorphine administration when compared to mature (6–8 months) rats. Since dopaminergic autoreceptors provide the neuron with feedback regarding the synaptic dopaminergic signal, this decreased feedback may provide a compensatory mechanism with which to counter age-related decreases in dopaminergic release. Furthermore, the decrease in dopaminergic autoreceptor density may also have functional consequences for dopamine uptake, as the latter process has been reported to be linked in part to D_2 dopaminergic autoreceptors (Cass and Gerhardt, 1994).

5. Reuptake/Uptake

As stated earlier, the number of dopamine transporters decrease with age in rats and humans. Declines in the number of dopamine transporters of up to 70% in aged humans' caudate nucleus (De Keyser *et al.*, 1990) and 50% in aged rats' striatum (Hebert *et al.*, 1999) have been reported. In addition, the amount of dopamine transporter mRNA has been shown to decline with age in rats ($\sim$22% decrease) and humans ($\sim$75% decrease) (Himi *et al.*, 1995; Bannon and Whitty, 1997). These decreases have generally not been accompanied by alterations in binding affinities (Allard and Marcusson, 1989; Shimizu and Prasad, 1991; Hebert *et al.*, 1999). Functional consequences of these changes have been measured through *in vivo* stimulus-evoked dopamine release and dopamine clearance (Friedemann, 1992; Friedemann and Gerhardt, 1992; Hebert and Gerhardt, 1999). For example, dopamine uptake capacity can be measured by examining the amplitude and decay of exogenously applied dopamine. Hebert and Gerhardt (1999) demonstrated that when 20 pmol of dopamine was locally applied in the dorsal striatum of 24-month-old F344 rats, the amplitude of the dopaminergic signal was almost 3 times greater than the signal recorded in a 6-month-old rat given the same dose. Furthermore, the uptake rate was slower in the aged rats than in the young rats. When uptake rate/μM dopaminergic signal amplitude was measured, this value was 39% slower in aged rats than in young rats. Likewise, the maximal uptake rates for aged rats were 72% slower than those for young rats. In studies which have utilized the dopamine uptake inhibitor nomifensine to measure dopamine transporter function, the effectiveness of the drug in blocking the clearance of dopamine in aged (24–30 months) rats was diminished by almost 50% when compared to young (6 months) rats (Friedemann, 1992; Friedemann and Gerhardt, 1992; Hebert and Gerhardt, 1999). These age-related changes in dopamine clearance may be regionally specific, as Hebert and Gerhardt (1999) reported a slowing of dopamine clearance in the dorsal striatum but not in the nucleus accumbens.

Whereas there is a decrease in striatal dopamine release with age, the decline in number of uptake sites may be a compensatory mechanism through which the dopaminergic neuron adapts to decreased dopaminergic signal. This compensation would be consistent with the remarkable adaptive capacity of the nigrostriatal dopamine system (Hornykiewicz and Kish, 1987; Zigmond *et al.*, 1990; Cass *et al.*, 1995). For example, the density of dopamine transporters has been reported to decrease in individuals with PD (Uhl *et al.*, 1994), suggesting that a downregulation of dopamine transporters may be an adaptive mechanism through which to increase the amount of extracellular dopamine. Likewise, there may be a downregulation of dopamine transporters to adapt to the decreased dopaminergic signal that has been demonstrated to occur in normal aging.

6. Monoamine Oxidase

The relationship between the catabolic enzyme monoamine oxidase (MAO) and age is controversial (Irwin *et al.*, 1997; Shih, 1975). Studies that have examined MAO levels in rodents during aging have generally reported a positive relationship between age and the level of the ''B''-type of MAO isoform (MAO-B) in the basal ganglia. By contrast, either no change or a decrease in the ''A''-type of MAO isoform (MAO-A) has been reported. Increases in MAO-B have been demonstrated to be from 124 to 170% higher in basal ganglia of aged rats (24–25 months) and mice (10–25 months) when compared to young controls (Benedetti and Keane, 1980; Arai and Kinemuchi, 1988; Venero *et al.*, 1991; Saura *et al.*, 1994; Irwin *et al.*, 1997). Conversely, no effect of age was seen in the levels of MAO types A and B as a function of age in squirrel monkeys (Irwin *et al.*, 1997). Irwin and colleagues (1997) argued that their failure to find age-related increases was a true species difference and not related to methodology, since they did find an age-related increase in mice. They further suggest that their results may be a more accurate reflection of age-related changes in MAO, since the results of human studies are potentially confounded by uncontrolled disease states as well as by postmortem interval.

B. Postsynaptic and Extracellular Changes

1. Cellular Electrophysiology

There is considerable evidence for altered electrophysiological properties of aged nigrostriatal neurons. Most studies have reported an increased threshold for elicited responses with age (Levine, 1988; Cepeda *et al.*, 1996; Gould *et al.*, 1996). The increased thresholds have been demonstrated under several conditions. Cepeda and colleagues (1996) reported that the average iontophoretic current intensities necessary to elicit a response to excitatory amino acid (glutamate, NMDA) ejections in *in vitro* striatal cells of aged (24–26 months) rats were 147–161% higher than in young rats. Likewise, Levine (1988) stimulated corticostriatal and nigrostriatal cells electrically while recording responses of cells in the caudate nucleus of aged (11- to 14-year-old) cats *in vivo*. In this preparation, the electrical current necessary to elicit responses from caudate nucleus cells in aged cats increased 44% for cortical stimulation and 41% for nigral stimulation (however, the current necessary to produce corticostriatal responses in the 6- to 7-year-old cats was 79% higher than young cats). Finally, using pressure ejections of selective D_1 and D_2 dopamine receptor agonists (SKF 38393 and quinpirole, respectively), Gould and colleagues (1996) measured the doses required to produce *in vivo* changes of 50% in striatal neuronal firing rate. They reported that the ED_{50} (measured in psi $\times$ sec) required were 315 and 289% higher for SKF 38393 and quinpirole, respectively, in 26- to 27-month-old versus 3-month-old rats. Somewhat unexpectedly, Levine (1988) found that the average latencies of orthodromically evoked action potentials decreased with age in the caudate nucleus of cats. Upon further examination, however, this decrease was not attributed to increased speed of conduction, but to a loss of long-latency responses in the aged cats. Also reported in some studies were age-related diminishments in paired-pulse facilitation (Levine, 1988; Walsh and Ou, 1994), a deficit that may underlie the relatively diminished capacity for aged rats to habituate to a familiar environment or repeated stimulus (Hebert and Gerhardt, 1998).

In addition to increased response thresholds following either pharmacological or orthodromic stimulation, there are a variety of other altered response characteristics of aged striatal neurons. These include the following: a general lack of response, decreased proportion of neurons responding in a distinctive manner, ''unusual'' responses, reduction in the components of a response, significant changes in the proportion of occurrence of different types of responses, increased durations of response inhibitions, and decreased average spontaneous firing rates of caudate nucleus neurons (Levine, 1988; Cepeda *et al.*, 1996).

2. Neurotransmitter Interactions

Despite this chapter's deliberately exclusive concentration on age-related dopaminergic function, dopamine does not exist and function in isolation in the basal ganglia. Potential changes in other neuronal systems that impact on dopaminergic neurons may indirectly alter the function of dopaminergic neuronal systems in aging. The principal output neuron of the striatum is the medium spiny GABAergic neuron which is modulated not only by nigrostriatal dopaminergic neurons, but also by corticostriatal glutamatergic neurons and striatal acetylcholinergic interneurons (Di Chiara *et al.*, 1994; Parent and Hazrati, 1995; Wilson, 1998). There have been very few studies to date reporting age-related changes in the interactions between different neurotransmitter systems in the basal ganglia. When examined in isolation, reports of age-related changes in corticostriatal glutamatergic function are equivocal. Some studies have found stability of corticostriatal glutamatergic function (e.g., Donzanti *et al.*, 1993; Porras *et al.*, 1993; Sanchez-Prieto *et al.*, 1994), while others have found age-related diminutions or alterations (Cepeda and Levine, 1991; Castorina *et al.*, 1994; Cepeda *et al.*, 1996). Striatal γ-aminobutyric acid (GABA) function has been reported to remain steady with age (McGeer and McGeer, 1975; Govoni *et al.*, 1980; Strong *et al.*, 1982). Studies of age-related acetylcholinergic function have reported declines in synthesis (Gibson *et al.*, 1981), loss of striatal muscarinic cholinergic receptors (Norman *et al.*, 1986; Briggs *et al.*, 1989), and diminished cholinergic receptor binding (Freund, 1980).

Although age-related changes in these non-dopaminergic striatal neurotransmitter systems appear to vary considerably, when age-related deficits occur in the nigrostriatal depaminergic system, the balance between these modulators of basal ganglia motor output may be compromised (Randall, 1980; Porras and Mora, 1995; Cepeda *et al.*, 1996). For example, in striatal brain slices from aged rats and cats the ability of dopamine to modulate excitatory amino acid receptor-mediated responses was reduced when compared to slices from young rats and cats (Cepeda *et al.*, 1996). Specifically, depending upon the type of receptor, the response to dopamine was either lost or the threshold for the response was increased by 232%. Likewise, in the young rat striatum it has been demonstrated that the mixed D_1–D_2 dopamine receptor agonist apomorphine produces concentration-dependent increases in

glutamate and GABA release. This modulation was altered in older rats. As the glutamatergic response to apomorphine was decreased the threshold for apomorphine-induced GABA release was increased (Porras and Mora, 1995). Finally, the importance of dopamine input to acetylcholinergic function is evidenced by previous studies which have demonstrated that acetylcholinergic neurons become hyperactive following loss of dopamine input (Stoof *et al.*, 1992). Likewise, it has been demonstrated that the colocalization of dopamine autoreceptors and muscarinic and/or nicotinic cholinergic receptors provides a means for cholinergic agonists to enhance striatal dopamine release by inhibiting dopamine autoreceptors (Chesselet, 1984). Previous studies have demonstrated that the capacity for cholinergic agonists to enhance potassium-evoked dopamine release is decreased in aged rats (Joseph *et al.*, 1988; Kametani *et al.*, 1995).

IV. Conclusions

In conclusion, the evidence for age-related decrements in basal ganglia function is considerable and the nigrostriatal dopaminergic system seems to incur the majority of age-related deterioration. However, additional studies are needed to investigate potential changes in dopaminergic systems in mesolimbic dopaminergic pathways and in other areas of the basal ganglia that contain dopaminergic fibers. Although there is evidence for loss of dopaminergic neurons with age, the degree of loss is not of sufficient magnitude to account for the decrements in movement that accompany senescence that are often termed parkinsonism (Bennett *et al.*, 1996). Of the age-related alterations in the nigrostriatal pathways that correlate with these decrements, most involve functional decrements of neuronal machinery. These include decreases in dopamine receptors, transporters, as well as dopamine release and reuptake. Further work, however, needs to be carried out in the areas of dopamine synthesis, storage, and release, as previous studies examining these processes have produced equivocal results.

With the increasing use of techniques that can study the dynamics of neuronal function, we have only begun to quantify the capacities of neural systems and how these capacities are affected by age. The fact that the weight of evidence indicates a primary role for alterations in the function of existing neurons—and not neuronal loss—in age-related movement disturbances is encouraging. Research into these processes may facilitate the development of treatments that can increase the functional capacity of dopaminergic neurons, resulting in the attenuation of age-related movement deficits.

References

Adolfsson, R., Gottfries, C. G., Roos, B. E., and Winblad, B. (1970). Post-mortem distribution of dopamine and homovanillic acid in human brain, variations related to age, and a review of the literature. *J. Neural Transm.* **45**, 81–105.

Algeri, S., Bonati, M., Brunello, N., and Ponzio, F. (1977). Dihydropteridine reductase and tyrosine hydroxylase activities in rat brain during development and senescence: A comparative study. *Brain Res.* **132**, 569–574.

Allard, P., and Marcusson, J. O. (1989). Age-correlated loss of dopamine uptake sites labeled with [3H]GBR-12935 in human putamen. *Neurobiol. Aging* **10**, 661–664.

Arai, Y., and Kinemuchi, H. (1988). Differences between monoamine oxidase concentrations in striatum and forebrain of aged and young rats. *J. Neural Transm.* **72**, 99–105.

Austad, S. N. (1997). Comparative aging and life histories in mammals. *Exp. Gerontol.* **32**, 23–38.

Bannon, M. J., and Whitty, C. J. (1997). Age-related and regional differences in dopamine transporter mRNA expression in human midbrain. *Neurology* **48**, 969–977.

Barbeau, A. (1973). Aging and the extrapyramidal system. *J. Am. Geriatr. Soc.* **21**, 145–149.

Beck, C. H. (1978). Functional implications of changes in the senescent brain: A review. *Can. J. Neurol. Sci.* **5**, 417–424.

Benedetti, M. S., and Keane, P. E. (1980). Differential changes in monoamine oxidase A and B activity in the aging rat brain. *J. Neurochem.* **35**, 1026–1032.

Bennett, D. A., Beckett, L. A., Murray, A. M., Shannon, K. M., Goetz, C. G., Pilgrim, D. M., and Evans, D. A. (1996). Prevalence of parkinsonian signs and associated mortality in a community population of older people. *N. Engl. J. Med.* **334**, 71–76.

Briggs, R. S., Petersen, M. M., and Cook, P. J. (1989). Muscarinic agonist receptor subtypes in aging rat brain. *Neurobiol. Aging* **12**, 193–199.

Brody, H. (1955). Organization of the cerebral cortex. III. A study of aging in the human cerebral cortex. *J. Comp. Neurol.* **102**, 511–556.

Buck, S. H., Deshmukh, P. P., Burks, T. F., and Yamamura, H. I. (1981). A survey of substance P, somatostatin, and neurotensin levels in aging in the rat and human central nervous system. *Neurobiol. Aging* **2**, 257–264.

Carlsson, A., and Winblad, B. (1976). Influence of age and time interval between death and autopsy on dopamine and 3-methoxytyramine levels in the human basal ganglia. *J. Neural Transm.* **38**, 271–276.

Cass, W. A., and Gerhardt, G. A. (1994). Direct in vivo evidence that D2 dopamine receptors can modulate dopamine uptake. *Neurosci. Lett.* **176**, 259–263.

Cass, W. A., Gerhardt, G. A., Zhang, Z., Ovadia, A., and Gash, D. M. (1995). Increased dopamine clearance in the non-lesioned striatum of rhesus monkeys with unilateral 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) striatal lesions. *Neurosci. Lett.* **185**, 52–55.

Castorina, M., Ambrosini, A. M., Pacifici, L., Ramacci, M. T., and Agelucci, L. (1994). Age-dependent loss of NMDA receptors in hippocampus, striatum, and frontal cortex of the rat: Prevention by acetyl-L-carnitine. *Neurochem. Res.* **19**, 795–798.

Cepeda, C., and Levine, M. S. (1991). Altered responsiveness of caudate neurons to amino acids and dopamine in aged cats. *Brain Dysfunct.* **4**, 17–27.

Cepeda, C., Li, Z., and Levine, M. S. (1996). Aging reduces neostriatal responsiveness to *N*-methyl-D-aspartate and dopamine: An in vitro electrophysiological study. *Neuroscience* **73**, 733–750.

Chesselet, M. F. (1984). Presynaptic regulation of neurotransmitter release in the brain: Facts and hypothesis. *Neuroscience* **12**, 347–375.

Colon, E. J. (1972). The elderly brain. A quantitative analysis in the cerebral cortex of two cases. *Psychiat. Neurol. Neurochir.* **75**, 261–270.

Cotzias, G. C., Van Woert, M. H., and Schiffer, L. M. (1967). Aromatic amino acids and modification of parkinsonism. *N. Engl. J. Med.* **276**, 374–378.

Cotzias, G. C., Papavasiliou, P. S., and Gellene, R. (1969). Modification of parkinsonism—chronic treatment with L-dopa. *N. Engl. J. Med.* **280**, 337–343.

Cruz-Sánchez, F. F., Cardozo, A., and Tolosa, E. (1995). Neuronal changes in the substantia nigra with aging: A Golgi study. *J. Neuropathol. Exp. Neurol.* **54**, 74–81.

Dayan, A. D. (1970). Quantitative histological studies on the aged human brain. I. Senile plaques and neurofibrillary tangles in "normal" patients. *Acta Neuropathol.* **16**, 85–94.

De Keyser, J., Ebinger, G., and Vauquelin, G. (1990). Age-related changes in the human nigrostriatal dopaminergic system. *Ann. Neurol.* **27**, 157–161.

Demarest, K. T., Riegle, G. D., and Moore, K. E. (1980). Characteristics of dopaminergic neurons in the aged male rat. *Neuroendocrinology* **31**, 222–227.

Di Chiara, G., Morelli, M., and Consolo, S. (1994). Modulatory functions of neurotransmitters in the striatum: Ach/dopamine/NMDA interactions. *Trends Neurosci.* **17**, 228–233.

Dluzen, D. E., McDermott, J. L., and Ramirez, V. D. (1991). Changes in dopamine release in vitro from the corpus striatum of young versus aged rats as a function of infusion modes of L-dopa, potassium, and amphetamine. *Exp. Neurol.* **112**, 153–160.

Dobrev, D., Bergstrasser, E., Fischer, H. D., and Andreas, K. (1995). Restriction and functional changes of dopamine release in rat striatum from young adult and old rats. *Mech. Ageing Dev.* **80**, 107–119.

Donzanti, B. A., Hite, J. F., and Yamamoto, B. K. (1993). Extracellular glutamate levels increase with age in the lateral striatum-potential involvement of presynaptic D-2 receptors. *Synapse* **13**, 378–382.

Ehringer, H., and Hornykiewicz, O. (1960). Verteilung von Noradrenalin und Dopamin (3-Hydroxytyramin) im Gehirn des Menschen und ihr Verhalten bei Erkrankungen des Extrapyramidalen Systems. *Klin. Wochenschr.* **38**, 1236–1239.

Emborg, M. E., Ma, S. Y., Mufson, E. J., Levey, A. I., Taylor, M. D., Brown, W. D., Holden, J. E., and Kordower, J. H. (1998). Age-related declines in nigral neuronal function correlate with motor impairments in rhesus monkeys. *J. Comp. Neurol.* **401**, 253–265.

Emerich, D. F., McDermott, P., Krueger, P., Banks, M., Zhao, J., Marszalkowski, J., Frydel, B., Winn, S. R., and Sanberg, P. R. (1993). Locomotion of aged rats: Relationship to neurochemical but not morphological changes in nigrostriatal dopaminergic neurons. *Brain Res. Bull.* **32**, 477–486.

Fearnley, J. M., and Lees, A. J. (1991). Ageing and parkinson's disease: Substantia nigra regional selectivity. *Brain* **114**, 2283–2301.

Finch, C. E. (1973). Catecholamine metabolism in the brains of ageing male mice. *Brain Res.* **52**, 261–276.

Flood, D. G., and Coleman, P. D. (1988). Neuron numbers and sizes in aging brain: Comparisons of human, monkey, and rodent data. *Neurobiol. Aging* **9**, 453–463.

Freund, G. (1980). Cholinergic receptor loss in brains of aging mice. *Life Sci.* **26**, 371–375.

Friedemann, M. N. (1992). *In vivo* electrochemical studies of dopamine diffusion and clearance in the striatum of young and aged Fischer-344 rats. *Age* **15**, 23–28.

Friedemann, M. N., and Gerhardt, G. A. (1992). Regional effects of aging on dopaminergic function in the Fischer-344 rat. *Neurobiol. Aging* **13**, 325–332.

Friedemann, M. N., and Gerhardt, G. A. (1996). In vivo electrochemical studies of the dynamic effects of locally applied excitatory amino acids in the striatum of the anesthetized rat. *Exp. Neurol.* **138**, 53–63.

Gerhardt, G. A., and Maloney, R. E., Jr. (1999). Microdialysis studies of basal levels and stimulusevoked overflow of dopamine and metabolites in the striatum of young and aged Fischer 344 rats. *Brain Res.* **816**, 68–77.

Gerhardt, G. A., Cass, W. A., Henson, M., Zhang, Z., Ovadia, A., Hoffer, B. J., and Gash, D. M. (1995). Age-related changes in potassium-evoked overflow of dopamine in the striatum of the rhesus monkey. *Neurobiol. Aging* **16**, 939–946.

Gibson, G. E., Peterson, C., and Jenden, D. J. (1981). Brain acetylcholine synthesis declines with senescence. *Science* **213**, 674–676.

Giorgi, O., Calderini, G., Toffano, G., and Biggio, G. (1987). D-1 dopamine receptors labelled with ^{3}H-SCH-23390: Decrease in the striatum of aged rats. *Neurobiol. Aging* **8**, 51–54.

Giros, B., Jaber, M., Jones, S. R., Wightman, R. M., and Caron, M. G. (1996). Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature (London)* **379**, 606–612.

Goldman-Rakic, P. S., and Brown, R. M. (1981). Regional changes of monoamines in cerebral cortex and subcortical structures of aging rhesus monkeys. *Neuroscience* **6**, 177–187.

Gordon, I., Weizman, R., Rosenne, E., and Rehavi, M. (1995). Developmental and age-related alterations in rat brain presynaptic dopaminergic mechanisms. *Dev. Brain Res.* **85**, 225–228.

Gould, T. J., Stromberg, I., and Bickford, P. C. (1996). Decline in striatal dopamine D1 and D2 receptor activation in aged F344 rats. *Neurobiol. Aging* **17**, 877–883.

Govoni, S., Loddo, P., Spano, P. F., and Trabucchi, M. (1977). Dopamine receptor supersensitiviy in brain and retina of rats during aging. *Brain Res.* **138**, 565–570.

Govoni, S., Memo, M., Saiani, L., Spano, P. F., and Trabucchi, M. (1980). Impairment of brain neurotransmitter receptors in aged rats. *Mech. Ageing Dev.* **12**, 39–46.

Gray, J. A. (1995). A model of the limbic system and basal ganglia: Applications to anxiety and schizophrenia. *In* "The Cognitive Neurosciences" (M.S. Gazzaniga, ed.), pp. 1165–1176. MIT Press, Cambridge, MA.

Gregerson, K. A., and Selmanoff, M. (1990). Changes in the kinetic of [3H]dopamine release from median eminence and striatal synaptosomes during aging. *Endocrinology (Baltimore)* **126**, 228–234.

Han, Z., Kuyatt, B. L., Kochman, K. A., DeSouza, E. B., and Roth, G. S. (1989). Effect of aging on concentrations of D2-receptor-containing neurons in the rat striatum. *Brain Res.* **498**, 299–307.

Hebert, M. A., and Gerhardt, G. A. (1998). Normal and drug-induced locomotor behavior in aging: Comparison to evoked DA release and tissue content in Fischer 344 rats. *Brain Res.* **797**, 42–54.

Hebert, M. A., and Gerhardt, G. A. (1999). Age-related changes in the capacity, rate, and modulation of dopamine uptake within the striatum and nucleus accumbens of Fischer 344 rats: And in vivo electrochemical study. *J. Pharmacol. Exp. Ther.* **288**, 879–887.

Hebert, M. A., Larson, G. A., Zahniser, N. R., and Gerhardt, G. A. (1999). Age-related reductions in [^{3}H]WIN 35, 428 binding to the dopamine transporter in nigrostriatal and mesolimbic brain regions of the Fischer 344 rat. *J. Pharmacol. Exp. Ther.* **288**, 1334–1339.

Himi, T., Cao, M., and Mori, N. (1995). Reduced expression of the molecular markers of dopaminergic neuronal atrophy in the aging rat brain. *J. Gerontol.* **50**, B193–B200.

Hornykiewicz, O. (1963). Die Topische Lokalisation und das Verhalten von Noradrenalin and Dopamin (3-hydroxytyramin) in der Substantia nigra des normalen und parkinsonkranken Menschen. *Wien. Klin. Wochenschr.* **75**, 309–312.

Hornykiewicz, O., and Kish, S. J. (1987). Biochemical pathophysiology of Parkinson's disease. *Adv. Neurol.* **45**, 19–34.

Hubble, J. P. (1998). Aging and the basal ganglia. *Neurol. Clin.* **16**, 649–657.

Ingram, D. K., Wiener, H. L., Chachich, M. E., Long, J. M., Hengemihle, J., and Gupta, M. (1993). Chronic treatment of aged mice with L-deprenyl produces marked striatal MAO-B inhibition but no beneficial effects on survival, motor performance, or nigral lipofuscin accumulation. *Neurobiol. Aging* **14**, 431–440.

Irwin, I., DeLanney, L. E., McNeill, T., Chan, P., Forno. L., Murphy, M. M., Jr., DiMonte, D. A., Sandy, M. S., and Langston, J. W. (1994). Aging and the nigrostriatal dopamine system: A nonhuman primate study. *Neurodegeneration* **3**, 251–265.

Irwin, I., DeLanney, L., Chan, P., Sandy, M. S., Di Monte, D. A., and Langston, J. W. (1997). Nigrostriatal monoamine oxidase A and B in aging squirrel monkeys and C57BL/6 mice. *Neurobiol. Aging* **18**, 235–241.

Joseph, J. A., and Roth, G. S. (1988a). Upregulation of striatal dopamine receptors and improvement of motor performance in senescence. *Ann. N.Y. Acad. Sci.* **515**, 355–362.

Joseph, J. A., and Roth, G. S. (1988b). Altered striatal dopaminergic and cholinergic reciprocal inhibitory control and motor behavioral decrements in senescence. *Ann. N.Y. Acad. Sci.* **521**, 110–122.

Joseph, J. A., Berger, R. E., Engel, B. T., and Roth, G. S. (1978). Age-related changes in the nigrostriatum: A behavioral and biochemical analysis. *J. Gerontol*. **33**, 643–649.

Joseph, J. A., Dalton, T. K., and Hunt, W. A. (1988). Age-related decrements in the muscarinic enhancement of K^+-evoked release of endogenous striatal dopamine: An indicator of altered cholinergic-dopaminergic reciprocal inhibitory control in senescence. *Brain Res*. **454**, 140–148.

Kametani, H., Iijima, S., Spangler, E. L., Ingram, D. K., and Joseph, J. A. (1995). In vivo assessment of striatal dopamine release in the aged male Fischer 344 rat. *Neurobiol. Aging* **16**, 639–646.

Kebabian, J. W., and Calne, D. B. (1979). Multiple receptors for dopamine. *Nature (London)* **277**, 93–96.

Kish, S. J., Shannak, K., Rajput, A., Deck, J. H. N., and Hornykiewicz, O. (1992). Aging produces a specific pattern of striatal dopamine loss: Implications for the etiology of idiopathic Parkinson's disease. *J. Neurochem*. **58**, 642–648.

Kopin, I. J. (1993). Parkinson's disease: Past, present, and future. *Neuropsychopharmacology* **9**, 1–12.

Lai, H., Bowden, D. M., and Horita, A. (1987). Age-related decreases in dopamine receptors in the caudate nucleus and putamen of the rhesus monkey *(Macaca mulatta). Neurobiol. Aging* **8**, 45–49.

Levine, M. S. (1988). Neurophysiological and morphological alterations in caudate neurons in aged cats. *Ann. N.Y. Acad. Sci*. **515**, 314–328.

Lloyd, K. G. (1977). CNS compensation to dopamine neuron loss in Parkinson's disease. *Adv. Exp. Med. Biol*. **90**, 255–266.

Makman, M. H., Ahn, H. S., Thal, L. J., Sharpless, N. S., Dvorkin, B., Horowitz, S. G., and Rosenfeld, M. (1979). Aging and monoamine receptors in brain. *Fed. Proc.* **38**, 1922–1926.

Makman, M. H., Ahn, H. S., Thal, L. J., Sharpless, N. S., Dvorkin, B., Horowitz, S. G., and Rosenfeld, M. (1980). Evidence for selective loss of brain dopamine- and histamine-stimulated adenylate cyclase activity in rabbits with aging. *Brain Res*. **192**, 177–183.

Mann, D. M. A., and Yates, P. O. (1982). Pathogenesis of Parkinson's disease. *Arch. Neurol. (Chicago)* **39**, 545–549.

Mann, D. M., and Yates, P. O. (1983). Possible role of neuromelanin in the pathogenesis of Parkinson's disease. *Mech. Ageing Dev.* **21**, 193–203.

Marin, O., Smeets, W. J. A. J., and Gonzalez, A. (1998). Evolution of the basal ganglia in tetrapods: A new perspective based on recent studies in amphibians. *Trends Neurosci*. **21**, 487–494.

Marshall, J. F., and Rosenstein, A. J. (1990). Age-related decline in rat striatal dopamine metabolism is regionally homogeneous. *Neurobiol. Aging* **11**, 131–137.

Masoro, E. J. (1990). Animal models in aging research. *In* ''Handbook of the Biology of Aging, (E. L. Schneider and J. W. Rowe, eds.), 3rd ed., pp. 72–94. Academic Press, San Diego, CA.

McGeer, E. G., and McGeer, P. L. (1975). Age changes in the human for some enzymes associated with metabolism of catecholamines, GABA and acetylcholine. *Adv. Behav. Biol*. **16**, 287–305.

McGeer, P. L., McGeer, E. G., and Suzuki, J. S. (1977). Aging and extrapyramidal function. *Arch. Neurol. (Chicago)* **34**, 33–35.

McNeill, T. H., and Koek, L. L. (1990). Differential effects of advancing age on neurotransmitter cell loss in the substantia nigra and striatum of C57BL/6N mice. *Brain Res*. **521**, 107–117.

McNeill, T. H., Koek, L. L., and Haycock, J. W. (1984). Age-correlated changes in dopaminergic nigrostriatal perikarya of the C57BL/6NNia mouse. *Mech. Ageing Dev.* **24**, 293–307.

McNeill, T. H., Koek, L. L., Brown, S. A., and Rafols, J. A. (1990). Quantitative analysis of age-related dendritic changes in medium spiny I (MSI) striatal neurons of C57BL/6N mice. *Neurobiol. Aging* **11**, 537–550.

Mesco, E. R., Joseph, J. A., Blake, M. J., and Roth, G. S. (1991). Loss of D_2 receptors during aging is partially due to decreased levels of mRNA. *Brain Res*. **545**, 355–357.

Mesco, E. R., Carlson, S. G., Joseph, J. A., and Roth, G. S. (1993). Decreased striatal D_2 dopamine receptor mRNA synthesis during aging. *Mol. Brain Res*. **170**, 160–162.

Morelli, M., Mennini, T., and Di Chiara, G. (1988). Nigral dopamine autoreceptors are exclusively of the D_2 type: Quantitative autoradiography of [^{125}I]iodosulpiride and [^{125}I]SCH 23982 in adjacent brain sections. *Neuroscience* **27**, 865–870.

Morgan, D. G., and Finch, C. E. (1988). Dopaminergic changes in the basal ganglia. *Ann. N.Y. Acad. Sci*. **515**, 145–159.

Morgan, D. G., Marcusson, J. O., Nyberg, P., Wester, P., Winblad, B., Gordon, M. N., and Finch, C. E. (1987). Divergent changes in D-1 and D-2 dopamine binding sites in human brain during aging. *Neurobiol. Aging* **8**, 195–201.

Morrison, J. H., and Hof, P. R. (1997). Life and death of neurons in the aging brain. *Science* **278**, 412–419.

Mortimer, J. A., and Webster, D. D. (1982). Comparison of extrapyramidal motor function in normal aging and parkinson's disease. *Adv. Neurol*. **3**, 217–241.

Nakano, M., and Mizuno, T. (1996). Age-related changes in the metabolism of neurotransmitters in rat striatum: A microdialysis study. *Mech. Ageing Dev.* **86**, 95–104.

Norman, A. B., Blaker, S. N., Thal., L., and Creese, I. (1986). Effects of aging and cholinergic deafferentation on putative muscarinic cholinergic receptor subtypes in rat cerebral cortex. *Neurosci. Lett*. **70**, 289–294.

Osterburg, H. H., Donahue, H. A., Severson, J. A., and Finch, C. E. (1981). Catecholamine levels and turnover during aging in brain regions of male C57BL/6J mice. *Brain Res*. **224**, 337–352.

Pakkenberg, H., Andersen, B. B., Burns, R. S., and Pakkenberg, B. (1995). A stereological study of substantia nigra in young and old rhesus monkeys. *Brain Res*. **693**, 201–206.

Parent, A., and Hazrati, L.-N. (1995). Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. *Brain Res. Rev.* **20**, 91–127.

Peters, A., Palay, S. L., and Webster, H. deF. (1991). ''The Fine Structure of the Nervous System.'' Oxford University Press, New York.

Ponzio, F., Brunello, N., and Algeri, S. (1978). Catecholamine synthesis in brain of aging rat. *J. Neurochem*. **30**, 1617–1620.

Ponzio, F., Calderini, G., Lomuscio, G., Vantini, G., Toffano, G., and Algeri, S. (1982). Changes in monoamines and their metabolite levels in some brain regions of aged rats. *Neurobiol. Aging* **3**, 23–29.

Porras, A., and Mora, F. (1995). Dopamine-glutamate-GABA interactions and ageing: Studies in the striatum of the conscious rat. *Eur. J. Neurosci*. **7**, 2183–2188.

Porras, A., Gisolfi, C. V., and Mora, F. (1993). Release of excitatory amino acid neurotransmitters and glutamine along age in the neostriatum of the conscious rat. *Soc. Neurosci. Abstr.* **19**, 1535.

Randall, P. K. (1980). Functional aging of the nigro-striatal system. *Peptides (N.Y.)* **1** (Suppl. 1), 177–184.

Reimann, W., Bartoszyk, G. D., Kollhofer, U., Schneider, F., and Schoenherr, U. (1993). Effects of ageing and long-term operant conditioning on behavior and presynaptic cholinergic and dopaminergic neuronal mechanisms in rats. *Arch. Int. Pharmacodyn*. **325**, 5–20.

Rogers, J., Zornetzer, S. F., and Bloom, F. E. (1981). Senescent pathology of cerebellum: Purkinje neurons and their parallel fiber afferents. *Neurobiol. Aging* **2**, 15–25.

Rose, G. M., Gerhardt, G. A., Conboy, G. L., and Hoffer, B. J. (1986). Age-related alterations in monoamine release from rat striatum: An in vivo electrochemical study. *Neurobiol. Aging* **7**, 77–82.

Sanchez-Prieto, J., Herrero, I., Miras-Portugal, M. T., and Mora, F. (1994). Unchanged exocytotic release of glutamic acid in the prefrontal cortex and striatum of the rat. *NeuroReport* **4**, 1194–1196.

Saura, J., Richards, J. G., and Mahy, N. (1994). Age-related changes in MAO in B1/C57 mouse tissues: A quantitative radioautographic study. *J. Neural. Transm., Suppl*. **41**, 89–94.

Severson, J. A., and Randall, P. K. (1985). D-2 dopamine receptors in aging mouse striatum: Determination of high- and low-affinity agonist binding sites. *J. Pharmacol. Exp. Ther.* **233**, 361–368.

Severson, J. A., Marcusson, J., Winblad, B., and Finch, C. E. (1982). Age correlated loss of dopaminergic binding sites in human basal ganglia. *J. Neurochem*. **39**, 1623–1631.

Shih, J. C. (1975). Multiple forms of monoamine oxidase and aging. *In* ''Aging'' (H. Brody, D. Harman, and J. M. Ordy, eds.), Vol. 1. pp. 191–198. Raven Press, New York.

Shimizu, I., and Prasad, C. (1991). Relationship between [^{3}H]mazindol binding to dopamine uptake sites and [^{3}H]dopamine uptake in rat striatum during aging. *J. Neurochem*. **56**, 575–579.

Sohal, R. S., and Wolfe, L. S. (1986). Lipofuscin: Characteristics and significance. *Prog. Brain Res*. **70**, 171–183.

Sprott, R. L., and Austad, S. N. (1996). Animal models for aging research *In* ''Handbook of the Biology of Aging'' (E. L. Schneider and J. W. Rowe, eds.), 4th ed., pp.3–23. Academic Press, San Diego, CA.

Stoessl, A. J., Martin-Iverson, M. T., Barth, T. M., Dourish, C. T., and Iversen, S. D. (1989). Effects of ageing on the behavioural responses to dopamine agonists: Decreased yawning and locomotion, but increased stereotypy. *Brain Res*. **495**, 20–30.

Stoof, J. C., Drukarch, B., de Boer, P., Westerink, B. H. C., and Groenewegen, H. J. (1992). Regulation of the activity of striatal cholinergic neurons by dopamine. *Neuroscience* **47**, 755–770.

Stricker, E. M., and Zigmond, M. J. (1976). Recovery of function following damage to central catecholamine-containing neurons: A neurochemical model for the lateral hypothalamic syndrome. *Prog. Psychobiol. Physiol. Psychol.* **6**, 121.

Strong, R., Samorajshi, T., and Gottesfeld, Z. (1982). Regional mapping of neostriatal neurotransmitter system as a function of aging. *J. Neurochem.* **39**, 831–836.

Strothjohann, M., Müller, H., Braak, E., and Braak, H. (1993). Stereological estimation of the total cell number of the human substantia nigra (pars compacta and pars reticulata). *Clin. Neuropathol.* **12**, 271.

Sugawa, M., and May, T. (1993). Age-related alteration in signal transduction: Involvement of the cAMP cascade. *Brain Res.* **618**, 57–62.

Teräväinen, H., and Calne, D. B. (1983). Motor system in normal aging and Parkinson's disease. *In* ''The Neurology of Aging'' (R. Katzman and R. Terry, eds.), pp. 85–109. Davis, Philadelphia.

Uhl, G. R., Walther, D., Mash, D., Faucheux, B., and Javoy-Agid, F. (1994). Dopamine transporter messenger RNA in Parkinson's disease and control substantia nigra neurons. *Ann Neurol.* **35**, 494–498.

Venero, J. L., Machado, A., and Cano, J. (1991). Turnover of dopamine and serotonin and their metabolites in the striatum of aged rats. *J. Neurochem.* **56**, 1940–1948.

Volkow, N. D., Wang, G. J., Fowler, J. S., Ding, Y. S., Gur, R. C., Gatley, J., Logan, J., Moberg, P. J., Hitzemann, R., Smith, G., and Pappas, N. (1998). Parallel loss of presynaptic and postsynaptic dopamine markers in normal aging. *Ann. Neurol.* **44**, 143–147.

Walsh, J. P., and Ou, X. (1994). Loss of paired-pulse facilitation at the corticostriatal synapse of the aged rat. *Synapse* **17**, 36–42.

Watanabe, H. (1987). Differential decrease in the rate of dopamine synthesis in several dopaminergic neurons of aged rat brain. *Exp. Gerontol.* **22**(1), 17–25.

West, M. J. (1993). New stereological methods for counting neurons. *Neurobiol. Aging* **14**, 275–285.

West, M. J., and Gundersen, H. J. (1990). Unbiased stereological estimation of the number of neurons in the human hippocampus. *J. Comp. Neurol.* **296**, 1–22.

West, M. J., Coleman, P. D., Flood, D. G., and Troncoso, J. C. (1994). Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. *Lancet* **344**, 769–772.

Wilson, C. J. (1998). Basal ganglia. *In* ''The Synaptic Organization of the Brain'' (G. M. Shepherd, ed.), pp. 329–375. Oxford University Press, New York.

Wong, D. F., Wagner, H. N., Jr., Dannals, R. F., Links, J. M., Frost, J. J., Ravert, H. T., Wilson, A. A., Rosenbaum, A. E., Gjedde, A., Douglass, K. H., Petronis, J. D., Folstein, M. F., Toung, J. K. T., Burns, H. D., and Kuhar, M. J. (1984). Effects of age on dopamine and serotonin receptors measured by positron tomography in the living human brain. *Science* **226**, 1393–1396.

Yurek, D. M., Hipkens, S. B., Hebert, M. A., Gash, D. M., and Gerhardt, G. A. (1998). Age-related decline in striatal dopamine release and motoric function in Brown Norway/Fischer 344 hybrid rats. *Brain Res.* **791**, 246–256.

Zigmond, M. J., Abercrombie, E. D., Berger, T. W., Grace, A. A., and Stricker, E. M. (1990). Compensations after lesions of central dopaminergic neurons: Some clinical and basic implications. *Trends Neurosci.* **12**, 290–296.

SECTION

V

Homeostasis: Hypothalamus and Related Systems

A. Reproduction and the Aging Brain
(CHAPTERS 51–57)

B. Metabolism and the Aging Brain
(CHAPTERS 58 AND 59)

C. Biological Rhythms and the Aging Brain
(CHAPTERS 60 AND 61)

D. Glucocorticoid Secretion and the Aging Brain
(CHAPTERS 62 AND 63)

E. Autonomic Nervous System and the Aging Brain
(CHAPTER 64)

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Helen Kuno, Michael Godschalk, and Thomas Mulligan

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Male Sexual Behavior during Aging

As men age, there are clear changes in sexual behavior. For example, young married men report a strong desire for sexual activity and typically engage in sexual intercourse more than once per week. In contrast, older age men report desire for sexual activity (albeit to a lesser degree), but do not engage in frequent sexual intercourse because of erectile dysfunction or health problems. The factors responsible for these changes include an age-associated decrement in the serum testosterone concentration (andropause) which likely results in diminished libido and various health disorders which cause erectile dysfunction. For example, older age is associated with atherosclerotic vascular disease, which is the most frequent cause of inadequate erections. There are very little data on the effect of aging on orgasmic function. In essence, older age (in the absence of disease) is associated with modest decrements in sexual interest, ability, and activity. However, the majority of Americans develop various diseases as they age (i.e., diabetes mellitus) which impair erectile function. Ideally, these diseases can and should be prevented, thereby allowing older married couples to enjoy at least a modicum of the sexual pleasures they enjoyed during their youth.

I. Introduction

As men proceed through the various stages of life, there is a decrease in the frequency of sexual intercourse with older age (Leigh *et al*., 1993). Twenty-nine percent of 18- to 29-year-old men report having intercourse three to four times per week, whereas only 7% of men ages 60–69 years and 2% of men ages $\geq$70 years report the same frequency (i.e., three to four times per week). Kinsey *et al*. (1948) reported that by age 80 years, the average frequency of intercourse decreases to once every 10 weeks. Pfeiffer *et al*. (1968) found that 95% of men ages 46–50 years had intercourse once a week, while in the 66- to 71-year-old group, only 28% were having intercourse once a week. In the Bretschneider and McCoy (1988) study of Caucasian, healthy, upper-middle-class men ages 80–102 years, 26% reported their frequency of sexual intercourse to be several times per month to several times per week. They found that prior importance of sex correlated positively with current frequency and enjoyment of intercourse in older men.

The prevalence of engaging in any sexual activity also declines with aging. Bretschneider and McCoy found that 63% of healthy men over the age of 80 still engaged in sexual intercourse (at least once per year). Pfeiffer *et al*. (1968) found that 50% of 60- to 71-year-old men still engaged in sexual intercourse, whereas only 15% of men older than 78 years reported any sexual activity. In a subgroup of this population, followed longitudinally for 10 years, 70% were sexually active at the starting age of 67, but only 25% were still active by age 77 (Pfeiffer *et al*., 1969). Likewise, Diokno *et al*. (1990) found sexual intercourse was present in 80% of married men ages 65–69 years but only 28% in those over age 80 years. In nursing home residents, 80% no longer engaged in coitus despite availability of a sexual partner, moderately strong libido, and preference for vaginal intercourse. However, 17% reported sexual intercourse while on home visits once a month (Mulligan and Palguta, 1991). Thus, although there is a decline in sexual activity with aging, there is no specific age of sudden cessation. Furthermore, sexual interest and desire often persist despite decreased activity.

In men ages 30–99 years old, Mulligan and Moss (1991) found that sexual interest decreases with age, but total absence of interest was never reported. Similarly, Pfeiffer *et al*. (1969) reported continued sexual interest in 80% of men 67 years old; 75% of the same subjects still reported sexual interest at age 76 years. In the Bretschneider and McCoy healthy males ages 80–102, 88% still fantasized about sexual activity, and 66% indicated sexual activity to be of importance in their current lives. Schiavi *et al*. (1990) surveyed healthy couples ages 45–74 years and reported frequency of desire for sexual contact to decrease from a mean score of 6 (based on a scale where 1 = never and 8 = daily) in 45- to 54-year-old men to a mean score of 3 for men age 65–74 years. In another study, of healthy, well-educated men attending a sexuality and aging lecture series (ages 56–85 years), 92% reported that they would like to have sexual activity at least once per week (Wiley and Bortz, 1996). However, only 33% currently engaged in sexual activity at least once per week. This desired frequency was similar to their recall of their frequency of sexual activity 10 years previously. In nursing home residents,

Mulligan and Palguta (1991) reported continued sexual interest, especially among those with partners; those without partners stated they would have greater interest if a partner was available. Overall, 66% of all their subjects reported sexual interest, albeit less than in younger years.

Even with old age, the preferred sexual activity remains vaginal intercourse. Mulligan and Moss (1991) reported that 82% of men preferred intercourse regardless of age. With the decline in intercourse, however, they did not find a compensatory increase in other forms of sexual contact, such as kissing, caressing, petting, oral sex, or masturbation. In contrast, Bretschneider and McCoy (1988) reported that 83% of their subjects engaged in touching or caressing without sexual intercourse at least several times per year. Furthermore, 74% expressed moderate to great enjoyment from this activity. In fact, touching or caressing without intercourse was the most common activity (83%). Similarly, Wiley and Bortz (1996) found in their self-selected group of attendees at sex lectures that sexual intercourse and orgasm were the highest rated forms of sexual activity 10 years previously. Currently, however, these forms of sexual expression declined but with an increase in expressions of intimacy without intercourse. The majority of nursing home residents in the Mulligan and Palguta study also preferred vaginal intercourse, but 21% preferred hugging or caressing, 5% preferred kissing, and 2% preferred masturbation.

Despite the lower level of sexual activity, many older males still express sexual satisfaction. Mulligan and Palguta (1991) found that nursing home residents were reasonably satisfied sexually with a mean score of 6 (scale 1 = not at all satisfied and 10 = extremely satisfied). However, the nursing home residents who had nonresident partners reported higher levels of sexual distress (mean score of 3 on scale from 1–10 with 1 = not at all distressed and 10 = extremely distressed); those without partners had a mean sexual distress score of 2. Age, functional status, and intercourse frequency were independently and positively correlated with sexual satisfaction in these nursing home residents. Schiavi *et al*. (1990) found in their study of couples that mean male marital sexual satisfaction scores were 6 (1 = not enjoyable and 7 = very enjoyable) for men ages 45–54 years and 6 for men ages 65–74 years.

An important social factor in sexual behavior during aging is the role of the sexual partner. Bretschneider and McCoy (1988) found that marital status significantly correlated with frequency and enjoyment of sexual activities. Diokno *et al*. (1990) also demonstrated that availability of a marital partner is an important factor with sexual activity occurring in 74% of married men and 31% of unmarried men. Among those with sexual partners, Pfeiffer *et al*. (1968) found that men tended to attribute cessation of sexual intercourse to themselves, while 74% of the women attributed it to their spouses. The main reasons cited were loss of potency, decreased libido, and medical illness. In those men who blamed their spouses for stopping, 62% attributed the cause to illness, 37% to loss of interest, and 5% to loss of potency. In addition, relationship incongruity and libido ''mismatches'' may inhibit sexual overtures toward a partner and further reduce sexual function and activity (Kaiser, 1996).

The causes of the age-associated changes in sexual behavior are multifactorial, including organic and social factors. To understand these causes, we will review the anatomy and physiology of male sexual function and then the pathophysiology of dysfunction.

II. Normal Physiology of Sexual Function

A. Anatomy

The penis consists of three components, two dorso-lateral corpora cavernosa and a ventral corpus spongiosum which surrounds the penile urethra and distally forms the glans penis. A thick fibrous sheath, the tunica albuginea, surrounds each of the corpora cavernosa, and all three corpora are bound together by Buck's fascia. The ischiocavernosus and bulbospongiosus muscles surround the proximal portions of the corpora cavernosa (Williams and Warwick, 1980). Each corpus consists of smooth muscle bundles, elastic fibers, collagen, and loose fibrous tissue which form the trabeculae. Between the trabeculae are blood-filled lacunar spaces which are lined by flat endothelial cells (Bossart *et al*., 1980).

The arterial supply to the penis is the internal pudendal arteries, which become the penile arteries. Each penile artery terminates in bulbar, urethral, dorsal, and cavernosal arteries. The paired cavernosal arteries penetrate the tunica albuginea and enter the crura of the corpora cavernosa. Each ends in multiple twisted branches called helicine arteries that supply the lacunae. There may be two circulatory routes in the human corpora. One route goes from the cavernosal artery to capillary networks underlying the tunica albuginea with the capillaries serving as nutritional vessels. This pathway is the main circulatory route during the flaccid state. The second route is via anastomoses from the cavernosal artery through the helicine arteries to the cavernosa, which then empties into the postcavernous venules and serves as the main vascular pathway in the mechanism of erection (Banya *et al*., 1989).

Venous return from the pendulous penis occurs through the deep and superficial dorsal veins of the penis. Subtunical venules located between the periphery of the erectile tissue and the tunica albuginea drain the lacunar spaces. They coalesce to form emissary veins which penetrate the tunica albuginea and drain into the deep dorsal vein or the circumflex system. Drainage from the proximal crura is mainly through the cavernosal and crural veins. Superficial dorsal veins communicate with the external pudendal vein and/or the saphenous vein to drain the skin and prepuce of the penis (Aboseif and Lue, 1988; Tudoriu and Bourmer, 1983).

B. Mechanism of Erection

1. Neural Component

Penile erection is a complex event, occurring as a result of the integration of central (cerebral and spinal) and local (smooth muscle and endothelium) factors (Anderson and Wagner, 1995). It arises in response to sensory stimuli, fantasy, or genital stimulation. Specialized areas in the hypothalamus and thalamus organize the autonomic response to these stimuli (Sachs, 1995).

Sympathetic preganglionic nerve fibers to the penis arise from neurons in the intermediolateral cell columns of T12–

L2 spinal cord segments, while parasympathetic input to the penis arises in the S2–S4 sacral spinal cord segments. Sympathetic impulses travel via the hypogastric nerve, and parasympathetic impulses travel via the pelvic nerve. The pelvic plexus serves as the peripheral integration center for autonomic input to the penis. The pelvic plexus then branches into the cavernous nerves that traverse the posterolateral aspect of the prostate and continue on both sides of the urethra as the cavernosal nerves (Steers, 1990).

In the flaccid state, there is tonic contraction of the arterial and corporal smooth muscles mediated by α 2 adrenergic receptors which maintains high penile arterial resistance (Wagner *et al*., 1989). With erotic stimulation, there is a decrease in sympathetic tone and an increase in parasympathetic activity. Central to erection is a hemodynamic change which decreases penile arterial resistance with resultant increased penile blood flow (Lue and Tanagho, 1987).

Parasympathetic stimulation activates cholinergic receptors via acetylcholine, stimulating endothelial cells to produce a nonadrenergic, noncholinergic transmitter, nitric oxide (NO). NO relaxes trabecular smooth muscle (Saenz de Tejada *et al*., 1988), and is considered the major neurotransmitter controlling relaxation of penile smooth muscle (Kim *et al*., 1991; Rajfer *et al*., 1992). NO is formed by conversion of L-arginine into L-citrulline by the enzyme, nitric oxide synthase (NOS) (Burnett, 1995a). NOS is activated by the influx of calcium ions that occurs with parasympathetic stimulation. The increased oxygen levels derived from the arterialization of cavernosal blood flow further activates NOS and thereby maintains erection.

NO moves from cell to cell through gap junctions and by diffusion into smooth muscle cells providing the rapidity of the response within the penis (Korenman, 1998). NO activates guanylate cyclase thereby increasing production of cyclic guanidine monophosphate (cGMP). cGMP depletes intracellular calcium and further induces smooth muscle relaxation with resultant penile vasodilation (Burnett, 1995b; Ignarro *et al*., 1990; Lugg *et al*., 1995a; Moncada, 1992).

Other chemical entities have been implicated in the control of erection, including prostaglandins E1 (PGE1) and E2 (PGE2) and vasoactive peptide. PGE1, PGE2, and vasoactive peptide stimulate the production of cyclic adenosine monophosphate (cAMP) which decreases intracellular calcium and induces smooth muscle relaxation. Vasoactive peptide may also interact with either endothelial or corporal smooth muscle cells to stimulate local formation of NO, and thereby sustaining penile erection (Makhlouf and Grider, 1993; Said, 1992).

Genital stimulation elicits neural impulses which traverse the dorsal nerve of the penis to the pudendal nerve. From the pudendal nerve, the impulses travel to the sacral spinal cord (S2–S4). Efferent impulses travel along the parasympathetic pelvic nerves and produce an erection as described above.

2. Vascular Component

Tonic sympathetic stimulation constricts the trabecular smooth muscle and helicine arteries keeping the penis in its flaccid state. During flaccidity, blood pressure in the cavernosal lacunae is similar to venous pressure. With sexual stimulation, sympathetic tone decreases and there is parasympathetic-mediated relaxation of arteriolar and trabecular smooth muscle. Penile arterial resistance decreases, resulting in increased blood flow into the corpora cavernosa. The increase in blood volume expands the lacunar spaces and compresses the subtunical venules between the expanding corpora cavernosa and the unyielding tunica albuginea. This results in reduction of venous outflow and trapping of blood within the penis. Penile rigidity develops as intracavernosal pressure rises to mean arterial pressure. Detumescence typically occurs after orgasm. During detumescence, there is a decrease in the arterial flow into the penis, decrease in intracavernosal pressure, increased venous drainage, and restoration of sympathetic nerve impulses returning the penis to the flaccid state (Newman and Northup, 1981; Wespes and Schulman, 1993).

C. Libido

Libido results from an interplay of psychological, social, physical, and endocrine factors. Animal research suggests that libido is centered in the medial preoptic area of the hypothalamus (Cunningham and Hirshkowitz, 1995). This area of the brain has androgen receptors and it appears that testosterone (and/or its metabolite dihydrotestosterone) is necessary for normal sexual desire. Udry *et al*. (1985) studied hormonal and social effects on adolescent male sexual behavior. Through self-administered questionnaires and serum hormone assays in 102 adolescent boys, they found that serum free-testosterone concentration was a strong predictor of sexual motivation and behavior. When the free-testosterone index was divided into quartiles, 16% of those with the lowest free-testosterone index reported having had intercourse, whereas 69% of boys in the highest free-testosterone index quartile reported intercourse. Similar findings occurred for noncoital sexual activity and libido. This suggests that the serum free-testosterone concentration appears to affect sexual activity and desire directly.

Bagatell *et al*. (1994) examined the role of physiological serum concentrations of testosterone in maintaining sexual behavior in eugonadal men. In a randomized, double-blind study of healthy, eugonadal men, they utilized a GnRH antagonist, Nal–Glu, without testosterone replacement to produce acute and profound reversible androgen deficiency. They found that subjects experienced decreased frequency of sexual desire, fantasies, and intercourse. There was also a decrease in noncoital sexual activity such as kissing, fondling, and masturbation. However, even low dose testosterone replacement was adequate to maintain normal sexual function and behavior. In a study of eugonadal men complaining of loss of sexual desire, O'Carroll and Bancroft (1984) found that with testosterone injection therapy, there was a modest increase in sexual desire, but no effect on erectile function. Anderson *et al*. (1992) examined the effects of supraphysiological levels of testosterone on sexual behavior and found that sexual awareness and arousability increased with the higher testosterone levels but these changes did not produce modifications of overt sexual behavior. This was in contrast to hypogonadal men in whom testosterone replacement stimulated both sexual interest and activity (Burris *et al*., 1992). From these and other studies, it appears that the major contribution of testosterone on sexuality is related to libido (Kwan *et al*., 1983).

D. Orgasm

Sexual intercourse usually terminates with the motor acts of emission and ejaculation, along with the sensory perception of orgasm. However, there is limited investigation and knowledge regarding orgasm. Orgasm occurs in conjunction with the physical events of contraction of smooth muscle of vas deferens, prostate, seminal vesicles, and the buildup of pressure within the penis. The pleasure of orgasm may derive from the development of this pressure, its release by relaxation of the distal sphincter, and the clonic striated muscle contractions of ejaculation (Newman *et al*., 1982). Electromyography reveals that the onset of the perception of orgasm precedes ejaculation by a few seconds (Kollberg *et al*., 1962). Orgasm may also be elicited cerebrally without afferent input from the penis. For example, orgasm has been reported during psychomotor seizures in patients with temporal lobe lesions (Blumer, 1970). Clearly, this area of human sexuality is in need of further research.

E. Emission and Ejaculation

Emission is the propulsion of semen into the posterior urethra. It is accomplished by peristaltic contractions of the vas deferens, ampulla, seminal vesicles, and prostatic smooth muscles. Emission is a spinal cord reflex in response to genital stimulation, but it may be voluntarily stopped, indicating partial cerebral control. It can also be elicited by cerebral erotic stimulation in the absence of afferent genital stimulation. Sympathetic fibers traversing the hypogastric nerves and plexi are the motor efferents producing emission. Parasympathetic fibers in the pelvic nerves mediate prostatic secretion.

Expulsion of semen from the urethra marks ejaculation. Intermittent relaxation of the distal sphincter allows semen to enter the bulbous urethra. Contractions of the bulbocavernosae muscles propel the semen through the pendulous urethra. Ejaculation is a reflex reaction in response to semen entrance into the bulbous urethra. Its neural center resides in the spinal cord between T12 and L2 and communicates with the ventral horn cells in the sacral cord controlling the perineal and pelvic striated muscles. The integrity of the vesicle sphincters determines the direction of seminal expulsion. Antegrade ejaculation is achieved by firm closure of the proximal sphincter and a functional distal sphincter. Dripping emission occurs if the distal sphincter is paralyzed, and retrograde ejaculation into the bladder occurs if the proximal sphincter is malfunctioning.

III. Erectile Dysfunction and Aging

The term *impotence* has been used to identify the inability of the male to achieve and maintain penile erection sufficient for sexual intercourse. However, this term has also been used to refer to anorgasmia, lack of libido, and inability to satisfy the partner. Therefore, the NIH consensus panel on impotence suggested that the term *erectile dysfunction* be used to describe the inability to achieve an erection (NIH Consensus Conference, 1993). It is estimated that 10–30 million American men have erectile dysfunction (Nelson and McLemore, 1988; National Center for Health Statistics, 1989). According to the Massachusetts Male Aging Study conducted on a community-based, random sample of men ages 40–70 years in the Boston area, the overall prevalence of erectile dysfunction was 52% (Feldman *et al*., 1994). Kaiser *et al*. (1988) found complete erectile failure in 41% of the men ages 60–79 years in their cross-sectional study. None of the men above the age of 70 years was able to achieve a full erection. Mulligan *et al*. (1988) also found high prevalence of sexual dysfunction in a veteran population, with 27% of men ages 65–75 years and 50% in those over 75 years reporting erectile dysfunction. This high prevalence of sexual dysfunction is important because men with erectile dysfunction report impaired quality of life when compared with unaffected men (Jonler *et al*., 1995).

A. Vascular Disease

The most common etiology of erectile dysfunction in aged men is vascular disease. In men over 60 years old who were being evaluated for inadequate penile rigidity, 21% of the cases were due to vascular disease (Mulligan and Katz, 1989). In a study by Virag *et al*. (1985) of 178 men with organic erectile dysfunction, arteriograms revealed arterial lesions in 68%. They also found that the risk of organic erectile dysfunction increased with the number of vascular risk factors (diabetes mellitus, smoking, hyperlipidemia, and hypertension); 100% of the patients with three or more risk factors had erectile dysfunction. Finally, Morley *et al*. (1988) showed that erectile dysfunction is a predictor of major atherosclerotic vascular disease (i.e., myocardial infarction and stroke).

Vascular disease results in erectile dysfunction by two mechanisms, arterial insufficiency and venous leakage. Obstruction from atherosclerotic arterial occlusive disease of the hypogastric-cavernous arterial bed decreases the perfusion pressure and arterial flow to the lacunar spaces which is necessary to achieve a rigid erection (Krane *et al*., 1989). In an animal model using New Zealand White male rabbits, Azadzoi and Goldstein (1992) demonstrated that hemodynamic alterations created by hypercholesterolemia and atherosclerotic occlusive disease of the iliac arteries caused erectile dysfunction. Additionally, atherosclerotic vascular disease may cause ischemia of trabecular smooth muscle and result in replacement of smooth muscle by connective tissue. Jevtich *et al*. (1990) examined corpora cavernosal tissue by electron microscopy. The tissue from impotent men showed marked thickening of the basal lamina, a paucity of contractile filaments, minimal or no glycogen, and fewer vesicles on the cell surface. The degree of smooth muscle cell alteration correlated with the severity of symptoms. Using New Zealand white rabbits, Nehra *et al*. (1998) found that the severity of arterial occlusion correlated with the decrease in trabecular smooth muscle content in the corpus cavernosum. This decrease in smooth muscle content impaired cavernosal expandability.

Venoocclusive dysfunction or venous leakage is characterized by excessive outflow through the subtunical venules, preventing the development of high pressure within the corpora cavernosa and, thereby, interfering with maintenance of a rigid erection. Venous leakage can result from La Peyronie's disease, arteriovenous fistula, or trauma-induced communication between the glans and the corpora (Azadzoi *et al*., 1996). In a study of men with erectile dysfunction who failed to achieve

an erection with intracorporeal papaverine, 86% had evidence of venous leakage (Rajfer *et al*., 1988). Tudoriu and Bourmer (1983) found an increase in both the size and the number of venous outflow channels with advancing age in human cadavers. The structural alteration in the fibroelastic components of the trabeculae causes a loss of compliance and inability to expand the trabeculae against the tunica albuginea, which is necessary to compress the subtunical venules. This decrease in fibroelasticity may be from increased cross-linking of collagen fibers induced by nonenzymatic glycosylation (Cerami *et al*., 1987). It may also result from vascular factors, such as hypercholesterolemia associated with altered collagen synthesis (Fischer *et al*., 1980). Finally, venoocclusive dysfunction can occur from insufficient relaxation of trabecular smooth muscle in an anxious patient who has excessive adrenergic-constrictor tone and in patients with injured parasympathetic dilator nerves (Krane *et al*., 1989).

B. Neurological Disease

Neurological disease accounts for the second most common cause of erectile dysfunction in elderly men. Partial or complete erectile dysfunction can result from disorders which affect the parasympathetic sacral spinal cord or the peripheral efferent autonomic fibers to the penis. Such disorders impair penile smooth muscle relaxation and prevent the vasodilation needed for erection. Suprasacral lesions may also cause erectile dysfunction through the lack of input from higher neural centers. In spinal cord injury patients the degree of erectile dysfunction is largely dependent on the completeness and the level of the spinal injury. Those patients with complete lesions or injury to the lower part of the spinal cord are more likely to have loss of erectile function. However, 25% of spinal cord injury patients with erectile capabilities have erections adequate for penetration. Multiple sclerosis is also associated with erectile dysfunction. Mattson *et al*. (1995) found that 78% of men with multiple sclerosis experienced sexual dysfunction including erectile problems, decreased sensation, and inability to reach orgasm. In the older man, diabetes mellitus, stroke, and Parkinson's disease can cause autonomic dysfunction resulting in erectile failure. Additionally, surgical procedures such as radical prostatectomy, cystoprostatectomy, and proctocolectomy frequently disrupt the autonomic nerve supply to the corporal bodies and result in postoperative erectile dysfunction (Quinlan *et al*., 1991).

C. Diabetes Mellitus

The prevalence of erectile dysfunction in diabetes mellitus has been reported to be as high as 75%. Erectile dysfunction occurs more frequently and at a younger age in the diabetic population when compared with the population as a whole. Greater than 50% of diabetic patients report erectile dysfunction within 10 years of the diagnosis of diabetes; for some it is the presenting symptom (Kaiser and Korenman, 1988). In addition to erectile dysfunction, diabetic patients report decreased sexual desire, activity, and satisfaction when compared to age-matched healthy controls (Schiavi *et al*., 1993).

Although the etiology of diabetic erectile dysfunction is multifactorial, the major cause in older diabetic patients is vascular disease; autonomic neuropathy plays a more important role in younger patients (Morley and Kaiser, 1989). In a survey of 200 diabetic men, 59% had erectile dysfunction and 88% of the impotent subjects had neuropathy. Only 12% of the potent diabetics had clinical signs of neuropathy (Ellenberg, 1971). Palmer *et al*. (1986) reported that the correlation with impotence was highest when neuropathic symptoms coexisted with slowing of motor nerve conduction velocity. In a later study, Buvat *et al*. (1985) found that motor and sensory median nerve conduction velocity was not useful in discriminating between potent and impotent diabetic patients. They concluded that most diabetic impotence arises from an abnormality of the autonomic nervous system and arterial factors.

This controversy in the importance of the neurological factors in diabetic erectile dysfunction may be confounded by the fact that many studies failed to categorize the diabetic patients into type I versus type II. Bemelmans *et al*. (1994) studied type I diabetic patients and found neuropathy in 85% of the impotent diabetic patients. Neuropathy was more severe in comparison to potent diabetic and impotent nondiabetic patients. They also found that 58% of impotent diabetic patients had vascular disease, as suggested by the inability to achieve an erection with intracavernosal injection of papaverine. They concluded that in type I diabetic patients, neurologic factors have a crucial role in the etiology of diabetic erectile dysfunction. Nevertheless, vascular disease may be the most common etiology of erectile dysfunction in diabetic men.

Impotent diabetic men may also have impaired penile cholinergic nerve synthesis and release of acetylcholine, resulting in decreased ability to relax trabecular smooth muscle (Blanco *et al*., 1990). *In vitro* study of human corpus cavernosum tissue from diabetic and nondiabetic impotent patients revealed that impotent diabetic men have impairment in both the autonomic and the endothelium-dependent mechanisms which facilitate relaxation of smooth muscle (Saenz de Tejada *et al*., 1989). Autonomically mediated contractions were maintained despite impairment in autonomically mediated relaxation of corporal tissue from diabetic subjects. Thus, there was an imbalance favoring detumescence rather than erection. The decreased response to acetylcholine in tissue from impotent diabetic men is likely due to decreased synthesis or release of NO, the endothelium-derived relaxing factor.

Another factor contributing to decreased vasodilation in diabetic impotent men may be the inactivation of endothelium-derived NO by basement membrane advanced glycosylation end products (Hogan *et al*., 1992). Advanced glycosylation end products which accumulate on tissue proteins such as basement membrane collagen, have been implicated in other long-term complications of diabetes mellitus such as vascular disease. Finally, Vernet *et al*. (1995) demonstrated a decrease in penile NOS in types I and II diabetic rats with erectile dysfunction and have suggested that this is due in part to androgen deficiency.

D. Testosterone and Erectile Dysfunction

The role of androgens in erection is controversial (Mulligan and Schmitt, 1993). Androgen receptors have been demonstrated in sacral parasympathetic nuclei and hypothalamic and limbic system neurons, suggesting potential hormonal reg-

ulation of centers involved in erectile function (Krane *et al*., 1989). However, patients with castrate levels of testosterone can attain erections in response to visual sexual stimuli (Bancroft and Wu, 1983). Hypogonadal patients show smaller and slower ability to develop erections in response to fantasy, and androgen replacement improved erectile response. These findings suggest that erections to certain types of sexual stimuli (i.e., direct penile stimulation) may be androgen independent while response to fantasy may be androgen dependent. In contrast, Kwan *et al*. (1983) found no difference in erectile response to erotic film and fantasy in hypogonadal versus eugonadal subjects. Davidson *et al*. (1979) showed that in hypogonadal men there were dose-related responses to androgen treatment in frequencies of nocturnal erection and coitus.

Androgen may indirectly affect penile smooth muscle relaxation and resultant rigidity through NOS. Chamness *et al*. (1995) demonstrated decrease in NOS activity and amount of NOS protein in the penis of adult rats after castration. These changes were reversed by testosterone replacement. The effect of testosterone was blocked by finasteride, a 5α-reductase inhibitor, suggesting that the active androgen may be dihydrotestosterone (Lugg *et al*., 1995b). These investigators also demonstrated a decrease in erectile response with castration and a concomitant decrease in total penile NOS activity. However, in men, despite a decline in serum testosterone concentration with aging, no age-related decline in dihydrotestosterone has been found (Gray *et al*., 1991). Overall, therefore, testosterone appears to play a minor role in erectile function in humans.

E. Drug-Induced Erectile Dysfunction

Many commonly used medications have been associated with erectile dysfunction (Anonymous, 1992). Slag *et al*. (1983) reported 25% incidence of drug-induced impotence in a medical outpatient clinic population. Almost all the data available on drug-induced erectile dysfunction are subjective, however, based on observations, case reports, patient and physician surveys, and pre- and postmarketing drug studies (Benet and Melman, 1995).

The mechanism of drug-mediated erectile dysfunction, for the most part, is unknown. Medications such as antidepressants, antipsychotics, and antihistamines have anticholinergic effects which may contribute to erectile dysfunction by blocking parasympathetic-mediated penile artery vasodilatation and trabecular smooth muscle relaxation (Godschalk *et al*., 1997). Virtually all antihypertensive agents have been associated with erectile dysfunction, but with a higher incidence for beta-blockers, thiazide diuretics, reserpine, clonidine, and alpha-methyldopa. The mechanism for erectile dysfunction with these medications may be the lowering of blood pressure below the critical threshold necessary to maintain sufficient blood flow for penile erection, particularly in patients who already have penile arterial disease. Antipsychotic medications such as phenothiazines, thioxanthines, and butyrophenones can cause erectile dysfunction through sedation, anticholinergic effects, central anti-dopaminergic effect, and elevation of prolactin serum concentrations (Wein and Van Arsdalen, 1988). Other medications which can cause erectile dysfunction through effects on the central nervous system include tricyclic antidepressants, lithium, and monoamine oxidase inhibitors.

F. Psychogenic Erectile Dysfunction

Reports on the prevalence of psychogenic erectile dysfunction vary from 10 to 90%, and the likelihood of psychogenic impotence inversely correlates with age; the younger patient has a greater likelihood that his erectile dysfunction is psychogenic (Carrier *et al*., 1993). Slag *et al*. (1983) reported 14% of their outpatient impotent males had a psychogenic etiology. Mulligan and Katz (1989) attributed psychopathology as the cause of erectile dysfunction in 9% of their aged impotent male veteran population. Psychogenic impotence may occur via increased sympathetic stimuli to the sacral cord inhibiting the parasympathetic dilator nerves to the penis, and thereby inhibiting erection. Common causes of psychogenic erectile dysfunction include performance anxiety, conflicts in relationships, sexual inhibition, childhood sexual abuse, and fear of sexually transmitted diseases (Smith, 1988). A classic psychogenic cause of erectile dysfunction in the older male is the ''widower's syndrome,'' where the older man involved in a new relationship feels guilt and develops impotence as a defense against his unfaithfulness to his dead spouse (Morley and Kaiser, 1989).

G. Other Factors in Erectile Dysfunction

In addition to hypogonadism, other endocrine abnormalities have been implicated in the etiology of erectile dysfunction, albeit in less than 5% (Johnson and Jarow, 1992). Hyperthyroidism, hypothyroidism, and hyperprolactinemia have been associated with impotence. In hyperprolactinemia, there is an associated decrease in serum testosterone concentration due to inhibition of gonadotropin-releasing-hormone secretion by the elevated prolactin concentration (Morley, 1986). However, normalizing the serum testosterone level does not restore potency in many patients with hyperprolactinemia. This suggests antagonism by prolactin to the peripheral action of testosterone. Low testosterone secretion and elevated prolactin may also contribute to the erectile dysfunction seen with hypothyroidism. Hyperthyroidism is more often associated with decline in libido rather than erectile dysfunction.

Chronic alcoholism with associated hypogonadism can impair erectile function. Hypogonadism in alcoholism occurs through alcohol toxicity at the gonadal and hypothalamic–pituitary levels (Van Thiel *et al*., 1982). Spontaneous reversal of erectile dysfunction has been shown to occur with sobriety for at least 1 year, but only if there is no gonadal atrophy. Alcoholic neuropathy may also contribute to erectile dysfunction. Chronic obstructive lung disease is thought to contribute to erectile dysfunction through hypoxia suppressing the hypothalamic–pituitary–gonadal axis (Semple *et al*., 1984).

IV. Libido and Aging

Despite the increased prevalence of erectile dysfunction, libido does not vary as much with advancing age. Mulligan *et al*. (1988) found that in an ambulatory geriatric population,

53% of those over the age of 75 years still reported intact libido. When a decline in libido occurs, it is often associated with androgen deficiency (Vermeulen, 1991). Burris *et al*. (1992) found low sexual interest in their hypogonadal subjects, and testosterone replacement increased libido in a dose-dependent manner. Davidson *et al*. (1983) found decreasing serum total and free-testosterone concentrations with aging and a parallel decline in sexual activity, libido, and potency. Schiavi *et al*. (1991) found that bioavailable testosterone showed positive correlation with sexual desire and arousal, but not with frequency of coitus in a healthy older population.

V. Alterations in Emission, Ejaculation, and Orgasm with Aging

In addition to alterations in erectile function, the four stages of sexual response, excitement, plateau, orgasm, and resolution, all change with aging (Masters and Johnson, 1970). During the excitement phase, there is a delay in erection, tensing of the scrotal sac decreases, and testicular elevation may not occur (Kaiser, 1991; Rowland *et al*, 1993). There is a prolonged duration of the plateau stage and decreased preejaculatory secretion from Cowper's gland. Orgasm is diminished in duration, and there are decreased or spastic prostatic contractions, decreased urethral contractions, and decreased force of emission. Orgasm is less intense with a smaller quantity of ejaculate being expelled. In the resolution stage, there is rapid detumescence and testicular descent. The refractory period between erections is prolonged with aging (Ludeman, 1981). Most young healthy males are capable of achieving an erection, engaging in intercourse, attaining a climax, and repeating this process within minutes. With aging, this rest or refractory period needed before erection and sexual intercourse can be repeated gradually lengthens. Masters and Johnson (1970) reported that at least several hours were usually needed before full erection could be attained again. This prolonged refractory period may contribute to the decline in the frequency of sexual intercourse in older men. Older males may experience retrograde ejaculation due to a damaged proximal sphincter following transurethral prostatic resection. Diabetic autonomic dysfunction can also cause retrograde ejaculation and a decline in orgasmic sensation. Last, there is a decrease in penile sensitivity to vibration and light touch with aging. Sexual function and penile sensitivity may be closely related since penile vibrotactile thresholds are higher in subjects with erectile dysfunction than in age-matched control subjects (Rowland *et al*., 1991). Rowland *et al*. (1989) found a higher penile threshold for vibratory stimulation in aged men.

VI. Summary

Sexuality remains an important issue in the older population (Ludeman, 1981). In spite of a decreased ability to achieve an erection, there clearly is continued sexual desire. Many studies suggest that erectile dysfunction in the aged is primarily due to age-associated chronic disease rather than normal, healthy aging. Therefore, preventive measures aimed at the underlying diseases should be sought. Nevertheless, effective treatment options are now available to successfully regain sexual function and thereby improve quality of life.

References

Aboseif, S. R., and Lue, T. F. (1988). Hemodynamics of penile erection. *Urol. Clin. North Am.* **15**, 1–7.

Anderson, R. A., Bancroft, J., and Wu, F. C. W. (1992). The effects of exogenous testosterone on sexuality and mood of normal men. *J. Clin. Endocrinol. Metab.* **75**, 1503–1507.

Andersson, K. E., and Wagner, G. (1995). Physiology of penile erection. *Physiol. Rev.* **75**, 191–236.

Anonymous. (1992). Drugs that cause sexual dysfunction: An update. *Med. Lett. Drugs Ther.* **34**, 73–78.

Azadzoi, K. M., and Goldstein, I. (1992). Erectile dysfunction due to atherosclerotic vascular disease: The development of an animal model. *J. Urol*. **147**, 1675–1681.

Azadzoi, K. M., Siroky, M. B., and Goldstein, I. (1996). Study of etiologic relationship of arterial atherosclerosis to corporal veno-occlusive dysfunction in the rabbit. *J. Urol*. **155**, 1795–1800.

Bagatell, C. J., Heiman, J. R., Rivier, J. E., and Bremner, W. J. (1994). Effects of endogenous testosterone and estradiol in sexual behavior in normal young men. *J. Clin. Endocrinol. Metab*. **78**, 711–716.

Bancroft, J., and Wu, F. C. W. (1983). Changes in erectile responsiveness during androgen replacement therapy. *Arch. Sex. Behav.* **12**, 59–66.

Banya, Y., Ushiki, T., Takagane, H., Aoki, H., Kubo, T., Ohhori, T., and Ide, C. (1989). Two circulatory routes within the human corpus cavernosum penis: A scanning electron microscopic study of corrosion casts. *J. Urol*. **142**, 879–883.

Bemelmans, B. L. H., Meuleman, E. J. H., Doesburg, W. H. *et al*. (1994). Erectile dysfunction in diabetic men: The neurological factor revisited. *J. Urol*. **151**, 884–889.

Benet, A. E., and Melman, A. (1995). The epidemiology of erectile dysfunction. *Urol. Clin. North Am*. **22**, 699–709.

Blanco, R., Saenz de Tejada, I., Goldstein, I., *et al*. (1990). Dysfunctional penile cholinergic nerves in diabetic impotent men. *J. Urol*. **144**, 278–280.

Blumer, D. (1970). Hypersexual episodes in temporal lobe epilepsy. *Am. J. Psychiatry* **126**, 1099–1106.

Bossart, M. I., Spjut, H. J., and Scott, F. B. (1980). Ultrastructural analysis of human penile corpus cavernosum. Its significance in tumescence and detumescence. *Urology* **15**, 448–456.

Bretschneider, J. G., and McCoy, N. (1988). Sexual interest and behavior in healthy 80-102-year-olds. *Arch. Sex. Behav.* **17**, 109–129.

Burnett, A. L. (1995a). Role of nitric oxide in the physiology of erection. *Biol. Reprod*. **52**, 485–489.

Burnett, A. L. (1995b). Nitric oxide control of lower genitourinary tract functions: A review. *Urology* **45**, 1071–1083.

Burris, A. S., Banks, S. M., Carter, C. S., Davidson, J. M., and Sherins, R. J. (1992). A long-term, prospective study of the physiologic and behavioral effects of hormone replacement in untreated hypogonadal men. *J. Androl*. **13**, 297–304.

Buvat, J., Lemaire, A., Buvat-Herbaut, M. *et al*. (1985). Comparative investigations in 26 impotent and 26 nonimpotent diabetic patients. *J. Urol*. **133**, 34–38.

Carrier, S., Brock, G., Kour, N. W., and Lue, T. F. (1993). Pathophysiology of erectile dysfunction. *Urology* **42**, 468–481.

Cerami, A., Vlassara, H., and Brownlee, M. (1987). Glucose and aging. *Sci. Am*. **256**, 90–96.

Chamness, S. L., Ricker, D. D., Crone, J. K., *et al*. (1995). The effect of androgen on nitric oxide synthase in the male reproductive tract of the rat. *Fertil. Steril*. **63**, 1101–1107.

Cunningham, G. R., and Hirshkowitz, M. (1995). Inhibition of steroid 5 alpha-reductase with finasteride: Sleep-related erections, potency, and libido in healthy men. *J. Clin. Endocrinol. Metab*. **80**, 1934–1940.

Davidson, J. M., Camargo, C. A., and Smith, E. R. (1979). Effects of androgen on sexual behavior in hypogonadal men. *J. Clin. Endocrinol. Metab.* **48**, 955–958.

Davidson, J. M., Chen, J. J., Crapo, L. *et al*. (1983). Hormonal changes and sexual function in aging men. *J. Clin. Endocrinol. Metab.* **57**, 71–77.

Diokno, A. C., Brown, M. B., and Herzog, A. R. (1990). Sexual function in the elderly. *Arch. Intern. Med*. **150**, 197–200.

Ellenberg, M. (1971). Impotence in diabetes: The neurologic factor. *Ann. Intern. Med*. **75**, 213–219.

Feldman, H. A., Goldstein, I. Hatzichristou, D. G., Krane, R. J., and McKinlay, J. B. (1994). Impotence and its medical and psychosocial correlates: Results of the Massachusetts male aging study. *J. Urol*. **151**, 54–61.

Fischer, G. M., Swain, M. L., and Cherian, K. (1980). Increased vascular collagen and elastin synthesis in experimental atherosclerosis in the rabbit: Variation in synthesis among major vessels. *Atherosclerosis* **35**, 11–20.

Godschalk, M. F., Sison, A., and Mulligan, T. (1997). Management of erectile dysfunction by the geriatrician. *J. Am. Geriatr. Soc*. **45**, 1240–1246.

Gray, A., Feldman, H. A., McKinlay, J. B., and Longscope, C. (1991). Age, disease, and changing sex hormone levels in middle-aged men: Results of the Massachusetts male aging study. *J. Clin. Endocrinol. Metab*. **73**, 1016–1025.

Hogan, M., Cerami, A., and Bucala, R. (1992). Advanced glycosylation endproducts block the antiproliferative effect of nitric oxide. *J. Clin. Invest*. **90**, 1110–1115.

Ignarro, L. J., Bush, P. A., Buga, G. M., *et al*. (1990). Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. *Biochem. Biophys. Res. Commun*. **170**, 843–850.

Jevtich, M. J., Khawand, N. Y., and Vidic, B. (1990). Clinical significance of ultrastructural findings in the corpora cavernosa of normal and impotent men. *J. Urol*. **143**, 289–293.

Johnson, A. R., III, and Jarow, J. P. (1992). Is routine endocrine testing of impotent men necessary? *J. Urol*. **147**, 1542–1543.

Jonler, M., Moon, T., Brannan, W., Stone, N. N., Heisey, D., and Bruskewitz, R. C. (1995). The effect of age, ethnicity and geographical location on impotence and quality of life. *B. J. Urol*. **75**, 651–655.

Kaiser, F. E. (1991). Sexuality and impotence in the aging man. *Clin. Geriatr. Med*. **7**, 63–72.

Kaiser, F. E. (1996). Sexuality in the elderly. *Urol. Clin. North Am*. **23**, 99–109.

Kaiser, F. E., and Korenman, S. G. (1988). Impotence in diabetic men. *Am. J. Med*. **85** (Suppl. 5A), 147–152.

Kaiser, F. E., Viosca, S. P., Morley, J. E., *et al*. (1988). Impotence and aging: Clinical and hormonal factors. *J. Am. Geriatr. Soc*. **36**, 511–519.

Kim, N., Azadzoi, K. M., Goldstein, I., and Saenz de Tejada, I. (1991). A nitric oxide-like factor mediates nonadrenergic-noncholinergic neurogenic relaxation of penile corpus cavernosum smooth muscle. *J. Clin. Invest*. **88**, 112–118.

Kinsey, A. C., Pomeroy, W. B., and Martin, C. E. (1948). ''Sexual Behavior in the Human Male.'' Saunders, Philadelphia.

Kollberg, S., Petersen, I., and Stener, I. (1962). Preliminary results of an electromyographic study of ejaculation. *Acta. Chir. Scand*. **123**, 478–483.

Korenman, S. G. (1998). New insights into erectile dysfunction: A practical approach. *Am. J. Med*. **105**, 135–144.

Krane, R. J., Goldstein, I., and Saenz de Tejada, I. (1989). Impotence. *N. Engl. J. Med*. **321**, 1648–1659.

Kwan, M., Greenleaf, W. J., Mann, J., Crapo, L., and Davidson, J. M. (1983). The nature of androgen action on male sexuality: A combined laboratory-self-report study on hypogonadal men. *J. Clin. Endocrinol. Metab*. **57**, 557–562.

Leigh, B. C., Temple, M. T., and Trocki, K. F. (1993). The sexual behavior of US adults: Results from a national survey. *Am. J. Public Health* **83**, 1400–1408.

Ludeman, K. (1981). The sexuality of the older person: Review of literature. *Gerontologist* **21**, 203–208.

Lue, T., and Tanagho, E. A. (1987). Physiology of erection and pharmacological management of impotence. *J. Urol*. **137**, 829–836.

Lugg, J. A., Gonzalez-Cadavid, N. F., and Rajfer, J. (1995a). The role of nitric oxide in erectile function. *J. Androl*. **16**, 2–4.

Lugg, J. A., Rajfer, J., and Gonzalez-Cadavid, N. F. (1995b). Dihydrotestosterone is the active androgen in the maintenance of nitric oxide-mediated penile erection in the rat. *Endocrinology (Baltimore)* **136**, 1495–1501.

Makhlouf, G. M., and Grider, J. R. (1993). Nonadrenergic noncholinergic inhibitory transmitters of the gut. *News Physiol. Sci*. **8**, 195–199.

Masters, W., and Johnson, V. (1970). ''Human Sexual Response.'' Little, Brown, Boston.

Mattson, D., Petrie, M., Srivastava, D. K., and McDermott, M. (1995). Multiple sclerosis. Sexual dysfunction and its response to medications. *Arch. Neurol. (Chicago)* **52**, 862–868.

Moncada, S. (1992). The L-arginine: Nitric oxide pathway. *Acta Physiol. Scand.* **145**, 201–227.

Morley, J. E. (1986). Impotence. *Am. J. Med*. **80**, 897–905.

Morley, J. E., and Kaiser, F. E. (1989). Sexual function with advancing age. *Med. Clin. North Am*. **73**, 1483–1495.

Morley, J. E., Korenman, S. G., Kaiser, F. E., *et al*. (1988). Relationship of penile brachial pressure index to myocardial infarction and cerebrovascular accidents in older men. *Am. J. Med*. **84**, 445–448.

Mulligan, T., and Katz, P. G. (1989). Why aged men become impotent. *Arch. Intern. Med*. **149**, 1365–1366.

Mulligan, T., and Moss, C. R. (1991). Sexuality and aging in male veterans: A cross-sectional study of interest, ability, and activity. *Arch. Sex. Behav*. **20**, 17–25.

Mulligan, T., and Palguta, R. F., Jr. (1991). Sexual interest, activity, and satisfaction among male nursing home residents. *Arch. Sex. Behav*. **20**, 199–204.

Mulligan, T., and Schmitt, B. (1993). Testosterone for erectile failure. *J. Gen. Intern. Med*. **8**, 517–521.

Mulligan, T., Retchin, S. M., Chinchilli, V. M., and Bettinger, C. B. (1988). The role of aging and chronic disease in sexual dysfunction. *J. Am. Geriatr. Soc*. **36**, 520–524.

National Center for Health Statistics. (1989). ''National Hospital Discharge Survey,'' DHHS Publ. No. 87-1751. U. S. Govt. Printing Office, Washington, Dc.

Nehra, A., Azadzoi, K. M., Moreland, R. B. *et al*. (1998). Cavernosal expandability is an erectile tissue mechanical property which predicts trabecular histology in an animal model of vascuolgenic erectile dysfunction. *J. Urol*. **159**, 2229–2236.

Nelson, C., and McLemore, T. (1988). ''The National Ambulatory Medical Care Survey: United States 1975-81 and 1985 Trends,'' Vital and Health Statistics, Ser. 13, No. 93, DHHS Publ. No. (PHS) 88-1754. U. S. Govt. Printing Office, Washington, DC.

Newman, H. F., and Northup, J. D. (1981). Mechanism of human penile erection: An overview. *Urology* **17**, 399–408.

Newman, H. F., Reiss, H., and Northup, J. D. (1982). Physical basis of emission, ejaculation, and orgasm in the male. *Urology* **19**, 341–350.

NIH Consensus Conference. (1993). Impotence. *JAMA, J. Am. Med. Assoc*. **270**, 83–90.

O'Carroll, R., and Bancroft, J. (1984). Testosterone therapy for low sexual interest and erectile dysfunction in men: A controlled study. *Br. J. Psychiatry* **145**, 146–151.

Palmer, J. D. K., Fink, S., and Burger, R. H. (1986). Diabetic secondary impotence: Neuropathic factor as measured by peripheral motor nerve conduction. *Urology* **28**, 197–200.

Pfeiffer, E., Verwoerdt, A., and Wang, H. S. (1968). Sexual behavior in aged men and women. *Arch. Gen. Psychiatry* **19**, 753–758.

Pfeiffer, E., Verwoerdt, A., and Wang, H. S. (1969). The natural history of sexual behavior in a biologically advantaged group of aged individuals. *J. Gerontol*. **24**, 193–198.

Quinlan, D. M., Epstein, J. I., Carter, B. S., and Walsh, P. C. (1991). Sexual function following radical prostatectomy: Influence of preservation of neurovascular bundles. *J. Urol*. **145**, 998–1002.

Rajfer, J., Rosciszewski, A., and Mehringer, M. (1988). Prevalence of corporeal venous leakage in impotent men. *J. Urol*. **140**, 69–71.

Rajfer, J., Aronson, W. J., Bush, P. A., Dorey, F. J., and Ignarro, L. J. (1992). Nitric oxide as a mediator of relaxation of the corpus cavernosum in response to nonadrenergic, noncholinergic neurotransmission. *N. Engl. J. Med*. **326**, 90–94.

Rowland, D. L., Greenleaf, W., Mas, M., Myers, L, and Davidson, J. M. (1989). Penile and finger sensory thresholds in young, aging, and diabetic males. *Arch. Sex. Behav.* **18**, 1–12.

Rowland, D. L., Leentvaar, E. J., Blom, J. H. M., and Slob, A. K. (1991). Changes in penile sensitivity following papaverine-induced erection in sexually functional and dysfunctional men. *J. Urol*. **146**, 1018–1021.

Rowland, D. L., Greenleaf, W. J., Dorfman, L. J., and Davidson, J. M. (1993). Aging and sexual function in men. *Arch. Sex. Behav.* **22**, 545–557.

Sachs, B. D. (1995). Placing erection in context: The reflexogenic-psychogenic dichotomy reconsidered. *Neurobiobehav. Rev.* **19(2)**, 211–224.

Saenz de Tejada, I., Blanco, R., Goldstein, I., *et al*. (1988). Cholinergic neurotransmission in human corpus cavernosum. I. Responses of isolated tissue. *Am. J. Physiol*. **254**, H459–H467.

Saenz de Tejada, I., Goldstein, I., Azadzoi, K. *et al*. (1989). Impaired neurogenic and endothelium-mediated relaxation of penile smooth muscle from diabetic men with impotence. *N. Engl. J. Med*. **320**, 1025–1030.

Said, S. I. (1992). Nitric oxide and vasoactive intestinal peptide: Cotransmitters of smooth muscle relaxation. *News Physiol. Sci*. **7**, 181–183.

Schiavi, R. C., Schreiner-Engel, P., Mandeli, J., Schanzer, H., and Cohen, E. (1990). Healthy aging and male sexual function. *Am. J. Psychiatry* **147**, 766–771.

Schiavi, R. C., Schreiner-Engel, P., White, D., and Mandeli, J. (1991). The relationship between pituitary-gonadal function and sexual behavior in healthy aging men. *Psychosom. Med*. **53**, 363–374.

Schiavi, R. C., Stimmel, B. B., Mandeli, J., and Rayfield, E. J. (1993). Diabetes mellitus and male sexual function: A controlled study. *Diabetologia* **36**, 745–751.

Semple, P. D., Beastall, G. H., Brown, T. M. *et al*. (1984). Sex hormone suppression and sexual impotence in hypoxic pulmonary fibrosis. *Thorax* **39**, 46–51.

Slag, M. F., Morley, J. E., Elson, M. K. *et al*. (1983). Impotence in medical clinic outpatients. *JAMA, J. Am. Med. Assoc*. **249**, 1736–1740.

Smith, A. D. (1988). Psychologic factors in the multidisciplinary evaluation and treatment of erectile dysfunction. *Urol. Clin. North Am*. **15**, 41–51.

Steers, W. D. (1990). Neural control of penile erection. *Semin. Urol*. **8**, 66–79.

Tudoriu, T., and Bourmer, H. (1983). The hemodynamics of erection at the level of the penis and its local deterioration. *J. Urol*. **129**, 741–745.

Udry, J. R., Billy, J. O. G., Morris, N. M., Groff, T. R., and Raj, M. H. (1985). Serum androgenic hormones motivate sexual behavior in adolescent boys. *Fertil. Steril*. **43**, 90–94.

Van Thiel, D. H., Gavaler, J. S., and Sanghvi, A. (1982). Recovery of sexual function in abstinent alcoholi men. *Gastroenterology* **84**, 677–682.

Vermeulen, A. (1991). Clinical review 24: Androgens in the aging male. *J. Clin. Endocrinol. Metab*. **73**, 221–224.

Vernet, D., Cai, L., Garban, H., *et al*. (1995). Reduction of penile nitric oxide synthase in diabetic BB/WOR dp (Type I) and BBZ/WOR dp (Type II) rats with erectile dysfunction. *Endocrinology (Baltimore)* **136**, 5709–5717.

Virag, R., Frydman, D., and Bouilly, P. (1985). Is impotence an arterial disorder? A study of arterial risk factors in 440 impotent men. *Lancet* **1**, 181–184.

Wagner, G., Gerstenberg, T., and Levin, R. J. (1989). Electrical activity of corpus cavernosum during flaccidity and erection of the human penis: A new diagnostic method? *J. Urol*. **142**, 723–725.

Wein, A. J., and Van Arsdalen, K. N. (1988). Drug-induced male sexual dysfunction. *Urol. Clin. North Am*. **15**, 23–31.

Wespes, E., and Schulman, C. (1993). Venous impotence: Pathophysiology, diagnosis, and treatment. *J. Urol*. **149**, 1238–1245.

Wiley, D., and Bortz, W. M., II. (1996). Sexuality and aging-usual and successful. *J. Gerontol*. **51A**, M142–M146.

Williams, P. L., and Warwick, R. (1980). "Gray's Anatomy," 36th ed. Saunders, Philadelphia.

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Nancy E. Avis

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Sexual Behavior in Aging Women

I. Introduction

The perimenopause is a time of significant hormonal changes. The hallmark of these hormonal changes is vasomotor symptoms such as night sweats and hot flashes. Other purported effects of changing hormones include sexual dysfunction, sleep disruption, mood disturbances, and a host of other ''symptoms.'' However, the evidence for these purported effects of menopause has not been clearly established. Complicating research in this area is the difficulty in separating direct and indirect effects of changing hormone levels. Vasomotor symptoms may impact sleep, which may in turn affect mood. All of these effects may impact sexual functioning. In addition, some of the effects of these hormonal changes may increase with age (e.g., vaginal dryness), whereas others appear to be transitory (e.g., mood effects). The issues addressed in this chapter concern the functional impact of these neuroendocrine changes and their interrelatedness.

The primary purpose of this chapter is to review the epidemiologic literature on changes in sexual functioning with age, with a particular focus on menopause. Because sexual functioning is interrelated with hormonal changes, hot flashes, and mood, these areas will be reviewed as well. Prior to reviewing this literature, it is important to first look at methodological issues in this area of research.

II. Methodological Issues

Several methodological issues are important to consider in reviewing this area of research. These include the distinction between clinic and population-based samples, defining menopause status, measuring mood and sexual functioning, and limitations of cross-sectional research.

A. Clinic versus Population-Based Samples

Much of the research on menopause and sexual functioning or mood is based on patient samples of women who report problems or women who have sought treatment for menopause-related problems. These patient samples present a biased view of sexual functioning and mood changes in the general population. Patient- or clinic-based samples are generally not representative of the general population, and it is well-established that people who seek treatment or generally use the health care system differ in a number of important ways from nonpatient samples; they are often less healthy, report greater psychological symptoms, and experience greater stress. Community or population-based studies are important because they provide information on the prevalence, incidence, and distribution of function and dysfunction in the general population. The population perspective also informs the clinician as to whether a condition is underdetected (i.e., more prevalent in the general population) or restricted to patient samples.

Data from such patient-based studies represent only women who seek medical care. This is particularly problematic for samples of women seeking treatment for acute menopausal symptoms. Studies have shown that fewer than half of menopausal women seek menopause-related treatment (Brown and Brown, 1976; Rose, 1977; Weideger, 1977; Ballinger, 1985; Morse *et al*., 1994; Avis *et al*., 1997) and those who do seek treatment tend to report more life stress and suffer more from clinical depression, anxiety, and psychological symptoms than women in nonpatient samples (Ballinger, 1985; Morse *et al*., 1994).

As data from population and community-based samples have become available, it is apparent that this research presents a very different picture of menopause than does patient-based research and challenges many of our long-held beliefs about menopause.

B. Defining Menopausal Status

The inconsistency with which researchers have defined menopause status presents a major challenge in comparing studies in this area (Holte, 1998). In relating any outcome to menopause status, it is important to use consistent definitions of pre-, peri-, and postmenopause. Some earlier studies (Bungay *et al*., 1980) used age (45–55) as a surrogate for menopause status. However, this is an imprecise measure of status as women between 45 and 55 can be pre-, peri-, or postmeno-

pausal. Pansini *et al*. (1994) have shown the considerable age overlap among menopause status groups.

The standard epidemiological definition of natural menopause is 12 consecutive months of amenorrhea in the absence of surgery or other pathological or physiological cause (e.g., pregnancy, lactation) that would terminate menstruation (World Health Organization, 1996). While perimenopause has been less consistently defined, the currently accepted epidemiological definition is having menses in the past 12 months with changes in regularity or no menstrual cycle in the past 3–11 months (Brambilla *et al*., 1994; Cooper and Baird, 1995). However, some studies have classified women as postmenopausal following only 6 months of amenorrhea. These women would be classified as perimenopausal by other investigators. Some studies combine pre- and perimenopausal or peri- and postmenopausal women for analysis.

An additional consideration is the distinction between surgical and natural menopause. Surgical menopause is defined as occurring when a surgical procedure stops menstruation. Women who have had a bilateral oophorectomy or a hysterectomy with or without removal of the ovaries are generally included in this category. Women who have a surgical menopause tend to be younger, of poorer health, and use health care more frequently (Roos, 1984; Kjerluff *et al*., 1993; Brett *et al*., 1997). Their menopause experience is also quite different, in that they experience more sudden hormonal changes, as well as a surgical procedure. A number of studies have found that women who have had a surgical menopause report more distress than women who experience a natural menopause (McKinlay *et al*., 1987; Kanfert *et al*., 1992; O'Connor *et al*., 1995; Kuh *et al*., 1997). Sexual functioning may also be more affected in this group (Dennerstein *et al*., 1977), but well-controlled prospective studies are rare. Therefore, women experiencing a surgical menopause must be considered separately from those experiencing a natural menopause.

C. Measurement of Sexual Functioning and Mood

Another challenge in this area is the wide variation in the measurement of these variables. This is most pronounced in the area of sexual functioning, but is also seen in the area of mood. With respect to sexual functioning, there are no standard scales used across studies. Researchers often use study-specific questions that range from a single item or two (Bungay *et al*., 1980; Køster and Garde, 1993; Dennerstein *et al*., 1994a), a group of items or symptoms (Hunter *et al*., 1986; Hunter, 1990), or several domains of sexual functioning (Hällström, 1977; Osborn *et al*., 1988; Dennerstein *et al*., 1997; Avis *et al*., in press). These same problems occur in the area of mood. Some studies use single symptoms from a symptom list such as feeling blue or depressed (Avis *et al*., 1993) or irritable (Jaszman *et al*., 1969; Boulet *et al*., 1994), while others studies use a set of symptoms derived from factor analyses of symptom lists that are labeled depressed mood or dysphoria (Dennerstein *et al*., 1993; Collins and Landgren, 1995) or psychological symptoms (Porter *et al*., 1996; Kuh *et al*., 1997). While still other studies have used a standard questionnaire such as the Center for Epidemiologic Studies Depression Scale, known as the CES-D (McKinlay *et al*., 1987; Avis *et al*., 1994; Woods and Mitchell, 1997). Clearly, one needs to be careful in comparing studies when such different outcomes are used.

Further, for those studies that use an index or scale consisting of several different aspects of sexual functioning or mood states, it is not possible to examine the impact of age or menopause on specific aspects of sex or mood. Use of factor scales with different symptoms or moods does not allow one to make distinctions among moods or aspects of sexual functioning, making it difficult to tease out specific changes associated with menopause.

D. Limitations of Cross-Sectional Research

The vast majority of research in this area is cross-sectional and therefore can look only at associations (real or spurious). Longitudinal cohort designs facilitate those associations that are most likely to reflect a cause–effect relationship, through observations of temporal sequences in events or rate changes. While cross-sectional research can point to associations, it is not possible to determine the causal ordering (McKinlay and McKinlay, 1973). Cross-sectional studies can neither control for premenopausal characteristics of women nor separate effects of aging from those of menopause. This is particularly problematic in establishing the relationship among variables.

Related to the difficulties of cross-sectional research is the problem of univariate analyses or incomplete models that do not control for important variables in analyses that are also related to the outcome of interest. For example, research on menopause and sexual functioning often does not control for other variables that also change with age, such as health and partner limitations. Models that do not include vasomotor symptoms and sleep cannot tease out direct versus indirect effects of menopause.

III. Sexual Functioning

It is generally recognized that sexual behavior changes with age. In particular, frequency of sexual activity declines with age (Kinsey *et al*., 1948, 1953; Pfeiffer *et al*., 1972; Laumann *et al*., 1994). Reasons for this decline among women include loss of or lack of a partner, partner's sexual problems, health changes, and physiological changes due to changing hormonal levels. The impact of menopause on declines in sexual functioning is of particular interest because of decreases in ovarian hormones at this time (which may be directly or indirectly related to aspects of sexual functioning) and because menopause can be viewed as a marker in the aging process. There is much debate, however, over the relative impact of menopause on sexual functioning. Much of the research in this area is based on patient samples, or women who are having sexual problems. Furthermore, changes in ovarian hormones often lead to vasomotor symptoms, sleep disruption, and possibly mood changes. All of these factors may also impact sexual functioning. Thus, any attempt to understand the role of menopause per se on sexual functioning needs to take into account these factors.

A. Aspects of Sexual Functioning

In general, sexual functioning is studied in terms of satisfaction, frequency of activity (intercourse, masturbation, orgasm),

desire (including interest and sexual thoughts or fantasies), arousal, attitudes toward sexuality, and difficulties such as pain during intercourse and problems reaching orgasm. These reflect the characterization of sexual functioning in terms of libido and potency (Davidson, 1985; Iddenden, 1987). Libido includes sexual interest, desire, drive, motivation, and pleasure. Potency is the physiologically measurable event during sexual arousal/activity—the sexual response (Masters and Johnson, 1966). Although postmenopausal declines in ovarian hormone production and reproductive atrophy may increase the incidence of dyspareunia and vaginal dryness (potency), it is less clear how menopause affects sexual interest or libido (Davidson, 1985).

B. Sexual Functioning and Age

The earliest studies of normative sexual behavior were conducted about 50 years ago by Kinsey and his colleagues (1948, 1953). While the Kinsey samples were volunteers and not randomly selected or population-based, much of their pioneering work still holds up today. Kinsey and his colleagues interviewed large numbers of men and women over a period of 15 years. The majority of their samples were between the ages of 16–25, while approximately 2600 women were between 26 and 60. Kinsey and colleagues found a decline with age in incidence and frequency of marital coitus and of coitus to the point of orgasm. However, they did not find a decline in women's solitary activity until well after 60 years of age. For men, they found impotence to be an age-dependent disorder with a prevalence of 1.9% at age 40 years and 25% at age 65 years.

Subsequent work by Pfeiffer and colleagues (1972; Pfeiffer and Davis, 1972) also showed a decline in sexual interest with age in their sample of men and women ages 46–71, but the reasons for this decline differed by gender. When those who had stopped having sexual relations were asked why, Pfeiffer *et al*. (1972) found that men were most likely to report that they were unable to perform sexually. On the other hand, women reported that cessation of sexual relations was due to the death of a spouse, illness of spouse, or spouse unable to perform sexually. Only 10% of women reported that cessation of activity was due to their own illness, loss of interest, or inability to perform sexually. Pfeiffer and Davis (1972) conducted multiple regression analyses of factors related to sexual interest, enjoyment, and frequency of intercourse for men and women. For men, the primary factor related to these aspects of sexual functioning was age, with older men reporting lower levels of all outcomes. Age was followed in importance by present health. For women, the primary factor related to sexual functioning was marital status, with married women reporting higher levels of these outcomes. Also related were age and education. For women, health was not related to sexual functioning. Being postmenopausal did contribute somewhat to lower sexual interest and frequency, but not to enjoyment.

These early results suggest that while age impacts sexual functioning for both men and women, the reasons for this may differ.

Perhaps the largest population-based survey of sexual behavior is the National Health and Social Life Survey (NLSLS) conducted in 1992 by researchers at the University of Chicago (Laumann *et al*., 1994). This in-person interview of a national probability sample of 1410 men and 1749 women ages 18–59 included an extensive set of questions on sexual function and dysfunction. The NLSLS confirms earlier findings of frequency of sex declining with age. This decline appears to begin in the late 30s and is greater for women than men. For both men and women, only a small percentage reported that sex was not pleasurable and this did not vary much by age (although after age 35, fewer women said that sex was not pleasurable). In additional analyses of the NHSLS, Laumann *et al*. (1999) found that lack of interest in sex significantly increased with age for men, but not for women.

Laumann *et al*. (1999) further examined aspects of sexual dysfunction and factors related to dysfunction. Interestingly, pain during sex, which is often associated with menopause, was lowest in the women ages 50–59. However, trouble lubricating increased with age and was highest among the 50–59 age group. For women, the significant factors related to lack of interest in sex were emotional problems or stress, a decrease in household income, and ever having a sexually transmitted disease. This study is noteworthy for its large and representative sample. Unfortunately the study sample stops at age 59. Further, the focus is mostly on dysfunction, rather than function.

C. Sexual Functioning and Menopause

1. Research on Menopause Status

Research among general populations of women does not show clear associations between menopause and declines in sexual functioning. While some studies have found lower sexual interest among peri- or postmenopausal women as compared to premenopausal women (Hällström, 1977; Hunter *et al*., 1986; Dennerstein *et al*., 1994a; Cawood and Bancroft, 1996), other studies have not found an association between menopause status and sexual functioning (Osborn *et al*., 1988; Køster and Garde, 1993; Hawton *et al*., 1994; Dennerstein *et al*., 1997). In a study of 567 Swedish women ages 38, 46, 50, and 54 randomly selected from the general population, Hällström (1977) found progressive decline in sexual interest across menopausal stages over and above the effects of age. Compared to pre- and perimenopausal women, postmenopausal women reported lower sexual interest in the previous 5 years. In a cross-sectional survey of 682 women ages 45–56 recruited from a general ovarian screening clinic, Hunter *et al*. (1986) also found that peri- and postmenopausal women reported lower sexual interest than premenopausal women, but sexual satisfaction did not differ by menopause status. Dennerstein *et al*. (1994a) report cross-sectional results of a large ($n = 1879$) population-based sample of women ages 45–55 in Melbourne, Australia. They asked women to report whether in the past 12 months there had been any change in sexual interest. While the majority of women reported no change in sexual interest, menopause status was related to declining interest, with postmenopausal women most likely to report less interest. Postmenopausal women were more likely to report unusual pain on intercourse. Cawood and Bancroft (1996) report results of 141 women ages 40–60 from Edinburgh where they found that postmenopausal women reported

a significantly greater loss of interest in sex. However, there was no correlation between menopause status and orgasm.

On the other hand, Osborn *et al*. (1988) did not find an association between menopause status and sexual dysfunction among 436 women ages 35–59 recruited from two general practices in Oxford England. Sexual interest was considered part of a general measure of sexual dysfunction including impaired interest in sexual activity, frequency of orgasm, dyspareunia, and vaginal dryness. In their sample of 472 Danish women ages 51, Køster and Garde (1993) did not find an association between menopause status and sexual desire in multivariate regressions.

Hawton *et al*. (1994) found no association between menopause status and frequency of sexual activity, frequency of orgasm, or satisfaction in an age-matched comparison of pre- and postmenopausal British women who had partners. In their smaller cohort study ($n = 200$), Dennerstein *et al*. (1997) looked at six aspects of sexual functioning: feelings for partner, sexual responsivity, frequency of sexual intercourse, libido, partner problems, and vaginal dryness. They found no significant differences on any of these variables by menopause status.

The Massachusetts Women's Health Study, one of the most comprehensive community-based studies of women transitioning through the menopause, found that menopause status was significantly related to lower sexual desire, a belief that interest in sexual activity declines with age, and women's reports of decreased arousal compared to when in their 40s (Avis *et al*., in press). Menopause status was unrelated to frequency of sexual intercourse, satisfaction with one's sexual relationship, difficulty reaching orgasm, and pain during or after intercourse in either unadjusted or multiple regression analyses.

In general, satisfaction with one's sexual relationship has not been found to be related to menopause or age (Hunter *et al*., 1986; Hawton *et al*., 1994; Avis *et al*., in press). There are at least three possible explanations for this: (1) frequency of activity is not related to satisfaction, (2) people accommodate to the age-related declines that occur, and (3) people's expectation with respect to sexual activity or desire declines with age.

The vast majority of research in this area is cross-sectional. McCoy and Davidson (1985) conducted the first longitudinal study of sexual activity among perimenopausal women in which perimenopausal women were followed at 4-month intervals until they were postmenopausal (one year without cycling). During this time, hormone levels and weekly rates of intercourse were measured. Results showed that following menopause, the women reported fewer sexual thoughts or fantasies, more lacked vaginal lubrication, and they were less satisfied with their partners than prior to menopause. Unfortunately, while 39 women began the study, their final sample size was only 16. As McCoy and Davidson point out, not only was the remaining sample extremely small, it was also potentially quite biased. Hunter (1990) followed 36 postmenopausal women who had been recruited from an ovarian screening clinic for 3 years. All women became peri- or postmenopausal during this time. She found some change in sexual behavior over the 3 years, but these differences were not statistically significant. Unfortunately, her small sample may not have provided enough power to detect statistically different changes. Thus, both of these prospective studies suffer from small and potentially biased samples. Further, they do not account for potential age-related changes.

Taken as a whole, these studies suggest that menopause may have an impact on some aspects of sexual functioning, but not others. Some inconsistencies in findings can be explained by the wide variation in the specific sexual functioning questions that are asked, the time frame used (e.g., past month, past year, etc.), whether women without partners are included in analyses, and nature of the study sample. Further, many of these studies only look at univariate associations and do not adjust for other variables in the analyses that may be related to sexual functioning (e.g., age, partner problems, health).

2. Factors Other Than Menopause Status

Psychosocial and aging factors are often reported as more important determinants than ovarian function of sexual functioning among middle-aged women (Leiblum *et al*., 1983; Hagstad, 1988; Køster and Garde, 1993; Hawton *et al*., 1994). Some of these factors include the availability of a partner (Pfeiffer *et al*., 1972; Leiblum *et al*., 1983; Køster and Garde, 1993), previous sexual behavior and enjoyment (Christensen and Gagnon, 1965; Pfeiffer *et al*., 1972), the marital relationship (Clark and Wallin, 1965; Bachmann *et al*., 1985; Hawton *et al*., 1994; Cawood and Bancroft, 1996), mental health (Dennerstein *et al*., 1994a; Hawton *et al*., 1994; Avis *et al*., in press), general physical health (Hunter, 1990; Køster and Garde, 1993), stress (Hunter, 1990), expectations (Køster and Garde, 1993), and male partner problems (Pfeiffer *et al*., 1972).

Hawton *et al*. (1994) found that psychiatric status and marital adjustment were variables most related to sexual functioning. Cawood and Bancroft (1996) found that quality of the marital relationship was quite strongly related to frequency of sexual intercourse and quality of sexual activity and that menopause status was not related in multivariate analysis. In multivariate models, Dennerstein *et al*. (1994a) found that declining sexual interest was significantly associated with lower emotional well-being; not working full-time; greater vasomotor, cardiopulmonary, and skeletal symptoms; and use of hormone replacement therapy, as well as menopause status. Van Keep and Kelherhals (1974) reported a significant association between social factors and sexual behavior. Koster and Garde (1993) found that significant predictors of low sexual desire were low frequency of sexual intercourse, being single, poor physical fitness, lower social status, and anticipation of decreased sexuality measured at age 40.

In the Massachusetts Women's Health Study (MWHS), Avis *et al*. (in press) found that health was a significant variable related to all aspects of sexual functioning. Depression and greater psychological symptoms were related to lower satisfaction, frequency, and desire. Interestingly, smoking was related to less desire and lower frequency of sexual intercourse. This finding is consistent with results reported by Greendale *et al*. (1996) and are consistent with other research on the negative effects of smoking on sex steroid levels (Johnnton *et al*., 1983; Merchnovitz and Bradlow, 1990).

3. Sexual Functioning and Hormones

If changes in sexual functioning and activity are directly related to menopause, one would expect to find an association

between sexual functioning and hormones, particularly estradiol, which significantly decreases during the menopause transition. While only a few studies have examined sexual functioning and hormones, they consistently do not find a relation between estrogen and sexual functioning (Leiblum *et al*., 1983; Bachmann *et al*., 1984, 1985; McCoy *et al*., 1985; Cawood and Bancroft, 1996; Dennerstein *et al*., 1997; Avis *et al*., in press). Some of these studies, however, look at frequency of sexual intercourse (Leiblum *et al*., 1983; McCoy *et al*., 1985), which may be a poor indicator of sexual functioning for women, since frequency of activity is often determined more by the male partner. The one exception to these negative findings was a study by Cutler *et al*. (1987) who found that a subset of perimenopausal women with especially low estradiol levels (< 35 mg/pl) had reduced coital frequency. However, their outcome was frequency of intercourse.

On the other hand, studies that have looked at other aspects of sexual functioning such as desire or interest also do not find an association with hormone levels (Bachmann *et al*., 1984, 1985; Cawood and 1996; Dennerstein *et al*., 1997; Avis *et al*., in press). In the MWHS, pain was the only aspect of sexual functioning that was related to E_2. Others have also found that lowered estrogen levels have been associated with vaginal dryness (Hutton *et al*., 1979; Morrel *et al*., 1984; Sarrel, 1987; Dennerstein *et al*., 1997).

Two small studies have looked at physiologic signs of arousal by using vaginal pulse amplitude in response to erotic films to compare physiologic response among pre- and postmenopausal women (Myers and Morokoff, 1986; Myers *et al*., 1990). Myers and Morokoff (1986) found no diminished vasocongestive response in postmenopausal women with low estradiol levels compared to other postmenopausal women on hormone replacement therapy and to premenopausal women. They also found no correlation between estradiol level and vaginal pulse amplitude, although estradiol level was significantly correlated with subjective reports of greater vaginal lubrication. Myers *et al*. (1990) found that hormone treatment did not significantly alter psychophysiologically measured sexual arousal or sexual behaviors.

In general, these studies suggest that elevated estrogen levels are not an important factor in libido or potency, but are related to levels of vaginal lubrication. Thus, while hormone levels affect vaginal dryness and dyspareunia, they do not appear directly related to sexual drive or interest.

Unfortunately, most of the studies measuring estrogen levels do not take into account the availability of E_2 to target tissues. For example, it has been well-established that body weight is correlated with circulating levels of estrone and estradiol (Judd *et al*., 1976; Vermeulen and Verdonck, 1978) and also influences the availability of E_2 to target tissues by an inverse relationship to the circulating concentration of sex-hormone blinding globulin (Nisker *et al*., 1980). Thus, univariate analyses of estrogen and sexual functioning may not be expected to yield significant results.

A number of researchers have argued that androgens, and not estrogens, are the relevant hormone in relation to sexual functioning. Investigators have found that androgens maintain sexual interest after surgical menopause, while estrogens do not (Sherwin and Gelfand, 1987; Sherwin, 1991). Most of this work has been conducted among women who have an oophorectomy and are given supraphysiologic levels of the hormone. Two recent, well-designed studies of sexual functioning and testosterone within normal physiologic ranges show inconsistent results. In their study of 40 postmenopausal women followed for 10 weeks, Myers *et al*. (1990) found a significant increase in pleasure from masturbation among women who received estrogen plus testosterone, compared to those who received estrogen alone or estrogen plus medroxyprogesterone acetate. However, there were no group or treatment effects in either pleasure from sexual activity or sexual desire. Davis *et al*. (1995) found that among 34 postmenopausal women randomly assigned to either estradiol or estradiol plus testosterone implant (with considerably higher levels of testosterone than Myers *et al*., 1990), the estradiol plus testosterone group showed significantly greater improvement for sexual activity, satisfaction, pleasure, and orgasm, but not for libido or fantasies. These effects tended to decrease at 24 months.

Only a few studies have examined the relation between natural androgens and sexual functioning (McCoy *et al*., 1985; Cawood and Bancroft, 1996; Dennerstein *et al*., 1997) and none of these studies found an association. In fact, Dennerstein *et al*. (1997) found only weak evidence for androgen involvement (FAI with libido), but in a negative direction. In their small longitudinal study, McCoy and Davidson (1985) did find a relation between coital frequency and testosterone, but as previously mentioned, their sample was small and potentially biased since it was based on only 16 of an initial 39 women. In their cross-sectional analyses of the full sample of 43 women, they did not find an association between testosterone and frequency of intercourse (McCoy *et al*., 1985). These differences could be due to sample differences or problems with using frequency of sexual activity as a measure. A review by Campbell and Udry (1994) concluded that while some amount of testosterone is important to maintain sexual motivation, there is no evidence that within the normal range of testosterone, there is an association between testosterone and libido.

4. Summary

In conclusion, menopause appears to have some impact on sexual functioning, which is as much psycho-social-cultural, as biological. Estradiol is primarily related to vaginal dryness and pain, but not libido. Studies of natural androgens have not yet demonstrated a significant effect. Other factors (e.g., health, partner availability, partner limitations, etc.) appear to have greater impact on sexual functioning than menopause.

It is important to point out that most studies of the impact of menopause on sexual functioning are conducted among women who are newly postmenopausal. It is possible that the effects of declines in ovarian functioning do not impact sexual functioning until later. We have very little data on sexual functioning among the general population of women in their 60s and 70s.

IV. Hot Flashes

Vasomotor symptoms such as hot flashes (or flushes) and night sweats are potential mediators of the association between

menopause and sexual activity. Women describe a hot flash as a sensation of heat, primarily on the upper body, the chest, neck, face, and scalp. A hot flash is generally accompanied by a flushing of the skin, primarily on the upper body, and by sweating. Often there is an increase in heart rate, sometimes experienced as palpitations (Kronenberg, 1990). Longitudinal data from the MWHS show that hot flashes are often most prevalent in the perimenopausal period and peak prior to the last menstrual cycle (McKinlay *et al*., 1992). Hot flushes typically last from 0.5 to 5.0 years after natural menopause, but may persist longer (Bachmann, 1990). Estimates of the incidence of hot flashes from population studies in United States and worldwide show huge variability with ranges from 24 to 93% of women (Kronenberg, 1990).

It is well-established that hot flashes are generally associated with declining estrogen levels (Kronenberg, 1990). Data show that women who report hot flashes, on average, have lower circulating estrogen levels than women who are asymptomatic (Erlik *et al*., 1982). However, there is not a one-to-one association between serum estrogen levels and the onset of hot flashes and the actual cause(s) of hot flashes remains speculative (Bachmann, 1990). For example, vasomotor flashes do not occur in prepubertal girls whose estrogen levels are low, there is no particular threshold level of serum estradiol below which hot flashes always occur. Many women experience a decrease in estrogen yet do not experience hot flashes. Hot flashes do tend to occur when there is an abrupt and significant drop in estrogen level. For example, hot flashes often occur in pre- or perimenopausal women soon after chemotherapy or an ovariectomy, and similarly they often occur in men soon after orchiectomy for the treatment of prostate cancer (Kronenberg, 1990). While hot flashes have been associated with a pulse of luteinizing hormone (Casper and Wilkes, 1979; Tataryn *et al*., 1980), there is not a causal association as hot flashes can occur in women who have no episodic LH release, such as after hypophysectomy (Mulley *et al*., 1977). Further, Casper and Wilkes (1979) found that the increase in LH occurred after the onset of symptoms, thus suggesting it is not responsible for the hot flashes. Other hormones found to increase in association with hot flashes include β-endorphin, β-lipotropin, adrenocorticotropic hormone, cortisol (Genazzani, *et al*., 1984; Meldrum *et al*., 1984), and epinephrine (Kronenberg *et al*., 1984).

For many women, hot flashes may be occasional, mild, transient sensations of warmth. Others may experience frequent night sweats that disrupt sleep and increase fatigue and irritability. Erlik *et al*. (1981) demonstrated a significant correlation between the occurrence of hot flashes and waking episodes among menopausal women as compared to premenopausal women. Hot flashes and night sweats may be related to sexual functioning in several ways: the discomfort of hot flashes might inhibit sexual behavior, sleep disturbances resulting from night sweats could lead to fatigue and irritability which may in turn affect sexual behavior, regular sexual activity might protect against lowered steroid levels, and/or hot flashes and sexual desire may both be hormonally related. Unfortunately, this area has not been well studied.

Correlational data have shown that regular weekly sexual behavior is highly associated with the absence of hot flashes (Cutter *et al*., 1983; McCoy *et al*., 1985). As previously mentioned, however, frequency of sexual intercourse is not a good indicator of desire or interest for women. In the Massachusetts Women's Health Study, we explored the association between hot flashes and three measures of sexual activity: satisfaction, frequency of intercourse, and arousal among 206 women who had a sexual partner (Johannes *et al*., 1995). While in univariate analysis, hot flashes were related to sporadic frequency of sexual intercourse, in multivariate analysis, the only significant predictors of frequency were partner limitations, recent pap test, and estrogen use. On the other hand, hot flashes were found to be significantly related to lowered sexual satisfaction and arousal, adjusting for estrogen use, partner limitations, and other health-related covariates. In longitudinal analyses, hot flashes at one interview were related to decreased satisfaction and arousal 1 year later, but were unrelated to frequency. Estradiol levels were unrelated to hot flashes or sexual activity. These results suggest that hot flashes may be related to lowered sexual satisfaction and arousal and may be independent of lowered estrogen levels.

Unfortunately, the majority of research in the area of sexual functioning has not included vasomotor symptoms in multivariate models. Clearly, future research needs to take these symptoms into consideration when studying the impact of menopause and or hormonal changes and sexual functioning. Research on vasomotor symptoms and mood is reviewed in the next section.

V. Mood

A. Cross-Sectional Research

Numerous cross-sectional studies have examined the association between menopause status and mood. Most of these studies do not find a relation between menopause status and depression or dysphoric or negative mood, using such measures as the CES-D (McKinlay *et al*., 1987; Kaufert *et al*., 1992; Porter *et al*., 1996; Woods and Mitchell, 1997), a psychiatric interview (Hällström and Samuelsson, 1985; Gath *et al*., 1987), dysphoric mood (Dennerstein *et al*., 1993), negative affect (Dennerstein *et al*., 1994b), nervousness and mood lability (Holte and Mikkelsen, 1991), anxiety/fears (Hunter *et al*., 1986), psychological symptoms (Porter *et al*., 1996; Kuh *et al*., 1997), and individual symptoms of irritability, anxiety, and/or depression (Ballinger, 1976; Cawood and Bancroft, 1996).

On the other hand, several studies have found a relation between menopause status and mood, mostly in the perimenopause (Ballinger, 1975; Hunter *et al*., 1986; Avis *et al*., 1993; Collins and Landgren, 1995). Hunter *et al*. (1986) found higher rates of depressed mood (including loss of interest in things, lack of enjoyment, feeling miserable and sad, no feelings of well-being, life not worth living, irritability, and loss of appetite) among peri- and postmenopausal women in a sample of 682 women ages 45–65 recruited from an ovarian screening clinic. They did not find a relation between menopause status and symptoms labeled anxiety/fears or sleep problems. While their sample was not recruited from a menopause clinic, it is a clinic sample, and not a population-based sample.

Avis *et al*. (1993), conducted an analysis of responses to feeling blue or depressed by menopause status among community-based samples of women in the United States, Japan, and Canada. They found that for women in the United States sample, peri- and surgical menopausal women reported higher rates of ''feeling blue or depressed'' in the past 2 weeks than pre- or postmenopausal women. There was no relation between status and symptom reporting for the Canadian or Japanese samples.

In a large Swedish sample of 1324 women, all age 48, Collins and Landgren (1995) found higher rates of negative mood (including tension, depression, spells of crying, early wakening, insomnia, and difficulty concentrating) among postmenopausal women. Collins and Landgren defined postmenopausal as 6 months amenorrhea and therefore this category of women probably included some women who would be classified as peri-menopausal, using the WHO criteria. In multivariate analysis, only vasomotor symptoms were significantly related to status. They did not analyze the group of perimenopausal women, which was quite small.

Thus, while the majority of cross-sectional studies do not find a significant association between menopause status and various measures of mood disturbances, several studies have reported slight increases among perimenopausal women. These studies, however, generally have not controlled for vasomotor symptoms. The one study that did control for symptoms found that the association disappeared when vasomotor symptoms was included in the model (Collins and Landgren, 1995).

B. Prospective and Longitudinal Studies

While, the majority of studies in this area have been cross-sectional, several prospective or longitudinal studies have been conducted (Hällström and Samuelsson, 1985; Hunter, 1990; Matthews *et al*., 1990; Holte, 1992; Kaufert *et al*., 1992; Avis *et al*., 1994; Woods and Mitchell, 1996; Kuh *et al*., 1997). Each of these studies analyzes their longitudinal data somewhat differently. Neither Kaufert *et al*. (1992) nor Woods and Mitchell (1996) found that the onset of menopause was related to increased depression using the CES-D scale. The Pittsburgh Healthy Women study compared psychological characteristics and symptoms between pre- and postmenopausal women, after 3 years of follow-up of a sample of 541 initially premenopausal women. Controlling for age and baseline levels of characteristics, they found that natural menopause did not adversely affect anxiety or depression (Matthews *et al*., 1990). Hällström and Samuelsson (1985) conducted a psychiatric interview on 899 women from the general population in Sweden, on two occasions, 6 years apart. They found no increase in onset of mental disorder at menopause. Holte (1992) reports a longitudinal follow-up of 59 women from his cross-sectional study who were premenopausal at the beginning of the study and postmenopausal at its end, 4 years later, and not taking hormone replacement therapy. Holte did not find an increase in anxiety or depression as measured by Goldberg's General Health Questionnaire.

Kuh *et al*. (1997) surveyed a nationally representative sample of over 1200 British women born in 1946 when they were 36 years of age and again at age 47. Psychological symptoms at age 47 were unrelated to natural menopause status, except for a slight rise in irritability among perimenopausal women. However, women who had had a hysterectomy or were on hormone replacement therapy reported significantly more psychological symptoms. They further found that psychological symptoms at age 47 were strongly related to current family life and work stress, anxiety, depression, and health problems at age 36.

Two exceptions to these findings are reported by Hunter (1990) and Avis *et al*. (1994). Using data from the Massachusetts Women's Health Study, Avis *et al*. (1994) addressed the effect of change in menopause status on depression as measured by the CES-D scale, while controlling for prior depression. To study change in menopause status, a menopause transition variable was created that took into account a woman's menopausal status at the two time points (27 months apart) at which depression was measured (referred to as T_1 and T_2). Across all menopause statuses, those women who were classified as depressed at T_1 had higher rates of depression at T_2. For women who were not depressed at T_1, the rate of depression at T_2 increased slightly as women moved from pre-premenopausal to pre-perimenopausal, and was highest for women who remained perimenopausal for at least 27 months. The rate of depression began to decrease as women moved from peri- to postmenopause, and was lowest for those women who were postmenopausal for at least 27 months. Controlling for premenopausal depression, there was still a slight increase in depression among the peri-perimenopausal women.

In further analyses of the MWHS data, Avis *et al*. examined whether this increased rate of depression among the perimenopausal-perimenopausal women could be attributed to symptoms associated with menopause (i.e., hot flashes, night sweats, and menstrual problems). When menopausal symptoms was added to the regression model, it became a significant predictor of T_2 depression, and menopausal transition was no longer statistically significant. Over all menopause transition categories, those women who reported experiencing hot flashes, night sweats, and/or menstrual problems consistently showed higher rates of depression.

Hunter (1990) reports follow-up data on 36 women who were initially premenopausal and became peri- or postmenopausal 3 years later. While she found that the women reported significantly more depressed mood at follow-up, she did not have a comparison group to control for age and she did not control for vasomotor symptoms in analysis. There was no change in anxiety. She found no evidence of an increase in psychiatric caseness as defined by the General Health Questionnaire.

In summary, it thus appears that from longitudinal studies there is no evidence that onset of perimenopause leads to increased depression. One study that examined length of the perimenopause (Avis *et al*., 1994) did find a small increase in depression associated with a long perimenopause, which was not significant when vasomotor symptoms were included in the model.

C. Psychosocial and Health Factors and Prior History

Cross-sectional studies of depression and menopause have shown that psychosocial factors account for more of the varia-

tion in depressed mood among women at the time of menopause than does menopause itself (Ballinger, 1975; Greene and Cooke, 1980; Dennerstein, 1987; McKinlay *et al*., 1987; Holte and Mikkelson, 1991; Kaufert *et al*., 1992; Kuh *et al*., 1997; Woods and Mitchell, 1997). For example, Greene and Cooke (1980) conducted a detailed cross-sectional study of postmenopausal women in Glasgow and found that stressful life events involving exits from a woman's social network were associated with reports of psychological symptoms. These events accounted for a greater proportion of the variation in reports of psychological symptoms than did menopause status. In a survey of women ages 40–55 from general practitioner lists, Ballinger (1975) found that the death of a parent and changing patterns of relations with children were related to psychiatric morbidity. In a cross-sectional analysis of 2500 women from the MWHS, McKinlay *et al*. (1987) looked at the relative contributions of health and social circumstances, and menopause to depression. They found that the variables most associated with depression were lower education, marital status (widowed, divorced, and separated women have higher rates), physical health, and stress from worry about others. Other studies have also shown that socioeconomic status (Hunter *et al*., 1986; Holte and Mikkelsen, 1991), stress (Hunter, 1990), negative attitudes toward menopause and aging (Dennerstein *et al*., 1993; Kuh *et al*., 1997), and negative expectations of menopause (Holte and Mikkelsen, 1991) are related to more negative mood during menopause. Other studies have found that health is significantly related to depression (McKinlay *et al*., 1987; Kaufert *et al*., 1992; Dennerstein *et al*., 1994b; Woods and Mitchell, 1996).

In longitudinal research, Hunter (1990) found that negative stereotyped beliefs about the menopause and being under stress before the menopause, together with not working outside the home and being working class, were related to depression. Together, these factors accounted for 51% of the variation in depressed mood when these women became peri- or postmenopausal. Hällström and Samuelsson (1985) found that the weighted sum of life events, but not menopause, was associated with onset risk of mental disorder. Kaufert *et al*. (1992) found that the likelihood of becoming depressed was increased for women with areas of current stress in their lives—particularly if it was related to husbands or children.

Longitudinal data suggest that prior depression is the primary factor related to depression during menopause. In addition to the previously reported findings of Avis *et al*. (1994), Hunter (1990) also found that depressed mood during the menopause was most strongly predicted by premenopausal depression. In their prospective study, Kuh *et al*. (1997) found depression at age 36 was highly related to depression at age 47. In their cross-sectional study, Porter *et al*. (1996) found that depression was higher among those women who reported a past history of depression or anxiety.

Studies have also found that women who report greater mood disturbances at menopause also report menstrual cycle or reproductive related problems. These include previous or current premenstrual symptoms or complaints (Dennerstein *et al*., 1993; Stewart and Boydell, 1993; Collins and Landgren, 1995; Woods and Mitchell, 1996), dysmenorrhea (Collins and Landgren, 1995), and postpartum depression (Stewart and Boydell, 1993). Stewart and Boydell (1993) divided women attending a menopausal clinic into those experiencing high or low psychological distress. Those reporting high psychological distress were significantly more likely to report past psychiatric history, dysphoric premenstrual syndrome, and postpartum depression. Woods and Mitchell (1996) found that both a history of premenstrual syndrome and postpartum depression differentiated women with consistently depressed mood (measured at two points in time, 1 year apart) from those recovering from depressed mood or not depressed. Holte and Mikkelson (1991) found that women who reported experiencing greater depression during their menstrual periods earlier in life were more likely to report nervous complaints and mood lability at ages 45–55. Menopause status was unrelated to either nervous complaints or mood lability. It is not clear from these findings, however, whether this pattern of symptom reporting related to reproductive events is hormonally based or a result of greater symptom sensitivity or greater symptom reporting in general.

D. Endogenous Hormones and Mood

The most direct evidence of a relation between hormones and mood would come from studies concurrently measuring both hormones (specifically estrogen) and mood among menopausal women. Unfortunately, very few such studies exist.

Chakravarti *et al*. (1979) studied 82 premenopausal women seen at a menopause clinic. They found that while mean plasma estradiol concentrations were associated with vasomotor symptoms, there was no clear association between hormone levels and symptoms (including depression and irritability).

In 1987, Ballinger *et al*. studied hormone profiles and psychological symptoms in 85 perimenopausal women ages 40–55. Over half of their sample consisted of women attending a gynecology outpatient clinic. In general, they found no differences in hormone profiles of women with high or low psychological symptoms. However, they did find that among 18 women in the early postmenopause group (last menstrual period 1 to 4 years ago) those with higher symptom scores had significantly higher levels of estradiol than women with lower scores. The 48 women who were recruited from the gynecology clinic were interviewed for depression using a clinical interview. There were no significant differences in hormone profiles between the depressed and nondepressed groups.

Cawood and Bancroft (1996) concurrently measured mood (according to the multiple affect adjective checklist) and hormones among 145 women ages 40–60 recruited from the community. Women completed an initial interview and four subsequent interviews 1 week apart. They found no relation between estrone or estradiol and depression or positive affect plus sensation seeking (the only moods analyzed). However, they did find that feelings of tiredness were significantly related to depression and positive affect. Tiredness was also positively correlated with hot flushes.

In the MWSH, we are examining the relation between hormones (specifically E_2), symptoms, and depression among women traversing the menopause. Preliminary evidence does not suggest a relation between hormones and depression. However, experiencing hot flashes or night sweats or having trouble sleeping do appear related to depression.

While biochemical research on the neurobiology of estrogen and mood is an active area of investigation, there is insufficient evidence at this time to support a biochemical hypothesis for menopause and mood disturbances. While researchers have shown that estrogen can impact brain neurotransmitter activity in several ways, the majority of this research has been conducted in animals and the extent to which neurotransmitter activity interacts with menopause related mood changes has yet to be determined (Schmidt and Rubinow, 1991). There is no clear understanding of the mechanisms whereby hormonal changes mediate change in mood (Brace and McCauley, 1997). A review by Blehar and Oren (1995) on the psychobiology of depression concluded that biological factors (compared to genetic, environmental, and psychological factors) are the weakest in terms of what research can tell us about depression.

E. Summary

Epidemiologic studies of menopause and mood do not show consistent evidence of a relation between menopause and depression or other negative moods in the general population. This conclusion was also reached by other recent reviews of the literature on menopause and depression (Nicol-Smith, 1996; Pearlstein *et al*., 1997). Factor analyses of symptom lists have quite consistently shown that vasomotor symptoms load on a factor separate from psychological symptoms (Avis *et al*., 1999). Community- or population-based studies consistently show that in the general population of women, only a minority of peri- or postmenopausal women evidence signs of depression or other mood disturbance. While some studies have found more negative mood among perimenopausal women, it appears that the majority of mood disturbance seen at the time of menopause can be attributed to prior depression, vasomotor symptoms, or non-menopause-related factors such as health problems or social circumstances.

While there is no evidence that menopause is associated with increased mood disturbance on a population level, an unanswered question is whether some women may be more vulnerable to mood effects of hormonal changes. Several researchers have proposed that while menopause does not cause mood disturbances in all women, there may be a subgroup of women at higher risk for depression or other mood changes (Steiner, 1992; Charney, 1996; Brace and McCauley, 1997). It has been suggested that women with a history of premenstrual syndrome have an increased sensitivity to hormone changes (Bancroft and Bäckström, 1985) and that women with previous affective disorders that are cyclic or associated with reproductive events are at higher risk (Pearlstein *et al*., 1997). While the postpartum period is not always associated with depression, women with previous depression, a history of premenstrual dysphoric disorder, or a history of bipolar disorder are at increased risk for postpartum depression (Blehar and Oren, 1995). However, it is premature to conclude that this pattern is related to a hormonal imbalance. Other factors that may be related are coping style or a greater sensitivity to symptoms. Further, since most studies involve retrospective reporting of reproductive and psychiatric history, there is the inherent problem of selective recall among women experiencing problems at the time of the study.

VI. Conclusions

Menopause is thought to alter central nervous system function as manifested by hot flashes, impaired sexual functioning, mood changes, and sleep disturbances. However, only vasomotor symptoms such as hot flashes and night sweats have been clearly and consistently associated with menopause. Epidemiologic studies of menopause and sexual functioning show some impact of menopause on sexual functioning, but this does not appear directly related to changing hormones. Estradiol has been related to vaginal dryness and painful intercourse, but not other aspects of sexual function. Androgens are thought to be important for libido, but have not been shown to be related to sexual functioning and natural menopause.

Epidemiologic studies of menopause and mood do not show consistent evidence of a relation between menopause and depression or other negative moods in the general population. While menopausal symptoms are related to depression, correlational studies of mood and hormone levels have failed to show a relation between depressed mood and estrogen levels. Evidence suggests that the rate of hormonal changes may be the important regulatory variable, rather than absolute hormonal level (Schmidt and Rubinow, 1991), which may explain why associations between hormones and mood disorders and sexual functioning are stronger for women undergoing a bilateral oophorectomy than for naturally menopausal women. Further, while all women experience a decline in estrogen following menopause, not all women report sexual problems, vasomotor symptoms, or mood disturbances. These results suggest that other factors mediate the relation between hormonal level and these variables.

The domino theory of menopausal symptoms proposes that vasomotor symptoms lead to other effects such as sexual dysfunction and mood disturbances. In the area of sexual functioning this has not been well studied. However, with respect to mood, the domino theory has some support. A number of studies have found a strong association between vasomotor complaints and depressed mood both cross-sectionally (Holte and Mikkelsen, 1991; Hunter, 1992; Palinkas and Barrett-Connor, 1992; Oldenhave *et al*., 1993; Collins and Lundgren, 1995) and longitudinally (Holte, 1992; Hunter, 1992; Avis *et al*., 1994). These findings suggest that studies need to control for vasomotor symptoms in analyses of any direct effect of menopause on mood. However, many studies do not find a relation between menopause and mood, even when vasomotor symptoms have not been controlled for. Further, other studies suggest that not all women who have vasomotor symptoms experience mood disturbances. Future research should examine more closely whether asymptomatic women show fluctuations in mood.

Other theorist argue that other factors concurrent with menopause have a greater impact on sexual functioning and mood than menopause per se. In the area of sexual functioning, this theory has considerable support. Research has shown that general mental health and male partner's health and limitations affect a woman's sexual functioning. Research also provides consistent evidence that social circumstances have a strong effect on mood during the menopause transition. Studies that have included measures of stress or life changes have typically found that social factors are highly related to mood, and often more so than menopause status. Thus, there is strong support

for the social circumstances perspective that menopause per se is less responsible for mood effects than life events of middle-aged women.

There is much needed research in this area. Future research should examine the longer term impact of the decline in ovarian function among women, as well as the role of androgens. Most research has been conducted among newly menopausal women. The effects of decreased estrogen on sexual functioning may be pronounced as women age. The role of androgens in women who experience a natural menopause has barely been looked at. While androgens may not be linearly associated with sexual functioning, it is quite possible that women with particularly low levels of testosterone may have lower levels of libido. We need research to determine if women vary in their rate of testosterone decline and if this rate changes as a result of menopause. Longitudinal research of large samples that can control for multiple covariates is greatly needed.

Future research needs to focus on developing a better understanding of women who may be at risk for mood effects of the menopause transition and whether there is a pattern in response to periods of hormonal fluctuations. Are such effects due to abnormal biochemical changes resulting from hormonal fluctuations, a woman's general sensitivity to symptoms, or a coping style? Prospective research needs to follow women during their reproductive years to examine continuity with respect to responses to times of hormonal change.

Finally, we need to understand how these various changes relate to each other. Are changing hormones, vasomotor symptoms, and sleep disturbances independently related to sexual functioning and mood or is there a causal chain? Researchers need to begin to develop and test models of these associations.

References

Avis, N. E., Kaufert, P. A., Lock, M., McKinlay, S. M., and Vass, K. (1993). The evolution of menopausal symptoms. *Baillière's Clin. Endocrinol. Metab.* **7**(1), 17–32.

Avis, N. E., Brambilla, D., McKinlay, S. M., and Vass, K. (1994). A longitudinal analysis of the association between menopause and depression: Results from the Massachusetts Women's Health Study. *Ann. Epidemiol.* **4**, 214–220.

Avis, N. E., Crawford, S. L., and McKinlay, S. M. (1997). Psychosocial, behavioral, and health factors related to menopause symptomatology. *Women's Health: Res. Gender. Behav. Policy* **3**, 103–120.

Avis, N., Stellato, R., Crawford, S., and Johannes, C. (in press). Is there an association between menopause status and sexual functioning? *menopause*.

Bachmann, G. (1990). Sexual issues at menopause. *Ann. N. Y. Acad. Sci.* **592**, 87–94.

Bachmann, G. (1999). Vasomotor flushes in menopausal women. *Am. J. Obstet. Gynecol.* **180**, S312–316.

Bachmann, G., Leiblum, S., Kemmann, E., Colburn, D., Swartzmann, L., and Shelden, R. (1984). Sexual expression and its determinants in the post-menopausal woman. *Maturitas* **6**, 19–29.

Bachmann, G., Leiblum, S., Sandler, B., Ainsley, W., Narcessian, R., Shelden, R., and Hymans, H. (1985). Correlates of sexual desire in postmenopausal women. *Maturitas* **7**, 211–216.

Ballinger, C. B. (1975). Psychiatric morbidity and the menopause: Screening of general population sample. *Br. Med. J.* **3**, 344–346.

Ballinger, C. B. (1976). Psychiatric morbidity and the menopause: Clinical features. *Br. Med. J.* **1**, 1183–1185.

Ballinger, C. B., Browning, M., and Smith, A. (1987). Hormone profiles and psychological symptoms in peri-menopausal women. *Maturitas* **9**, 235–251.

Ballinger, S. E. (1985). Psychosocial stress and symptoms of menopause: A comparative study of menopause clinic patients and non-patients. *Maturitas* **7**, 315-327.

Bancroft, J., and Bäckström, T. (1985). Premenstrual syndrome: A review. *Clin. Endocrinol.* **22**, 313–336.

Blehar, M. C., and Oren, D. A. (1995). Women's increased vulnerability to mood disorders: Integrating psychobiology and epidemiology. *Depression* **3**, 3–12.

Boulet, M., Oddens, B., Lehert, P., Vemer, H., and Visser, A. (1994). Climacteric and menopause in seven South-East Asian countries. *Maturitas* **19**, 157–176.

Brace, M., and McCauley, E. (1997). Estrogens and psychological well-being. *Ann. Med.* **29**, 283–290.

Brambilla, D., McKinlay, S., and Johannes, C. (1994). Defining the perimenopause for application in epidemiologic investigations. *Am. J. Epidemiol.* **140**, 1091–1095.

Brett, K. M., Marsh, J. V. R., and Madans, J. H. (1997). Epidemiology of hysterectomy in the U. S.: Demographic and reproductive factors in a nationally representative sample. *J. Women's Health* **6**, 309–316.

Brown, J. R. W. C., and Brown, M. E. A. (1976). Psychiatric disorders associated with the menopause. *In* "The Menopause: A Guide to Current Research and Practice" (R. J. Beard, ed.). University Park Press, Baltimore, MD.

Bungay, G. T., Vessey, M. P., and McPherson, C. K. (1980). Study of symptoms in middle life with special reference to the menopause. *Br. Med. J.* **281**, 181–183.

Campbell, B., and Udry, J. (1994). Implications of hormonal influences on sexual behavior for demographic models of reproduction. *Ann. N. Y. Acad. Sci.* **706**, 117–127.

Casper, R., C, Y., and Wilkes, M. (1979). Menopausal flushes: A neuroendocrine link with pulsatile hormone secretion. *Science* **205**, 823–825.

Cawood, E., and Bancroft, J. (1996). Steroid hormones, the menopause, sexuality and well-being of women. *Psychol. Med.* **26**, 925–936.

Chakravarti, S., Collins, W. P., Thom, M. H., and Studd, J. W. W. (1979). Relation between plasma hormone profiles, symptoms, and response to estrogen treatment in women approaching the menopause. *Br. Med. J.* **1**, 983–985.

Charney, D. A. (1996). The psychoendocrinology of menopause in cross-cultural perspective. *Transcult. Psychiatr. Res. Rev.* **33**, 413–434.

Christensen, C., and Gagnon, J. (1965). Sexual behavior in a group of older women. *J. Gerontol.* **20**, 351–356.

Clark, A., and Wallin, P. (1965). Women's sexual responsiveness and the duration and quality of their marriage. *Am. J. Sociol.* **71**, 187–196.

Collins, A., and Landgren, B. (1995). Reproductive health, use of estrogen and experience of symptoms in perimenopausal women: A population-based study. *Maturitas* **20**, 101–111.

Cooper, G. S., and Baird, D. D. (1995). The use of questionnaire data to classify peri- and premenopausal status. *Epidemiology* **6**, 625–628.

Cutler, W., McCoy, N., and Davidson, J. (1983). Sexual behavior, steroids, and hot flashes are associated during the perimenopause. *Neuroendocrinology* **5**, 185.

Cutler, W., Garcia, C., and McCoy, N. (1987). Perimenopausal sexuality. *Arch. Sex. Behav.* **16**, 225–234.

Davidson, J. (1985). Sexual behavior and its relationship to ovarian hormones in the menopause. *Maturitas* **7**, 193–201.

Davis, S., McCloud, P., Strauss, B., and Burger, H. (1995). Testosterone enhances estradiol's effects on postmenopausal bone density and sexuality. *Maturitas* **21**, 227–236

Dennerstein, L. (1987). Depression in the menopause. *Obstet. Gynecol. Clin. North Am.* **4**, 33–48.

Dennerstein, L., Wood, C., and Burrows, G. (1977). Sexual response following hysterectomy and oophorectomy. *Obstet. Gynecol.* **49**, 92–96.

Dennerstein, L., Smith, A. M. A., Morse, C., Burger, H., Green, A., Hopper, J., and Ryan, M. (1993). Menopausal symptoms in Australian women. *Med. J. Aust*. **159**, 232–236

Dennerstein, L., Smith, A., Morse, C., and Burger, H. (1994a). Sexuality and the menopause. *J. Psychosom. Obstet. Gynecol*. **15**, 59–66.

Dennerstein, L., Smith, A. M. A., and Morse, C. (1994b). Psychological well-being, mid-life and the menopause. *Maturitas* **20**, 1–11.

Dennerstein, L., Dudley, E., Hopper, J., and Burger, H. (1997). Sexuality, hormones, and the menopausal transition. *Maturitas* **26**, 83–93.

Erlik, Y., Tataryn, V., Meldrum, D., Lomax, P., Bajorek, J., and Judd, H. (1981). Association of waking episodes with menopausal hot flashes. *JAMA, J. Am. Med. Assoc*. **245**, 1741–1744.

Erlik, Y., Meldrum, D., and Judd, H. (1982). Estrogen levels in postmenopausal women with hot flashes. *J. Obstet. Gynecol*. **59**, 403–407.

Gath, D., Osborn, M., Bungay, G., Iles, S., Day, A., Bond, A., and Passingham, C. (1987). Psychiatric disorder and gynaecological symptoms in middle aged women: A community survey. *Br. Med. J*. **294**, 213–218.

Genazzani, A., Petraglia, F., and Facchinetti, F. (1984). Increase of proopiomelanocortin-related peptides during subjective menopausal flushes. *Am. J. Obstet. Gynecol*. **149**, 775–779.

Greendale, G., Hogan, P., and Shumaker, S. (1996). Sexual functioning in postmenopausal women. The postmenopausal estrogen/progestin intervention (PEPI) trial. *J. Women's Health* **5**, 445–458.

Greene, J. G., and Cooke, D. J. (1980). Life stress and symptoms at the climacterium. *Br. J. Psychiatry* **136**, 486–491.

Hagstad, A. (1988). Gynecology and sexuality in middle-aged women. *Women Health* **13**, 57–80.

Hällström, T. (1977). Sexuality in the climacteric. *Clin. Obstet. Gynecol*. **4**, 227–239.

Hällström, T., and Samuelsson, S. (1985). Mental health in the climacteric: The longitudinal study of women in Gothenburg. *Acta Obstet. Gynecol. Scand.* **130** (Suppl.), 13–18.

Hawton, K., Gath, D., and Day, A. (1994). Sexual function in a community sample of middle-aged women with partners: Effects of age, marital, socioeconomic, psychiatric, gynecological, and menopausal factors. *Arch. Sex. Behav.* **23**(4), 375–395.

Holte, A. (1992). Influences of natural menopause on health complaints: A prospective study of healthy Norwegian women. *Maturitas* **14**, 127–141.

Holte, A. (1998). Menopause, mood and hormone replacement therapy: Methodological issues. *Maturitas* **29**, 5–18.

Holte, A., and Mikkelsen, A. (1991). Psychosocial determinants of climacteric complaints. *Maturitas* **13**, 205–215.

Hunter, M. S. (1990). Somatic experience of the menopause: A prospective study. *Psychosom. Med*. **52**, 357–367.

Hunter, M. S. (1992). The South-East England longitudinal study of the climacteric and postmenopause. *Maturitas* **14**, 117–126.

Hunter, M. S. Battersby, R., and Whitehead, M. (1986). Relationships between psychological symptoms, somatic complaints and menopausal status. *Maturitas* **8**, 217–228.

Hutton, J., Jacobs, H., and James, V. (1979). Steroid endocrinology after the menopause—A review. *J. R. Soc. Med*. **72**, 835–841.

Iddenden, D. (1987). Sexuality during the menopause. *Med. Clin. North Am*. **71**, 87–94.

Jaszmann, L., van Lith, N. D., and Zaat, J. C. A. (1969). The perimenopausal symtoms: The statistical analysis of a survey. *Med. Gynaecol. Sociol*. **4**, 268–277.

Johannes, C., Avis, N., Crawford, S., and Stellato, R. (1995). Are hot flashes related to decreased sexual activity in postmenopausal women. *Menopause* **2**, 272.

Johnston, C., Hui, S., Witt, R., Appledorn, R., Baker, R., and Longscope, C. (1983). Early menopausal changes in bone mass and sex steroids. *J. Clin. Endrocrinol. Metab.* **61**, 905.

Judd, H., Lucas, W., and Yen, S. (1976). Serum 17β estradiol and estrone levels in postmenopausal women with and without endometrial cancer. *J. Clin. Endocrinol. Metab*. **43**, 272.

Kaufert, P. A., Gilbert, P., and Tate, R. (1992). The Manitoba Project: A re-examination of the link between menopause and depression. *Maturitas* **14**, 143–155.

Kinsey, A., Pomeroy, W., and Martin, C. (1948). "Sexual Behavior in the Human Male." Saunders, Philadelphia.

Kinsey, A., Pomeroy, W., and Martin, C. (1953). "Sexual Behavior in the Human Female." Sanders, Philadelphia.

Kjerluff, K., Langenberg, P., and Guzinski, G. (1993). The socioeconomic correlates of hysterectomies in the United States. *Am. J. Public Health* **83**, 106–108.

Køster, A., and Garde, K. (1993). Sexual desire and menopausal developement. A prospective study of Danish women born in 1936. *Maturitas* **16**, 49–60.

Kronenberg, F. (1990). Hot flashes: Epidemiology and physiology. *Ann. N. Y. Acad. Sci*. **592**, 52–86.

Kronenberg, F., Côté, L., Linkie, D. M., Dyrenfurth, I., and Downey, J. (1984). Menopausal hot flashes: Thermoregulatory, cardiovascular, and circulating catecholamine and LH changes. *Maturitas* **6**, 31–43.

Kuh, D. L., Wadsworth, M., and Hardy, R. (1997). Women's health in midlife: The influence of the menopause, social factors and health in earlier life. *Br. J. Obstet. Gynaecol*. **104**, 923–933.

Laumann, E., Gagnon, J., Michael, R., and Michaels, S. (1994). "The Social Organization of Sexuality: Sexual Practices in the United States." University of Chicago Press, Chicago.

Laumann, E., Paik, A., and Rosen, R. (1999). Sexual dysfunction in the United States. *JAMA, J. Am. Med. Assoc*. **281**, 537–544.

Leiblum, S., Bachmann, G., Kemmann, E., Colburn, D., and Swartzmann, L. (1983). Vaginal astrophy in the post menopausal women: The importance of sexual activity and hormones. *JAMA, J. Am. Med. Assoc*. **249**, 2195.

Masters, W., and Johnson, V. (1966). "Human Sexual Response." Little Brown, Boston.

Matthews, K. A., Wing, R. R., Kuller, L. H., Meilahn, E. N., Kelsey, S. F., Costello, E. J., and Caggiula, A. W. (1990). Influences of natural menopause on psychological characteristics and symptoms of middle-aged healthy women. *J. Consult. Clin. Psychol*. **58**, 345–351.

McCoy, N., and Davidson, J. (1985). A longitudinal study of the effects of menopause on sexuality. *Maturitas* **7**, 203–210.

McCoy, N., Culter, W., and Davidson, J. (1985). Relationship among sexual behavior, hot flashes, and hormone levels in perimenopausal women. *Arch. Sex. Behav.* **14**, 385–394.

McKinlay, J. B., McKinlay, S. M., and Brambilla, D. (1987). The relative contributions of endocrine changes and social circumstances to depression in mid-aged women. *J. Health Soc. Behav.* **28**, 345–363.

McKinlay, S. M., and McKinlay, J. B. (1973). Selected studies of the menopause—A methodological critique. *J. Biosoc. Sci*. **5**, 533–554.

McKinlay, S. M., Brambilla, D., and Posner, J. (1992). The normal menopause transition. *Am. J. Hum. Biol*. **4**, 37–46.

Meldrum, D., DeFazio, J., Erlik, Y., Lu, J., Wolfsen, A., Carlson, H., Hershmen, J., and Judd, H. (1984). Pituitary hormones during menopausal hot flash. *Obstet. Gynecol*. **64**, 752–756.

Merchnovitz, J., and Bradlow, H. (1990). Dietary and pharmacological control of estradiol metabolism in humans. *Ann. N. Y. Acad. Sci*. **595**, 291.

Morrel, M., JM, D., CS, C., and Davidson, J. (1984). The influences of age and cycling status on sexual arousability in women. *Am. J. Obstet. Gynecol.* **148**, 66–71.

Morse, C., Smith, A., Dennerstein, L., Green, A., Hopper, J., and Burger, H. (1994). The treatment seeking women at menopause. *Maturitas* **18**, 3.

Mulley, G., Mitchell, J., and Tattersall, R. (1977). Hot flushes after hypophysectomy. *Br. Med. J*. **2**, 1062.

Myers, L., and Morokoff, P. (1986). Physiological and subjective sexual arousal in pre- and postmenopausal women taking hormone replacement therapy. *Psychophysiology* **23**, 283–292.

Myers, L., Dixen, J., Morrissette, D., Carmichael, M., and Davidson, J. (1990). Effects of estrogen, androgen, and progestin on sexual

psychophysiology and behavior in postmenopausal women. *J. Clin. Endocrinol. Metab.* **70**, 1124–1131.

Nicol-Smith, L. (1996). Causality, menopause, and depression: A critical review of the literature. *Br. Med. J.* **313**, 1229–1232.

Nisker, J., Hammond, G., Davidson, B., *et al*. (1980). Serum sex hormone-binding globulin capacity and the percentage of free estradiol in postmenopausal women with and without endometrial carcinoma. *Am. J. Obstet. Gynecol*. **138**, 637.

O'Connor, V. M., Del Mar, C. B., Sheehan, M., Siskind, V., Fox-Young, S., and Cragg, C. (1995). Do psycho-social factors contribute more to symptom reporting by middle-aged women than hormonal status? *Maturitas* **20**, 63–69.

Oldenhave, A., Jaszmann, L. J. B., Haspels, A. A., and Everaerd, W. T. A. M. (1993). Impact of climacteric on well-being. *Am. J. Obstet. Gynecol*. **168**, 772–780.

Osborn, M., Hawton, K., and Gath, D. (1988). Sexual dysfunction among middle aged women in the community. *Br. Med. J.* **296**, 959–962.

Palinkas, L. A.,and Barrett-Connor, E. (1992). Estrogen use and depressive symptoms in postmenopausal women. *Obstet. Gynecol*. **80**, 30–36.

Pansini, F., Albertazzi, P., Bonaccorsi, G., Calisesi, M., Campobasso, C., Zanotti, L., Bagni, B., and Mollica, G. (1994). The menopausal transition: A dynamic approach to the pathogenesis of neurovegetative complaints. *Eur. J. Obstet. Gynaecol. Reprod. Biol*. **57**, 103–109.

Pearlstein, M. D., Rosen, K., and Stone, A. B. (1997). Mood disorders and menopause. *Endocrinol. Metab. Clin. North Am*. **26**(2), 279–294.

Pfeiffer, E., and Davis, G. (1972). Determinants of sexual behavior in middle and old age. *J. Am. Geriatr. Soc*. **20**, 151–158.

Pfeiffer, E., Verwoerdt, A., and Davis, G. (1972). Sexual behavior in middle life. *Am. J. Psychiatry* **128**, 1262–1267.

Porter, M., Penney, G. C., Russell, D., Russell, E., and Templeton, A. (1996). A population based survey of women's experience of the menopause. *Br. J. Obstet. Gynaecol*. **103**, 1025–1028.

Roos, N. P. (1984). Hysterectomies in one Canadian province: A new look at risks and benefits. *Am. J. Public Health* **74**, 39–46.

Rose, L. (1977) What is menopause? *In* "The Menopause Book" (L. Rose, ed.), pp. 201–245. Hawthorne Books, New York.

Sarrel, P. (1987). Sexuality in the middle years. *Obstet. Gynecol. Clin. North Am.* **14**, 49–62.

Schmidt, P. J., and Rubinow, D. R. (1991). Menopause-related affective disorders: A justification for further study. *Am. J. Psychiatry* **148**, 844–852.

Sherwin, B. (1991). The impact of different doses of estrogen and progesting on mood and sexual behavior in postmenopausal women. *J. Clin. Endocrinol. Metab.* **72**, 336–343.

Sherwin, B., and Gelfand, G. (1987). The role of androgens in the maintenance of sexual functioning in oophorectomized women. *Psychosom. Med.* **49**, 397–409.

Steiner, M. (1992). Female-specific mood disorders. *Clin. Obstet. Gynecol*. **35**, 599–611.

Stewart, D. E., and Boydell, K. M. (1993). Psychologic distress during menopause: Associations across the reproductive life cycle. *Int. J. Psychiatry Med*. **23**, 157–162.

Tataryn, I., Meldrum, D., Frumar, A., LU, K., Judd, J., Bajorek, W., Chesarek, W., and Lomax, P. (1980). The hormonal and thermoregulatory changes in postmenopausal hot flashes. *In* "Thermoregulatory Mechanisms and their Therapeutic Implication" (B. Cox, P. Lomax, A. S. Milton, and E. Schonbaum, eds.), pp. 202–207. Karger, Basel.

van Keep, P., and Kelherhals, J. (1974). The impact of socio-cultural factors on symptom formation. *Psychother. Psychosom*. **23**, 251–263.

Vermeulen, A., and Verdonck, L. (1978). Sex hormones concentrations in postmenopausal women. Relation to obesity, fat mass, age and years post menopause. *Clin. Endocrinol*. **9**, 59–66.

Weideger, P. (1977) "Menstruation and Menopause." Delta, New York.

Woods, N. F., and Mitchell, E. S. (1996). Patterns of depressed mood in midlife women: Observations from the Seattle Midlife Women's Health Study. *Res. Nurs. Health* **19**, 111–123.

Woods, N. F., and Mitchell, E. S. (1997). Pathways to depressed mood for midlife women: Observations from the Seattle Midlife Women's Health Study. *Res. Nurs. Health* **20**, 119–129.

World Health Organization Scientific Group. (1996). Research on the menopause in the 1990's. *W. H. O. Tech. Rep. Ser.* **866**.

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Factors Influencing the Onset of Female Reproductive Senescence

Striking similarities exist between the mechanisms governing neuroendocrine and ovarian functions in the rat and in the human. Furthermore, age-related declines in reproductive cyclicity and fertility are also similar between the two species. Thus, the middle-aged female rat provides a relevant and practical model for studying the mechanisms controlling reproductive functions. Researchers have utilized aging rodent models to examine how advanced age affects the interactions between ovarian function and the neuroendocrine control of gonadotropin secretion. In young female rats, increased estradiol secretion by maturing follicles elicits a surge of luteinizing hormone on proestrus, which stimulates progesterone secretion by preovulatory follicles and induces ovulation the next morning. As the female rat ages, the magnitudes of the preovulatory surge of luteinizing hormone and associated progesterone secretion gradually decrease, serving as an early marker of the imminent loss of regular ovulatory cycles. The decline in the neuroendocrine regulation of luteinizing hormone secretion is associated with alterations in hypothalamic functions, involving both stimulatory and inhibitory pathways regulating gonadotropin-releasing hormone release. In addition, clear changes in the number and function of ovarian follicles occurs during aging, associated with declines in oocyte quality. Interestingly, the temporal onset of reproductive declines during aging can be modulated by factors such as exposure to ovarian steroid hormones, caloric intake, parity, and genetic influences. This chapter reviews previous and recent studies demonstrating that such extrinsic factors can significantly delay or advance the effects of aging on neuroendocrine and reproductive functions.

I. Introduction

The maintenance of normal reproductive function in mammals is dependent upon tightly balanced feedback interactions between the hypothalamus, pituitary, and gonads. It is clear from many studies in female rodents that age-related declines in reproductive competence are associated with not only alterations in the neural mechanisms regulating gonadotropin secretion, but also changes in pituitary and ovarian functions. Due to the highly interactive nature of the hypothalamic-pituitary-ovarian axis, it has been difficult to ascertain the initial locus at which such age-related changes occur. For example, just as aging-associated declines in the neural regulation of gonadotropin secretion surely influence ovarian function, it is also clear that altered gonadal steroid and peptide secretion can influence neuroendocrine aging. Indeed, studies by several investigators indicate that both intrinsic and extrinsic factors modulate the onset of reproductive senescence. This chapter will review our current understanding of age-related changes in hypothalamic, pituitary, and ovarian functions in the female rodent model, focusing on the influences of factors such as ovarian steroid exposure, parity, diet, and genetics on the progression of reproductive aging.

II. Female Rodents as a Model of Reproductive Aging

A. Neuroendocrine Regulation of Reproductive Functions

Although the ovulatory cycle of the female rat is relatively short (4 to 5 days long) compared to that of women, the basic interactions and mechanisms governing neuroendocrine and ovarian functions in the rat are in general similar to that in the human. Furthermore, age-related changes in reproductive cyclicity and fertility during the transition to reproductive senescence appear to be similar between the two species. Thus, the middle-aged female rat has provided investigators with a relevant, practical model in which to study the effects of aging on neuroendocrine and ovarian functions, and to examine the dynamic interactions between hypothalamic, pituitary, and ovarian functions.

In female rats of the Long-Evans strain, regular estrous cycles have a duration of 4 days (termed diestrus day 1, diestrus day 2, proestrus, and estrus) and repeat every 4 days in the absence of mating with a male. During each cycle, gonadotropins released from the pituitary stimulate the development,

growth, maturation, and steroidogenesis of ovarian follicles, and follicular growth is accompanied by a gradual, marked increase in estradiol (E2) secretion. On proestrus afternoon, high levels of plasma E2 act upon the neuroendocrine axis, triggering the hypothalamic release of gonadotropin-releasing hormone (GnRH), which stimulates the surge release of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary. Soon after initiation of the LH surge, rising plasma levels of LH stimulate ovarian expression of cytochrome P450 side chain cleavage (P450scc), the rate-limiting enzyme in progesterone (P4) biosynthesis (Strauss and Miller, 1991), in preovulatory follicles. This results in high circulating P4 levels on proestrus afternoon and evening, which facilitate and potentiate the preovulatory LH surge (Mahesh and Brann, 1992). In addition, the LH surge is essential in inducing ovulation of preovulatory follicles in the early morning of estrus. Under the luteinizing action of LH, the ruptured follicles form corpora lutea, which secrete substantial amounts of P4 during diestrus days 1 and 2 of the subsequent cycle. In addition to the preovulatory LH surge, the FSH surge on proestrus afternoon may also contribute to the processes of ovulation and luteinization of preovulatory follicles (Galway *et al*., 1990). The primary FSH surge on proestrus is followed immediately by a second increase in FSH release in the early morning of estrus. This secondary FSH surge is believed to be GnRH-independent and to play an important role in the selection and growth of preovulatory follicles for the next cycle (Hirshfield and De Paolo, 1981).

B. Characteristics of Reproductive Aging in Female Rodents

In the female rat, reproductive senescence is characterized by a gradual loss of regular ovulatory cycles and declines in fertility and fecundity (Ingram, 1959; Meites and Huang, 1976; Lu *et al*., 1979; Matt *et al*., 1987a). By 9–11 months of age, increasing numbers of multiparous rats (retired breeders) exhibit attenuated preovulatory LH surges on proestrus during regular estrous cycles (Cooper *et al*., 1980; Wise, 1982a; Nass *et al*., 1984), associated with a reduced rise in plasma P4, decreased ovulation rate, decreased fertility, and a declining reserve of ovarian follicles (Mandl and Shelton, 1959; Gray *et al*., 1980; Wise, 1982b; Matt *et al*., 1987a; LaPolt *et al*., 1998). Four to 6 weeks after exhibiting attenuated proestrous LH surges, middle-aged females begin to display lengthened, irregular estrous cycles characterized by a significant delay or absence of preovulatory LH surge and ovulation. Once aging female rats have entered an irregularly cyclic state, most are no longer able to maintain fertile gestations (Matt *et al*., 1987a). In middle-aged women, a similar transition from regular menstrual cycles to irregular ovulatory cycles, with an accompanying decline in fertility, occurs during the perimenopausal phase of reproductive life (Treloar *et al*., 1967; Sherman and Korenman, 1975), suggesting that common mechanisms may contribute to reproductive senescence in both humans and rodents.

After a brief period of displaying lengthened, irregular estrous cycles, aging female rats enter an acyclic, spontaneously persistent-estrous state, often lasting for 6 months or longer. During the prolonged period of acyclicity, the ovaries of middle-aged persistent-estrous rats continue to exhibit some degree of follicle growth and E2 secretion, but do not exhibit spontaneous LH surges or follicular ovulation (Lu *et al*., 1979). Thus, in aging persistent-estrous rats, the prolonged acyclic state is associated with a moderately elevated plasma E2 but persistently diminished circulating P4 levels, due to the absence of spontaneous LH surges. Interestingly, persistent-estrous rats will display LH surges and ovulate in response to mating with males, although their ovulation rates and subsequent fertility are very low (Matt *et al*., 1987b; Day *et al*., 1988). It should be noted that the acyclic persistent-estrous state in aging rats is not analogous to the menopause in middle-aged women, as there is some degree of follicular development and steroidogenesis taking place in the ovaries of persistent-estrous rats, as opposed to the relative depletion of ovarian follicles and loss of E2 production in postmenopausal women.

C. Changes in Ovarian Function in Middle-Aged Rats

As in the human, there is a marked decline in the numbers of ovarian follicles during aging in the rat (Mandl and Shelton, 1959; Block, 1968; Richardson and Nelson, 1990). Presumably, a minimal threshold of follicles is required to maintain normal patterns of feedback interactions with the neuroendocrine axis. During the reproductive life span, there is a constant, gonadotropin-independent recruitment of resting primordial follicles that begin growth, resulting in a gradual, continuous depletion of the ovarian follicular pool. The majority of these recruited follicles become atretic, with only a few being selected and stimulated by FSH for continued development to the preovulatory stage. The ovarian and/or endocrine substances which trigger a resting primordial follicle to begin growth are unknown, but hold the key to determining the rate of follicular depletion and, hence, reproductive senescence. Interestingly, there is an accelerated loss of ovarian follicles during the decade preceding menopause in women (Richardson and Nelson, 1990; Faddy *et al*., 1992), although the cause of such increased follicular depletion remains speculative. The importance of the ovary in reproductive aging of rodents is suggested by studies utilizing experimental manipulations that decrease the rate of follicular depletion. Treatments such as food restriction or progesterone treatments early in life (see below) are associated with a delayed onset of reproductive senescence and decelerated loss of ovarian follicles (Lintern-Moore and Everett, 1978; Merry and Holehan, 1979; Nelson *et al*., 1985; LaPolt *et al*., 1998). In contrast, experimental approaches that reduce the follicle pool, such as hemiovariectomy, shorten reproductive life span in rodents (Sopelak and Butcher, 1982; Meredith and Butcher, 1985). Thus, age-related declines in follicle number appear to contribute to the onset of reproductive senescence in rodent species, similar to that in humans.

In middle-aged cyclic female rats, the decline in the ovarian follicular pool results in fewer developing follicles early in each cycle (Peluso *et al*., 1979). However, there is accelerated follicle growth and E2 production by individual follicles (Lerner *et al*., 1990), apparently resulting in similar numbers of large, preovulatory follicles on proestrus between middle-aged and young cyclic females. Although the numbers of preovulatory follicles appear similar in young and middle-aged rats, it is not known whether age-related differences in follicu-

lar and oocyte function may occur. For example, the reduced increase in P4 secretion on proestrus afternoon in middle-aged rats (Gray *et al*., 1980; Wise, 1982b) may be related to a decreased follicular responsiveness to gonadotropin stimulation. In addition, although young and middle-aged rats have similar numbers of preovulatory follicles, there are age-related declines in ovulation rate (Matt *et al*., 1987a; Day *et al*., 1989) and oocyte quality (Peluso and Hutz, 1980; Peluso *et al*., 1980) which suggest differences in follicular function. In this regard, our recent studies examined the ovarian ovulatory responsiveness to gonadotropin stimulation in middle-aged cyclic as compared to young rats (Anzalone *et al*., 1998). Young and middle-aged proestrous rats were treated with sodium pentobarbital to block endogenous LH surges. That afternoon, rats were treated with various doses of human chorionic gonadotropin (hCG) and resulting ovulation rates were determined. Both young and middle-aged cyclic rats exhibited dose-dependent increases in ovulation rate with increasing doses of hCG. In addition, there was no significant difference in the ovulatory response to hCG between young and middle-aged proestrous rats (Anzalone *et al*., 1998). Thus, decreased ovulation rates in middle-aged cyclic females likely result from insufficient gonadotropin stimulation due to attenuated preovulatory LH surges, rather than a decreased ovarian sensitivity to LH. However, middle-aged acyclic persistent-estrous females did exhibit impaired ovulatory responses to exogenous gonadotropin stimulation, indicating decreased sensitivity to the stimulatory effects of LH in these acyclic females (Anzalone *et al*., 1998). Thus, the ovarian ovulatory responsiveness to gonadotropin stimulation in aging rats is dependent upon the reproductive status of the animal studied.

Declines in fertility are associated with decreased oocyte quality in both human and rodent species. However, it has not been clear whether declines in oocyte quality during aging reflect inherent defects in the last oocytes to be recruited during the reproductive life span, or changes in the pattern of follicular development and oocyte maturation that contribute to such defects. Recent studies by Eppig and O'Brien (1995) reveal that oocytes from aged mice matured *in vitro* have normal rates of *in vitro* fertilization and embryonic development. In contrast, induction of oocyte maturation *in vivo* resulted in decreased rates of embryonic development. This finding suggests that impaired oocyte quality during aging does not result from inherent defects in the oocyte, but rather from an altered course of oocyte development and maturation in the aging ovary. In this regard, alterations in follicular development rates have been documented in aging rodents (Lerner *et al*., 1990), and it is feasible that concomitant changes in oocyte maturation occur as well. Whether such age-related changes in follicle development result from altered levels of gonadotropin secretion during follicular development are unknown. Clearly, further studies are required to elucidate specific age-related changes in ovarian follicular growth, maturation, and steroidogenesis that contribute to the onset and progression of reproductive senescence and neuroendocrine aging.

D. Effect of Aging on Neuroendocrine Regulation of Gonadotropin Secretion

Alterations in ovarian function in middle-aged rats may reflect well-described effects of aging on the neuroendocrine regulation of gonadotropin secretion, particularly the preovulatory LH surge on proestrus. The preovulatory rise in circulating E2 is essential in eliciting the positive feedback stimulation of gonadotropin surges on proestrus. In this regard, previous studies demonstrated that the proestrous LH surge is abolished in young cyclic females after ovariectomy or administration of E2 antibodies or antagonists (Brom and Schwartz, 1968; Ferin *et al*., 1969; Labhsetwar, 1970). In addition to the stimulatory effect of E2 on gonadotropin surges, it is also clear that a major rise in circulating P4 on proestrus afternoon also acts upon the neuroendocrine axis to facilitate and potentiate the LH surge (Peduto and Mahesh, 1985; Lee *et al*., 1990; Mahesh and Brann, 1992). Thus, in young rats both E2 and P4 contribute to the initiation and full potentiation of proestrous gonadotropin surges.

Beginning around 9–11 months of age, increasing numbers of regularly cyclic, multiparous female rats exhibit attenuated preovulatory LH surges on proestrus (Cooper *et al*., 1980; Wise, 1982a; Nass *et al*., 1984), associated with smaller rises in plasma P4, reduced ovulation rates, and decreased fertility. There is considerable heterogeneity among aging females regarding the temporal onset of neuroendocrine aging. While some middle-aged rats show significantly attenuated proestrous LH surges and subsequently lose regular cyclicity within 2 months, other females of the same age exhibit a normal profile of the LH surge and continue to display regular cycles for at least 2 more months (Nass *et al*., 1984). These findings indicate that attenuation of the preovulatory LH surge serves as an early marker of neuroendocrine aging and is a reliable predictor of the imminent loss of regular ovulatory cycles.

Age-related declines in proestrous LH surge magnitude are believed to reflect altered hypothalamic function, as evidenced by decreased neuroendocrine response to the positive feedback effects of E2 (Lu *et al*., 1981; Wise, 1984), altered hypothalamic neurotransmitter turnover (Wise, 1984), changes in diurnal rhythmicity (Cohen and Wise, 1988; Weiland and Wise, 1990; Krajnak *et al*., 1998), and altered expression of immediate–early genes, such as Fos, in GnRH neurons, indicating deficits in neuronal activation and subsequent release of GnRH (Rubin *et al*., 1994, 1995; Lloyd *et al*., 1994). A previous study has demonstrated that middle-aged cyclic rats that are bilaterally ovx and primed with E2 exhibit attenuated LH surges, but normal surges of prolactin (Wise, 1984). These findings indicate that the effects of aging on the neuroendocrine control of pituitary hormone secretion are specific and differential. Inasmuch as an increased plasma P4 can facilitate and potentiate the E2 induced LH surge (Peduto and Mahesh, 1985; Lee *et al*., 1990), further studies are required to assess the causal relationship between an attenuated proestrous LH surge and a reduced rise in plasma P4 in middle-aged cyclic rats.

The neuroendocrine induction of the proestrous LH surge is associated with increased activity of several hypothalamic neurotransmitters, such as norepinephrine, serotonin, and neuropeptide Y (NPY). In young female rats, the steroid-induced LH surges are associated with a concomitant diurnal rise in the turnover rates and/or levels of these neurotransmitters (Barraclough, 1983; Vitale and Chiocchio, 1993; Sahu *et al*., 1995; Sahu and Kalra, 1998). Furthermore, administration of these factors stimulates GnRH release (Hery *et al*., 1976; Nowak and Swerdloff, 1985; Besecke *et al*., 1994). During aging, the patterns of stimulatory neurotransmitter activity and recep-

tor expression are altered (Wise, 1984; Cohen and Wise, 1988; Weiland and Wise, 1990; Sahu and Kalra, 1998). Together, such studies indicate that impaired positive feedback stimulation of LH release in aging rats is related to altered activity of several hypothalamic neurotransmitters which stimulate GnRH release.

In addition to stimulatory pathways, GnRH release is also regulated by inhibitory neurotransmitters as well. Thus, it is likely that alterations in inhibitory pathways also contribute to age-related declines in LH release, although relatively little research has been performed in this regard. It has been shown that endogenous opioid tone inhibits LH release in the rat, and there is evidence that a decline in opioid tone is partially responsible for the onset of the proestrous LH surge in young cyclic rats (Allen and Kalra, 1986). Treatment of middle-aged, acyclic female rats with the opioid antagonist naltrexone is sufficient to successfully reinstate estrous cycles and LH surges (Field and Kuhn, 1989), suggesting a potential limiting role of the opioid system in neuroendocrine aging. While some reports indicate a decline in expression of proopiomelanocortin, the precursor to β-endorphin, during aging (Lloyd *et al*., 1991), there is little direct information about the levels of various opioid receptor ligands (such as endormorphins, enkephalines, and endorphins) in aging rats. The effects of E2 and P4 on opioid inhibition-associated LH surges appear to also reflect a decline in opioid receptor binding (Jacobson and Kalra, 1989; Maggi *et al*., 1993). To date, no studies have been performed to address the effects of aging on hypothalamic regulation of opioid receptors. Thus, the role of the opioid system in altered neuroendocrine function during aging requires further investigations. Similarly, the neurotransmitter γ-aminobutyric acid (GABA) also exerts inhibitory effects on GnRH release (Adler and Crowley, 1986; Leonhardt *et al*., 1995) and there is a decline in hypothalamic GABA release during steroid-induced LH surges (Jarry *et al*., 1988, 1995). However, there is little information regarding potential changes in GABA expression or receptor levels in aging female rats. Thus, the role of potential age-related changes in inhibitory neurotransmitter pathways requires further consideration.

In addition to well-characterized influences of age on the hypothalamus, there is evidence that attenuated preovulatory LH surges on proestrus in middle-aged cyclic rats reflect in part a decline in pituitary LH response to GnRH stimulation. Previous reports by Smith *et al*. (1982) described decreased GnRH-stimulated LH release from hemipituitaries taken from middle-aged proestrous rats as compared to that from young rats. More recently, a study by Brito *et al*. (1994) examined the relation of pituitary responsiveness to LH surge magnitude in middle-aged cyclic rats, demonstrating that middle-aged rats with normal patterns of proestrous LH surge (comparable to those in young females) subsequently exhibit normal pituitary responsiveness to exogenous GnRH stimulation. In contrast, middle-aged cyclic females exhibiting attenuated LH surges subsequently showed decreased LH response to GnRH. Thus, declines in pituitary responsiveness to GnRH do occur in aging female rats displaying regular estrous cycles, but only in those aging females exhibiting attenuated proestrous LH surges. Whether such a decline in pituitary responsiveness to GnRH is secondary to a tonic decrease in hypothalamic GnRH release and/or reflects alterations in GnRH receptor expression or signal transduction remains to be determined.

III. Factors Influencing the Onset of Reproductive Senescence in Rodents

As with other outcomes of aging, there is considerable heterogeneity regarding the temporal onset of reproductive senescence among populations. Based on a number of studies, reviewed below, it is clear that a combination of environmental factors and genetic influences affect the timing of reproductive decline for a given individual.

A. Influences of Ovarian Steroid Exposure and Parity on the Onset of Reproductive Aging

The temporal onset of reproductive senescence in female rodents is strongly influenced by the duration and pattern of exposure to circulating levels of E2 and P4 during their lifetime. This conclusion has evolved from a number of studies. Early findings demonstrated that injection of supraphysiological levels (2 to 5 mg) of estrogen (estradiol valerate) to young female rats markedly impaired the neuroendocrine regulation of LH secretion, associated with chronic anovulation and the induction of a polycystic ovarian state (Brown-Grant and ter Haar, 1977; Brawer *et al*., 1978; Mobbs *et al*., 1984a). Whether the effects of these high doses of E2 on reproductive function represent an acceleration of normal aging processes is not clear. However, subsequent studies utilized subcutaneous implants and oral administration of E2, revealing that more physiological levels of E2 also caused the loss of regular ovulatory cycles and impaired neuroendocrine function (Lu *et al*., 1981; Kohama *et al*., 1989). The relationship between exposure to E2 and neuroendocrine aging is more clearly demonstrated in the studies of Lu and colleagues (1981). They observed that old (over 18 months) persistent-estrous rats maintain moderately elevated plasma E2 (around 30 pg/ml), slightly lower than the peak levels observed on proestrus afternoon. Treatment of acutely ovariectomized, old persistent-estrous rats with E2 followed by P4 failed to elicit an LH surge, in contrast to that observed in young females. However, five weeks after ovariectomy, an LH surge could be induced in formerly persistent-estrous rats by E2 and P4 treatment. Thus, positive feedback responses were reinstated in aged persistent-estrous rats following removal of the source of endogenous E2 production. To confirm that a chronic rise in circulating E2 is the factor inhibiting neuroendocrine responsiveness, ovariectomized young rats were treated with E2 implants subcutaneously for 7 weeks, resulting in circulating E2 levels similar to that in intact persistent-estrous females. This treatment of young rats with E2 implants effectively abolished the stimulatory actions of E2 and progesterone on LH release. Subsequent studies advanced further the hypothesis that estrogen exposure contributes to neuroendocrine dysfunction during aging. In both young rats and mice, long-term ovariectomy delays age-related declines in the neuroendocrine regulation of LH secretion, indicating that repetitive, cyclic increases in circulating E2 exert a cumulative, inhibitory effect on neuroendocrine function (Blake *et al*., 1983; Mobbs *et al*., 1984b). In accord

with these findings, it has been shown that, in mice, oral administration of E2 hastens the loss of ovulatory cycles, an effect partially counteracted by ovariectomy (Kohama *et al*., 1989). Together, these findings indicate that prolonged exposure to circulating E2 inhibits the neuroendocrine mechanisms regulating positive feedback of E2 on LH secretion, contributing to age-related infertility.

Other related studies from our laboratory have shown that parity of the female can influence the onset of reproductive aging, and that age-related declines in regular ovulatory cycles and normal fertility occur considerably earlier in virgin than in multiparous rats (LaPolt *et al*., 1986; Matt *et al*., 1987a). Similarly, continued caging of retired breeder females with fertile males significantly delays the onset of reproductive senescence, compared with females that do not have continued contact with males (Nass *et al*., 1982). During pregnancy, plasma levels of P4 are persistently elevated, whereas E2 levels remain low throughout the first two-thirds of gestation (LaPolt *et al*., 1986; Matt *et al*., 1986). Thus, the effects of repeated pregnancy in delaying reproductive aging may be due to the elevation of P4 levels and/or inhibition of E2 production during pregnancy. To test this hypothesis, young virgin rats and middle-aged retired breeder females received repeated treatments with subcutaneous P4 implants, which suppress ovarian E2 production and mimic the endocrine environment of pregnancy in the rat. During P4 implant treatments, the ovarian production of E2 is suppressed for a long period, compared with the repetitive cyclic increases in plasma E2 seen every few days in control females (LaPolt *et al*., 1986, 1988). The findings from these studies reveal that successive treatments of young and middle-aged rats with P4 implants effectively prevent attenuation of the proestrous LH surge, and delay the age-related loss of regular ovulatory cycles and fertility (Lu *et al*., 1985; LaPolt *et al*., 1986). To clarify whether the effects of P4 implants in delaying reproductive aging are due to elevated P4 levels or to an inhibition of E2 production, subsequent studies were performed. Concomitant treatments of young rats with P4 and E2 implants abolished the beneficial effects of P4 treatment on reproductive life span (LaPolt *et al*., 1988), indicating that the effects of P4 implants are dependent upon inhibition of E2 production. Based on these observations and the studies discussed above, it has been proposed that cyclic increases in plasma E2 (once every 4 days) during successive ovulatory cycles cumulatively inhibit the neuroendocrine mechanisms regulating gonadotropin release, resulting in the attenuation and eventual loss of preovulatory LH surges. While our recent study reveals that P4 implant treatments delay the ovarian depletion of the follicular pool (LaPolt *et al*., 1998), further studies are required to elucidate the specific mechanisms by which exposure to ovarian steroids influences the onset of reproductive senescence.

B. Caloric Restriction and the Prolongation of Reproductive Life Span

One of the most widely studied environmental influences on reproductive aging and life span is caloric intake, as examined with a number of studies utilizing various food restriction models. Several reports have shown that food restriction in mice and rats not only results in an increased longevity, but also delays reproductive senescence (Holehan and Merry, 1985; Nelson *et al*., 1995; McShane and Wise, 1996). The effects of caloric restriction on reproductive aging are associated with slower declines in the ovarian follicular reserve (Lintern-Moore and Everett, 1978; Nelson *et al*., 1985) and subsequent maintenance of the neural mechanisms regulating LH secretion after normal diet is resumed (McShane and Wise, 1996; McShane *et al*., 1999). During many regimens of food restriction, there is a temporary cessation of ovulatory cycles. During such treatments, the expression of gonadotropin subunit mRNAs in the anterior pituitary gland is reportedly decreased (Han *et al*., 1998), although positive feedback of LH and FSH responses to ovarian steroids are maintained (Sprangers and Piacsek, 1997). The efficacy of food restriction in delaying reproductive senescence does not, however, appear to be dependent upon the cessation of estrous cycles during reduced caloric intake. McShane and Wise (1996) have shown that female rats subjected to a moderately restricted diet (60% of fed rats ad libitum) continued displaying estrous cycles during experimental caloric reduction, and yet subsequently showed a delayed loss of regular estrous cycles and enhanced pulsatile secretion of LH. Thus, the beneficial effects of caloric restriction on reproductive life span are not dependent upon the cessation of cyclicity during the period of reduced caloric intake. The detailed mechanisms by which reduced caloric intake maintain normal reproductive functions during aging remain to be determined.

C. The Influence of Genetics on Reproductive Aging

Previous studies comparing different strains of inbred and wild-caught mice have revealed distinctive differences in the rate of ovarian follicular depletion and loss of estrous cycles and fertility with age, indicating genetic influences on the progression of reproductive aging. The temporal onset of aging differs in inbred mice of different strains, and is apparently influenced by genes associated with the H-2 major histocompatibility complex (Lerner *et al*., 1988) and other loci (Nebert *et al*., 1984). Several studies have demonstrated that it is possible to selectively breed mice to develop strains with increased or decreased reproductive longevity, demonstrating a genetic influence on reproductive life span (Miyamoto *et al*., 1995; Nagai *et al*., 1995). In addition, selection of mice to develop strains with high plasma magnesium levels and low magnesium levels resulted in strains with differing reproductive longevity as well; the low magnesium level strain exhibits a higher reproductive capacity than the high magnesium level strain (Motta *et al*., 1998). Other strain differences in reproductive longevity have also been described. One recently reported strain of mice exhibits a delayed onset of both fertility and reproductive senescence, continuing to breed into the second and third years of life (Biddle *et al*., 1997). In all of these cases, the specific interactions between genetics and the neural and ovarian mechanisms regulating reproductive longevity remain enigmatic. Furthermore, it is evident that even within a cohort of animals of a given genetic background, there is marked heterogeneity in the reproductive life span. Thus, while there are clear genetic influences on the progression of reproductive aging, much further research remains to be done to elucidate the specific genes modulating reproduc-

tive senescence and to understand the interactions between genetic and environmental factors.

IV. Conclusions

It is evident from this review that female reproductive senescence involves alterations occurring at the hypothalamic, pituitary, and ovarian levels, as would be predicted from the close feedback interactions that link these endocrine organs. Furthermore, the progression of reproductive aging is highly variable among individuals in a population and is influenced by genetic and endocrine factors. Whereas the finite numbers of ovarian follicles represent one definite constraint on reproductive life span, the identities of other factors which alter variables such as maintenance of diurnal rhythmicity, appropriate neurotransmitter release, the rate of follicular depletion, and the magnitude of steroid hormone action remain to be elucidated. Further studies on animal models with increased reproductive life span, whether due to food restriction, progesterone treatments, parity, or genetic influences, may provide new insights into the identity of primary factors responsible for reproductive senescence.

References

Adler, B. A., and Crowley, W. R. (1986). Evidence for gamma-aminobutyric acid modulation of ovarian hormonal effects on luteinizing hormone secretion and hypothalamic catecholamine activity in the female rat. *Endocrinology (Baltimore)* **118**, 91–97.

Allen, L. G., and Kalra, S. P. (1986). Evidence that a decrease in opioid tone may evoke preovulatory luteinizing hormone release in the rat. *Endocrinology (Baltimore)* **118**, 2375–2381.

Anzalone, C. R., Lu, J. K. H., and LaPolt, P. S. (1998). Influences of age and reproductive status on ovarian ovulatory responsiveness to gonadotropin stimulation. *Proc. Soc. Exp. Biol. Med.* **217**, 455–460.

Barraclough, C. A. (1983). The role of catecholamines in the regulation of gonadotropin secretion. *Acta Morphol. Hung.* **31**, 101–115.

Besecke, L. M., Wolfe, A. M., Pierce, M. E., Takahashi, J. S., and Levine, J. E. (1994). Neuropeptide Y stimulates luteinizing hormone-releasing hormone release from superfused hypothalamic GT1-7 cells. *Endocrinology (Baltimore)* **135**, 1621–1627.

Biddle, F. G., Eden, S. A., Rossler, J. S., and Eales, B. A. (1997). Sex and death in the mouse: genetically delayed reproduction and senescence. *Genome* **40**, 229–235.

Blake, C. A., Elias, K. A., and Huffman L. J. (1983). Ovariectomy of young adult rats has a sparing effect on the ability of aged rats to release luteinizing hormone. *Biol. Reprod.* **28**, 575–585.

Block, E. (1968). Quantitative morphological investigations of the follicular system in women, variations at different ages. *Acta Anat.* **14**, 108–123.

Brawer, J. R., Naftolin F., Martin, J., and Sonnenschein, C. (1978). Effects of a single injection of estradiol valerate on the hypothalamic arcuate nucleus and on reproductive function in the female rat. *Endocrinology (Baltimore)* **103**, 501–512.

Brito, A. N., Sayles, T. E., Krieg, R. J., Jr., and Matt, D. W. (1994). Relation of attenuated proestrous luteinizing hormone surges in middle-aged female rats to in vitro pituitary gonadotropin-releasing hormone responsiveness. *Eur. J. Endocrinol.* **130**, 540–544.

Brom, S. G., and Schwartz, N. B. (1968). Acute changes in the estrous cycle following ovariectomy in the golden hamster. *Neuroendocrinology* **3**, 366–377.

Brown-Grant, K., and ter Haar, M. B. (1977). Impairment of the control of gonadotrophin secretion after oestrogen administration to adult female rats. *J. Endocrinol.* **73**, 497–505.

Cohen, I. R., and Wise, P. M. (1988). Age-related changes in the diurnal rhythm of serotonin turnover in microdissected brain areas of estradiol-treated ovariectomized rats. *Endocrinology (Baltimore)* **122**, 2626–2633.

Cooper, R. L., Conn, P. M., and Walker, R. F. (1980). Characterization of the LH surge in middle-aged female rats. *Biol. Reprod.* **23**, 611–615.

Day, J. R. Morales, T. H., and Lu, J. K. (1988). Male stimulation of luteinizing hormone surge, progesterone secretion and ovulation in spontaneously persistent-estrous, aging rats. *Biol. Reprod.* **38**, 1019–1026.

Day, J. R., La Polt, P. S., Morales, T. H., and Lu, J. K. H. (1989). An abnormal pattern of embryonic development during early pregnancy in aging rats. *Biol. Reprod.* **41**, 933–939.

Eppig, J. J., and O'Brien, M. (1995). In vitro maturation and fertilization of oocytes isolated from aged mice: A strategy to rescue valuable genetic resources. *J. Assist. Reprod. Genet.* **12**, 269–273.

Faddy, M. J., Gosden, R. G., Gougeon, A., Richardson, S. J., and Nelson, J. F. (1992). Accelerated disappearance of ovarian follicles in mid-life: Implications for forecasting menopause. *Hum.* **7**, 1342–1346.

Ferin, M., Tempone, A., Zimmertag, P. E., and Wiele, R. L. (1969). Effects of antibodies to 17β-estradiol and progesterone on the estrus cycle of the rat. *Endocrinology (Baltimore)* **85**, 1070–1078.

Field, E. A., and Kuhn, C. M. (1989). Opiate antagonist treatment reinstates estrous cycles in middle-aged persistent-estrous rats. *Biol. Reprod.* **40**, 714–719.

Galway, A. B., LaPolt, P. S., Tsafriri, A., Dargan, C. M., Boime, I., and Hsueh, A. J. W. (1990). Recombinant FSH induces ovulation and tissue plasminogen activator expression in hypophysectomized rats. *Endocrinology (Baltimore)* **127**, 3023–3028.

Gray, G. D., Tennent, G., Smith, E. R., and Davidson, J. M. (1980). Luteinizing hormone regulation and sexual behavior in middle-aged female rats. *Endocrinology (Baltimore)* **107**, 187–194.

Han, E. S., Lu, D. H., and Nelson, J. F. (1998). Food restriction differentially affects mRNAs encoding the major anterior pituitary tropic hormones. *J. Gerontol. A* **53**, B322–329.

Hery, M., Laplante, E., and Kordon, C. (1976). Participation of serotonin in the phasic release of LH. I Evidence from pharmacological experiments. *Endocrinology (Baltimore)* **99**, 496–503.

Hirshfield, A. N., and De Paolo, L. V. (1981). Effect of suppression of the surge of follicle-stimulating hormone with porcine follicular fluid on follicular development in the rat. *J. Endocrinol.* **88**, 67–71.

Holehan, A. M., and Merry, B. J. (1985). Modification of the oestrous cycle hormonal profile by dietary restriction. *Mech. Ageing. Dev.* **32**, 63–76.

Ingram, D. K. (1959). The vaginal smear of senile laboratory rats. *J. Endocrinol.* **19**, 182–188.

Jacobson, W., and Kalra, S. P. (1989). Decreases in mediobasal hypothalamic and preoptic area opioid ([^{3}H]naloxone) binding are associated with the progesterone-induced luteinizing hormone surge. *Endocrinology (Baltimore)* **124**, 199–206.

Jarry, H., Perschl, A., and Wuttke, W. (1988). Further evidence that preoptic anterior hypothalamic GABAergic neurons are part of the GnRH pulse and surge generator. *Acta Endocrinol. (Copenhagen)* **118**, 573–579.

Jarry, H., Leonhardt, S., Schwarze, T., and Wuttke, W. (1995). Preoptic rather than mediobasal hypothalamic amino acid neurotransmitter release regulates GnRH secretion during the estrogen-induced LH surge in the ovariectomized rat. *Neuroendocrinology* **62**, 479–486.

Kohama, S. G., Anderson, C. P., Osterburg, H. H., May, P. C., and Finch, C. E. (1989). Oral administration of estradiol to young C57BL/6J mice induces age-like neuroendocrine dysfunctions in the regulation of estrous cycles. *Biol. Reprod.* **41**, 227–232.

Krajnak, K., Kashon, M. L., Rosewell, K. L., and Wise, P. M. (1998). Aging alters the rhythmic expression of vasoactive intestinal polypep-

tide mRNA but not arginine vasopressin mRNA in the suprachiasmatic nuclei of female rats. *J. Neurosci*. **18**, 4767–4774.

Labhsetwar, A. P. (1970). Role of estrogens in ovulation: A study using the estrogen antagonist ICI 46,474. *Endocrinology (Baltimore)* **87**, 542–551.

LaPolt, P. S., Matt, D. W., Judd, H. L., and Lu, J. K. H. (1986). The relation of ovarian steroid levels in young female rats to subsequent estrous cyclicity and reproductive function during aging. *Biol. Reprod*. **35**, 1131–1139.

LaPolt, P. S., Yu, S. M., and Lu, J. K. H. (1988). Early treatment of young female rats with progesterone delays the aging-associated reproductive decline: A counteraction by estradiol. *Biol. Reprod*. **38**, 987–995.

LaPolt, P. S., Matt, D. W., and Lu, J. K. H. (1998). Progesterone implants delay age-related declines in regular estrous cyclicity and the ovarian follicular reserve in Long-Evans rats. *Biol. Reprod*. **59**, 197–201.

Lee, W., Smith, M., and Hoffmann, G. (1990). Progesterone enhances the surge of luteinizing hormone by increasing the activation of luteinizing hormone-releasing hormone neurons. *Endocrinology (Baltimore)* **127**, 2604–2606.

Leonhardt, S., Seong, J. Y., Kim, K., Thorun, Y., Wuttke, W., and Jarry, H. (1995). Activation of central GABAA-but not of GABAB-receptors rapidly reduces pituitary LH release and GnRH gene expression in the preoptic/anterior hypothalamic area of ovariectomized rats. *Neuroendocrinology* **61**, 655–662.

Lerner, S. P., Anderson, C. P., Walford, R. L., and Finch, C. E. (1988). Genotypic influences on reproductive aging of inbred female mice: Effects of H-2 and non-H-2 alleles. *Biol. Reprod*. **38**, 1035–1043.

Lerner, S. P., Meredith, S., Thayne, W. V., and Butcher, R. L. (1990). Age-related alterations in follicular development and hormonal profiles in rats with 4-day estrous cycles. *Biol. Reprod*. **42**, 633–638.

Lintern-Moore, S., and Everett, A. V. (1978). The effect of restricted food intake on the size and composition of the ovarian follicle population in the Wistar rat. *Biol. Reprod*. **19**, 688–691.

Lloyd, J. M., Scarbrough, K., Weiland, N. G., and Wise, P. M. (1991). Age-related changes in proopiomelanocortin (POMC) gene expression in the periarcuate region of ovariectomized rats. *Endocrinology* **129**, 1896–1902.

Lloyd, J. M., Hoffman, G. E., and Wise, P. M. (1994). Decline in immediate early gene expression in gonadotropin-releasing hormone neurons during proestrus in regularly cycling, middle-aged rats. *Endocrinology (Baltimore)* **134**, 1800–1805.

Lu, K. H., Hopper, B. R., Vargo, T. M., and Yen, S. S. C. (1979). Chronological changes in sex steroid, gonadotropin and prolactin secretion in aging female rats displaying different reproductive states. *Biol. Reprod*. **21**, 193–203.

Lu, J. K. H., Gilman, D. P., Meldrum, D. R., Judd, H. L., and Sawyer, C. H. (1981). Relationship between circulating estrogens and the central mechanisms by which ovarian steroids stimulate luteinizing hormone secretion in aged and young rats. *Endocrinology (Baltimore)* **108**, 836–841.

Lu, J. K. H., LaPolt, P. S., Nass, T. E., Matt, D. W., and Judd, H. L. (1985). Relation of circulating estradiol and progesterone to gonadotropin secretion and estrous cyclicity in aging female rats. *Endocrinology (Baltimore)* **116**, 1953–1959.

Maggi, R., Dondi, D., Rovati, G. E., Martini, L., Piva, F., and Limonta, P. (1993). Binding characteristics of hypothalamic mu opioid receptors throughout the estrous cycle in the rat. *Neuroendocrinology* **58**, 366–372.

Mahesh, V. B., and Brann, D. W. (1992). Interaction between ovarian and adrenal steroids in the regulation of gonadotropin secretion. *J. Steroid Biochem. Mol. Biol*. **41**, 495–513.

Mandl, A. M., and Shelton, M. (1959). A quantitative study of oocytes in young and old nulliparous laboratory rats. *J. Endocrinol*. **18**, 444–450.

Matt, D. W., Lee, J., Sarver, P. L., Judd, H. L., and Lu, J. K. H. (1986). Chronological changes in fertility, fecundity and steroid hormone secretion during consecutive pregnancies in aging rats. *Biol. Reprod*. **34**, 478–487.

Matt, D. W., Sarver, P. L., Lu, J. K. H. (1987a). Relation of parity and estrous cyclicity to the biology of pregnancy in aging female rats. *Biol. Reprod*. **37**, 421–430.

Matt, D. W., Coquelin, A., and Lu, J. K. (1987b). Neuroendocrine control of luteinizing hormone secretion and reproductive function in spontaneously persistent-estrous aging rats. *Biol. Reprod*. **37**, 1198–1206.

McShane, T. M., and Wise, P. M. (1996). Life-long caloric restriction prolongs reproductive life span in rats without interrupting estrous cyclicity: Effects on the gonadotropin-releasing hormone/luteinizing hormone axis. *Biol. Reprod*. **54**, 70–75.

McShane, T. M., Wilson, M. E., and Wise, P. M. (1999). Effects of lifelong moderate caloric restriction on levels of neuropeptide Y, proopiomelanocortin, and galanin mRNA. *J. Gerontol.* **54**, B14–B21.

Meites, J., and Huang, H. H. (1976). Relation of neuroendocrine system to loss of reproductive function in aging rats. *In* ''Neuroendocrine Regulation of Fertility'' (A. Kumar, ed.). Karger, Basel.

Meridith, S., and Butcher, R. L. (1985). Role of decreased numbers of follicles on reproductive performance in young and aged rats. *Biol. Reprod.* **32**, 788–794.

Merry, B. J., and Holehan, A. M. (1979). Onset of puberty and duration of fertility in rats fed a restricted diet. *J. Reprod. Fertil*. **57**, 253–259.

Miyamoto, H., Manabe, N., Mitani, Y., Sugimoto, N., Watanabe, T., Aruga, C., and Sato, E. (1995). Female reproductive properties and prenatal development of a senescence-accelerated mouse strain. *J. Exp. Zool*. **272**, 116–122.

Mobbs, C. V., Flurkey K., Gee, D. M., Yamamoto, K., Sinha, Y. N., and Finch, C. E. (1984a). Estradiol-induced adult anovulatory syndrome in female C57BL/6J mice: Age-like neuroendocrine, but not ovarian, impairments. *Biol. Reprod*. **30**, 556–563.

Mobbs, C. V., Gee, D. M., and Finch, C. E. (1984b). Reproductive senescence in female C57BL/6J mice: Ovarian impairments and neuroendocrine impairments that are partially reversible and delayable by ovariectomy. *Endocrinology (Baltimore)* **115**, 1653–1662.

Motta, R., Lonis J. P., Frank, G., and Henrotte, J. G. (1998). Unexpected association between reproductive longevity and blood magnesium levels in a new model of selected mouse strains. *Growth Dev. Aging* **62**, 37–45.

Nagai, J., Lin, C. Y., and Sabour, M. P. (1995). Lines of mice selected for reproductive longevity. *Growth Dev. Aging* **59**, 79–91.

Nass, T. E., LaPolt, P. S., and Lu, J. K. H. (1982). The effects of prolonged caging with fertile males on reproductive functions in aging female rats. *Biol. Reprod.* **27**, 609–615.

Nass, T. E., LaPolt, P. S., Judd, H. L., and Lu, J. K. H. (1984). Alterations in ovarian steroid and gonadotrophin secretion preceding the cessation of regular oestrous cycles in ageing female rats. *J. Endocrinol*. **100**, 43–50.

Nebert, D. W., Brown, D. D., Towne, D. W., and Eisen, H. J. (1984). Association of fertility, fitness and longevity with the murine Ah locus among (C57BL/6N) (C3H/HeN) recombinant inbred lines. *Biol. Reprod*. **30**, 363–373.

Nelson, J. F., Gosden, R. G., and Felicio, L. S. (1985). Effect of dietary restriction on estrous cyclicity and follicular reserves in aging C57BL/6J mice. *Biol. Reprod.* 515–522.

Nelson, J. F., Karelus, K., Bergman, M. D., and Felicio, L. S. (1995). Neuroendocrine involvement in aging: Evidence from studies of reproductive aging and caloric restriction. *Neurobiol. Aging* **16**, 837–843.

Nowak, F. V., and Swerdloff, R. S. (1985). Gonadotropin-releasing hormone release by superfused hypothalami in response to norepinephrine. *Biol. Reprod*. **33**, 790–796.

Peduto, J. C., and Mahesh, V. G. (1985). Effect of progesterone on hypothalamic and plasma LHRH. *Neuroendocrinology* **40**, 238–245.

Peluso, J. J., and Hutz, R. (1980). The effect of age on the ability of oocytes to synthesize RNA and proteins during in vitro maturation. *Cell Tissue Res*. **213**, 29–35.

Peluso, J. J., Steger, R. W., Huang, H., and Meites, J. (1979). Pattern of follicular growth and steroidogenesis in the ovary of aging cycling rats. *Exp. Aging Res*. **5**, 319–333.

Peluso, J. J., England-Charlesworth, C., and Hutz, R. (1980). Effect of age and of follicular aging on the preovulatory oocyte. *Biol. Reprod.* **22**, 999–1005.

Richardson, S. J., and Nelson, J. F. (1990). Follicular depletion during the menopausal transition. *Ann. N. Y. Acad. Sci*. **592**, 13–20.

Rubin, B. S., Lee, C. E., and King, J. C. (1994). A reduced proportion of luteinizing hormone (LH)-releasing hormone neurons express Fos protein during the preovulatory or steroid-induced LH surge in middle-aged rats. *Biol. Reprod.* **51**, 1264–1272.

Rubin, B. S., Mitchell, S., Lee, C. E., and King, J. C. (1995). Reconstructions of populations of luteinizing hormone releasing hormone neurons in young and middle-aged rats reveal progressive increases in subgroups expressing Fos protein on proestrus and age-related deficits. *Endocrinology (Baltimore)* **136**, 3823–3830.

Sahu, A., and Kalra, S. P. (1998). Absence of increased neuropeptide Y neuronal activity before and during the luteinizing hormone (LH) surge may underlie the attenuated preovulatory LH surge in middle-aged rats. *Endocrinology (Baltimore)* **139**, 696–702.

Sahu, A., Crowley, W. R., and Kalra, S. P. (1995). Evidence that hypothalamic neuropeptide Y gene expression increases before the onset of the preovulatory LH surge. *J. Neuroendocrinol*. **7**, 291–296.

Sherman, B. M., and Korenman, S. G. (1975). Hormonal characteristics of the human menstrual cycle throughout reproductive life. *J. Clin. Invest*. **55**, 669–706.

Smith, W. A., Cooper, R. L., and Conn, P. M. (1982). Altered pituitary responsiveness to gonadotropin-releasing hormone in middle-aged rats with 4-day estrous cycles. *Endocrinology (Baltimore)* **111**, 1843–1848.

Sopelak, V. M., and Butcher, R. L. (1982). Decreased amount of ovarian tissue and maternal age affect embryonic development in old rats. *Biol. Reprod*. **27**, 449–455.

Sprangers, S. A., and Piacsek, B. E. (1997). Chronic underfeeding increases the positive efficacy of estrogen on gonadotropin secretion. *Proc. Soc. Exp. Biol. Med*. **216**, 398–403.

Strauss, J. F., and Miller, W. L. (1991). Molecular basis of ovarian steroid synthesis. *In* ''Ovarian Endocrinology'' (S. G. Hillier, ed.), pp. 25–72. Blackwell, London.

Treloar, A. E., Boynton, R. E., Borghild, G. B., and Brown, B. W. (1967). Variation of the human menstrual cycle through reproductive life. *Int. J. Fertil*. **12**, 77–126.

Vitale, M. L., and Chiocchio, S. R. (1993). Serotonin, a neurotransmitter involved in the regulation of luteinizing hormone release. *Endocr. Rev.* **14**, 480–493.

Weiland, N. G., and Wise, P. M. (1990). Aging progressively alters the diurnal rhythms and decreases the densities of alpha-1-adrenergic receptors in selected hypothalamic regions. *Endocrinology (Baltimore)* **126**, 2392–2397.

Wise, P. M. (1982a). Alterations in proestrous LH, FSH, and prolactin surges in middle-aged rats. *Proc. Soc. Exp. Biol. Med*. **169**, 348–354.

Wise, P. M. (1982b). Alterations in the proestrous pattern of median eminence LHRH, serum LH, FSH, estradiol and progesterone concentrations in middle-aged rats. *Life Sci*. **31**, 165–173.

Wise, P. M. (1984). Estradiol-induced daily luteinizing hormone and prolactin surges in young and middle-aged rats: Correlations with age-related changes in pituitary responsiveness and catecholamine turnover rates in microdissected brain areas. *Endocrinology (Baltimore)* **115**, 801–809.

Norma L. McCoy

54

Female Sexuality during Aging

Studies of sexuality and aging are few in number but generally are consistent in finding a decline in sexual interest and activity for both sexes with sexual activity declining faster than interest. Undeniably, one major purpose of sexual behavior is reproduction and survival of the species; thus, at the end of women's fertility and reproductive lives, when menstrual cycling ceases and sex hormone production decreases, it should not be surprising that female sexuality begins to decline.

Our attempts to understand what happens to female sexuality and its behavioral expression during aging are facilitated by utilizing the three basic concepts of sexual behavior in female mammals that were proposed by Beach in 1976. These characteristics of the sexuality of female mammals as applied to the human female are *attractivity*, or a woman's value as a sexual stimulus—her sexiness, *proceptivity*, or a woman's sexual motivation or desire and the behavior reflecting it, and *receptivity*, or a woman's willingness and ability to respond sexually (lubrication, orgasm, etc.). While surveys find that both reported sexual desire and sexual response inferred from reported frequency of sexual activity decline during aging, sexual attractiveness as a major component of female sexuality worthy of research has been largely ignored by all but popular women's magazines.

Understanding how sexual interactions involving these three components change with age requires an understanding of sexual interaction during the reproductive period. Perper (1985) has provided a detailed analysis of dyadic sexual behavior using data derived from observation of young adults in natural settings. What is important to our understanding of female sexuality during aging is that these data indicate that the human female initiates sexual encounters. Acting proceptively, in a progressive step-by-step escalation toward sexual intimacy in this dyadic courtship sequence, she signals her interest and arouses the male by her behavior. If the female is sexually attractive and succeeds in arousing the male, then the couple reaches what Perper terms a ''power transition'' in which the responsibility for the action changes to the male who must initiate the sexual foreplay/behavior and arouse the female (Perper, 1985, p. 181). If one accepts the existence of this pattern, then it suggests that declines in female sexuality during aging may involve decreased readiness to engage in proceptive behavior by the female and, more importantly, a decline in female attractivity or sexual attractiveness which results in decreased male interest and an end to the interaction prior to the initiation of sexual behavior/foreplay by the male. While we do not have similar data concerning such sexual interactions in the aging female, Perper's description of the courtship sequence is a rich source of hypotheses concerning changes in the sexual interaction of the aging couple. Knowledge of the courtship sequence also offers an explanation for the findings from survey research in which both women and men say that it is the man who is responsible for cessation of sexual activity (Pfeiffer *et al*., 1968, 1972; George and Weiler, 1981). If it is the responsibility of the man to initiate the sexual foreplay/behavior, then regardless of the reason, when such behavior does not occur, it follows that he is the one held accountable.

Related to this issue, Sarrel (1982) has published data strongly suggesting that sexual problems related to estrogen deficiency in the female (dryness, dyspareunia) often give rise to sexual problems such as erectile difficulties and premature ejaculation in the male and these may ultimately bring about a cessation of sexual activity.

I. Sexuality Research with Age as the Major Variable

A. Sex, Age, Marital Status, and Religiosity

Kinsey and his colleagues (1953) carried out some of the earliest scientific work on women's sexuality gathering data from almost 6000 subjects ages 16 to over 71. Although they considered their sample inadequate beyond age 50, their data clearly showed a lower frequency of sexual activity to orgasm in women compared to men at all ages and a decline with age in the number of both single and married women who were sexually active. They also observed that married women engaged in more sexual activity than single, widowed, divorced, or separated women and that religious devoutness was negatively associated with sexual activity. Table 54.1 lists subsequent studies of aging and sexuality and the variables each identified as associated with women's sexual activity. Clearly,

TABLE 54.1. Variables Associated with Sexuality in Women from Studies of Aging and Sexuality

Study	No. of subjects	Age of subjects (years)	Variables
Duke Study I			
Newman and Nichols (1960)	250; ? Women, 47 married women	60–93	Sex, age, marital status, past sexual urge, physical health
Pfeiffer *et al*., (1968)	254; 131 Women	60–94	Sex, age
Verwoerdt *et al*., (1969a)	Same	Same	Sex, age, marital status
Verwoerdt *et al*., (1969b)	Same	Same	Sex, age, marital status
Christenson and Gagnon (1965)	241 Women	50–70; 73% between 50–60	Age, marital status, early sexual activity, relative age of husband, religious devoutness
Duke Study II			
Pfeiffer *et al*., (1972)	502; 241 Women	45–69	Sex, age
Pfeiffer and Davis (1972)	Same	46–71	Sex. For women: Marital status, age, past sexual enjoyment
George and Weiler (1981)	278 Married; 108 married women	46–71 6-year longitudinal	Sex, no significant change in sex in 6 years for 46- to 55-, 56- to 65-, and 66- to 71-year-old women, decline across age group cohorts
Christenson and Johnson (1973)	71 Never-married women	50–69; 80% between 50–60	Age, early sexual activity, religiosity
De Nicola and Peruzza (1974)	85; 32 Women	62–81	Sex, age
Persson (1980)	432; 266 Women 219 married; 91 married women	70-Year-olds	Sex, marital status, Married women: physical/mental health, premarital sexuality, younger healthy husband, coital pleasure, attitude to sexual activity in aged, quality of marriage
Todarello and Boscia (1985)	300; 141 Women	55–90	Sex, age, marital status, noninstitutional living
Bretschneider and McCoy (1988)	202; 102 Women	80–102 Mean=86.1	Sex, marital status, past importance of sex, past extramarital sex, regular church attendence
Lindgren *et al*., (1993)	1867 Women	55, 57, 59, 65	Age, years since menopause, partner status, lack of desire, natural vs surgical menopause

all studies obtaining data from both sexes found a sex difference in favor of men. Some have pointed out that the sex difference in activity favoring men and seen at all ages may be confounded with the age of the partner given that women's partners tend to be several years older and men's partners several years younger than they are.

All studies found a decline in women's sexuality with age and that married women engaged in more sexual activity than other women. Some researchers have noted an exception to the decline with age with their oldest group of women reporting less or no decline in interest and/or activity compared with the preceding younger age group (Verwoerdt *et al*., 1969b; Pfeiffer *et al*., 1972; Bretschneider and McCoy, 1988). Pfeiffer and his colleagues (1972) explained this by suggesting that women of advanced age are "elite survivors from whose midst less highly advantaged individuals had already been removed." In addition to age, a comparatively recent study (Lindgren *et al*., 1993) has also identified years since the menopause, lack of desire, and surgical menopause as factors contributing to declining sexual activity.

Several of these studies as well as Kinsey *et al*. (1953) found religiosity to be negatively associated with sexual activity. Physical and mental health as positive factors were reported by two of these studies, but clearly, such variables have not turned up often, because individuals in poor physical and mental health have tended not to participate in such research and samples have tended to be rather homogeneous for these variables.

B. Past Sexuality

Several studies have found measures of past sexual interest or sexuality in the younger years to be positively associated with current levels of sexual activity. Table 54.1 reveals a number of aspects of past sexual behavior significantly associated with current sexuality. Such variables are past sexual urge, early sexual activity, past sexual enjoyment, premarital sexuality, past extramarital sex, and past importance of sex. Because sexual data concerning the past and the present are usually obtained from subjects at the same time, it is possible that relationships found between them are spurious. I would argue against this view given that such relationships are found routinely and make a great deal of sense based upon what we know about the general consistency of individual behavior across the life span.

C. Sexual Activity

Another factor in women's sexuality suggested by Masters and Johnson (1966) and clearly related to marital status is continued activity with age—a "use it or lose it" hypothesis. Employing direct observation of the sexual response cycle, Masters and Johnson studied only 34 women over the age of 50, but observed three remarkable women subjects, two in their 60s and one in her 70s, who responded to sexual stimulation with vaginal lubrication "in a manner expected of a 20–30-year-old woman" (p. 234). The explanation they offered for this was that these three women had "maintained active sexual connections once or twice a week throughout their mature lives" (p. 234).

Several studies using a vaginal atrophy index derived from direct examination by a trained physician have found that sexually active older women had significantly less vaginal atrophy or greater vaginal health than sexually inactive women of the same age (Leiblum *et al*., 1983; Bachmann *et al*., 1984; Bachmann and Leiblum, 1991). Leiblum and her colleagues (1983) found that active women had significantly higher levels of androstenedione, testosterone, and luteinizing hormone (LH). In the 1984 study by Bachmann and her colleagues, the active women had significantly higher levels of LH, but there were no significant differences in hormone levels between active and inactive women in the 1991 study by Bachmann and Leiblum. While sexual activity could have played a causal role in reducing vaginal atrophy, it is also possible that a third variable such as the level of sex hormones may have acted to increase both sexual activity and vaginal health.

II. Sexuality Research with Menopause as the Major Variable

During the 1980s, menopause appeared as a significant variable in research concerning female sexuality during aging. Much earlier, Kinsey and his colleagues (1953) had examined the effects of menopause but discounted its importance. They examined the data from a group of 127 postmenopausal women who had experienced orgasm within the past year; 48% stated they believed that their sexual response and activity had been decreased by menopause, and 53% stated they believed that menopause had brought about a decrease in the frequency of their sexual behavior (total outlet). Nonetheless Kinsey *et al*. (1953) continued to view declines in women's sexuality as related to the male. They stated:

> . . .the declining frequencies of sociosexual activities among females are not primarily dependent on an aging process in the female, but upon an aging process in the male which reduces his interest in having frequent coitus (p. 735).

Commenting further they stated:

> Some of the decreased frequencies also depended upon the fact that some of these women had seized upon menopause or their ovarian operations as an excuse for discontinuing sexual relationships in which they were never particularly interested (p. 736).

In another older study, Pfeiffer and Davis (1972) (see Table 54.1) conducted a regression analysis with postmenopausal status as a predictor variable. The inclusion of this variable accounted for less than 2% of the explained variance for both sexual interest and frequency of sexual intercourse in women; the major part of the variance was explained by marital status and age. In their study of never-married women, Christenson and Johnson (1973) also examined menopause as a variable but reported that 50% of the 17 postmenopausal women reported no change in erotic feelings after menopause; 25% each reported an increase and a decrease, respectively.

Before menopause could be acknowledged as a significant variable in understanding sexuality during aging, it was necessary to show that menopause and not age was the significant variable. Conducting research in Sweden, Hallström (1979) demonstrated this with a large sample of 800 women ages either 38, 46, 50, or 54. By grouping the 50-year-old women on the basis of pre-, peri-, early post-, and late postmenopausal phase, he was able to show significant decreases in sexual interest (see Fig. 54.1), capacity for orgasm, and coital frequency with advancing menopausal status while holding age constant. These data clearly implicate menopause rather than age as the dominant causal variable.

A. Menopause and Sex Hormones

Women have as many as 400,000 ovarian follicles at birth of which 400 will actually produce eggs (Richardson and Nelson, 1990). "Menopause" refers to the last menstrual cycle and is preceded by a period of 2 to 8 years with an average of 4 years commonly referred to as the menopausal transition period (McKinlay *et al*., 1992). This period typically begins in the early to mid 40s when the number of the remaining follicles drops below a certain threshold level (Soules *et al*., 1998). This situation results in an increase in gonadotropin levels

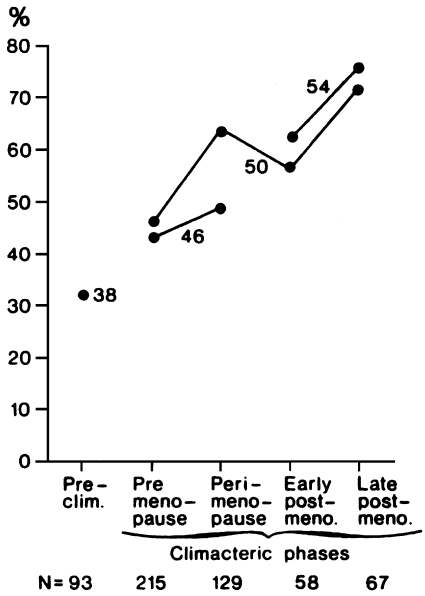

FIG. 54.1. Incidence of declining sexual interest by age and menopausal phase. (From Hallström, 1979 with permission of Combridge University Press.)

and an increase in the number of follicles recruited which then gives rise to higher estrogen levels (Santoro *et al*., 1996). Change in menstrual cycling during the transition proceeds from shorter regular cycles to irregular cycles or cycles of different lengths to skipping cycles to cessation of cycling (Treloar, 1981). Menopause occurs at the average age of 51 or 52 when the supply of ovarian follicles is depleted and follicles that remain are insensitive to stimulation (Costoff and Mahesh, 1975). Once menstrual cycling has stopped, production of sex hormones drops approximately 85% for estradiol (E2), 58% for estrone (E1), 67% for androstenedione, and only 29% for T (Longcope *et al*., 1981). There is little to no circulating progesterone. Significant production of E2 by the postmenopausal overies has ceased by one year past the last cycle (Vermeulen, 1980; Metcalf *et al*., 1982), although theca-like cells in the stromal tissues of the postmenopausal ovary produce significant amounts of testosterone in response to LH in at least 50% of women (Judd *et al*., 1974; Andreyko *et al*., 1992; Stevens and Lowe, 1992). Consistent with these findings, Sluijmer *et al*. (1998) have reported that the amount of stromal hyperplasia in the ovary was positively correlated with ovarian vein levels of androstenedione and testosterone in postmenopausal women.

The adrenal cortex is a second source of sex hormones in women. Androgens produced by the adrenal cortex peak in women between the ages of 16 and 19 and decline steadily thereafter, reaching a low point around the time of menopause or a few years following it (Orentreich *et al*., 1984). After the last cycle, adrenal dihydroepiandrosterone (DHA) continues to be a source of androstenedione and E1 as well as small amounts of testosterone and E2 through aromatization.

Women who have had their ovaries removed will obviously have no postmenopausal hormone production by their ovaries and, in addition, the loss of the ovaries apparently results in a decrease in the production of adrenal androgens (Cumming *et al*., 1982). These sequelae will also tend to occur in women whose ovaries have been damaged by chemotherapeutic agents (Kaplan, 1992). Women who had their ovaries removed had significantly lower levels of androstenedione, testosterone, dihydrotestosterone, and DHA (Vermeulen, 1976). If one assumes a relationship between sex hormones and sexuality, ovariectomized women should experience larger deficits in their postmenopausal sexuality than intact women of the same age. This prediction is consistent with the findings of Sherwin and her colleagues (Sherwin *et al*., 1985; Sherwin and Gelfand, 1987) and others (Lindgren *et al*., 1993).

B. Sexuality and Sex Hormones

Clearly, if the menopause plays a significant role in a decline in female sexuality during aging, then such a decline must be related directly or indirectly to the decrease in hormone production associated with menopause. An understanding of the relationship between menopause and female sexuality requires an understanding of the role that sex hormones play in female sexuality. Unfortunately our understanding in this area is incomplete.

1. Estrogens

The reproductive organs, the breast, and the vagina, vulva, urethra, and neck of the bladder have many estrogen receptors and are supported by estrogens (Smith and Judd, 1994). Over time following menopause and the drop in sex hormone production, low estrogen levels cause atrophic changes to take place in these structures. Masters and Johnson (1966) have provided a detailed account of the physical changes in sexual response. Through direct observation they were able to compare the sexual response of their sample of 348 women ages 18 to 50 with their sample of 34 women ranging in age from 51 to 78. After age 51 they found a marked reduction of the vasocongestion reactive potential of the breasts. Engorgement of the areolae occurred but typically with diminished intensity. Myotonia or general muscle-tension elevation in response to sexual stimulation was lessened and sex flush was rare. Clitoral response continued unchanged, but in women over 60, obvious tumescence of the clitoral glans was also rare. The flattening, separation, and elevation of the labia majora and the vasocongestive thickening of the labia minora that normally develop with increased sexual tension during the sexual response cycle in younger women were not observed. The walls of the vagina were described as tissue paper thin, light pinkish in color, and had lost their usual rough, corrugated appearance. The vagina was shorter in length, width, and some of its expansive ability was lost. Women over 60 took 1 to 3 minutes to develop significant vaginal lubrication compared to 10 to 30 sec in young women. Masters and Johnson (1966) concluded:

> Generally, the intensity of physiologic reaction and duration of anatomic response to effective sexual stimulation are reduced through all 4 phases of the sexual cycle with the advancing years. . .steroid starvation has the primary influence of reducing rapidity and intensity of physiologic response. (p. 238)

Thus, following menopause the decline in sex hormone production particularly that of estrogens appears to attenuate sexual ''receptivity'' or women's ability if not willingness to respond to sexual stimulation.

Estrogens are probably also involved in female attractivity or sexual attractiveness. In studies with nonhuman primates, females with the highest E2 peaks during the cycle were the most attractive to males during the entire cycle (McCoy, 1991). Female pheromones may be associated with ovulatory menstrual cycles and play a significant role in women's sexual attractiveness, but we still know comparatively little about this (Cutler, 1999).

2. Androgens

It is generally accepted that androgens are the hormone of sexual desire in both men and women (Sherwin, 1988). Nonetheless it has been difficult to demonstrate this relationship in naturally cycling women (Campbell and Udry, 1994). The best evidence we have for this relationship has been a prospective crossover study of women who underwent total hysterectomy with bilateral salpingo-oophorectomy (Sherwin *et al*., 1985). Women's sexual desire, frequency of sexual fantasies, and arousal increased significantly over preoperative baselines when women received androgens alone or androgen with estrogen following their surgery. This was not the case for women receiving estrogen alone or placebo who had significantly lower scores on all three measures than they had prior to surgery.

In a study of intact cycling women, Persky *et al*. (1978) studied 11 couples intensively over three menstrual cycles. Women gave blood for sex hormone assays twice a week, and each day completed a six-item self-gratification scale with questions concerning the extent of daily sexual arousal and activity. These researchers found significant correlations between average testosterone and average daily self-gratification scores for each of the three cycles (r_s=0.54, 0.63, 0.66). Thus, average testosterone does appear to be related to reported levels of sexual arousal and activity.

We might assume that average testosterone would be related to frequency of sexual intercourse, but that does not seem to be the case. Interestingly, Persky and his colleagues (1976, 1978) have reported that sexual frequency per cycle was related to women's peak testosterone level around midcycle, a finding that was replicated by Morris and her colleagues in 1987. Such puzzling findings suggest that peak testosterone level may be serving as a marker for the quality of that menstrual cycle as a whole.

3. Progestagens

Progesterone is the major progestagen and is produced in significant amounts during the luteal phase of the menstrual cycle and during pregnancy. Progesterone is an anti-androgen because it competes with androgens for androgen receptors and an anti-estrogen because it downregulates estrogen receptors (Johnson and Everitt, 1995). In a study of oral contraceptive users, the findings of Udry and his colleagues (1973) suggested the possibility that progesterone made women less sexually attractive to men. Progesterone does not play a significant role in the sexuality of naturally postmenopausal women, but its effects become relevant when it is used continuously as part of hormone replacement therapy.

C. Menopause and Sexuality

Studies concerned with the menopause are many, but studies concerning the effects of menopause on sexuality are comparatively few. In order for a study to implicate the menopause as playing a role in changes in sexual behavior, it is necessary to have data at a minimum of two different points during the menopausal transition. Many studies have done this through the less than satisfactory practice of using retrospective data. In studies of menopause and sexuality, sexual information has usually been obtained using questionnaires or interviews; daily recording of sexual activity on calenders was used by only one group of investigators (McCoy and Davidson, 1985; McCoy *et al*., 1985). Sexual variables that have been studied the most are sexual interest, vaginal dryness, and frequency of sexual intercourse. Fewer studies have examined vaginal discomfort (dyspareunia), capacity for orgasm, and satisfaction with the sex partner or relationship.

A description of 16 studies that have examined sexual variables at a minimum of two points during the menopausal transition is presented in Table 54.2. Although these studies have certainly suffered from methodological problems (McCoy, 1998), their findings are consistent. Ten of 11 studies found a decline in sexual interest from an earlier to a later point in the menopausal transition. Eight of eight found a decline in frequency of sexual intercourse, and six of six an increase in vaginal dryness. Three of five studies found some decline in orgasmic capacity, two of three an increase in pain or discomfort with sexual intercourse, and one of two found less satisfaction with the sexual partner.

In the only longitudinal study from pre- to postmenopause with daily recording of cycling and sexual behavior (McCoy and Davidson, 1985), frequency of sexual behavior dropped significantly in relationship to the last menstrual period (see Fig. 54.2). However, there was already a considerable decline 1 year prior to the last cycle. Using one cycle per month as a standard, reasearchers found that during the 12 to 24 month period prior to the last cycle, women were cycling an average of 80% of the time. During the year prior to the last cycle they were cycling an average of only half the time which may be related to this decline in frequency of sexual intercourse.

III. Research on Hormone Replacement Therapy and Sexuality

The fact that cessation of cycling results in decreases in sex hormone production and is associated with a decline in sexual interest, vaginal lubrication, and frequency of sexual intercourse suggests the possibility that hormone replacement therapy will effectively treat sexual complaints related to menopause. What evidence do we have on this issue? As was true for studies concerning menopause, there are many studies concerning hormone replacement therapy but only relatively few that have examined the effects of hormone replacement therapy on sexuality and fewer still that were placebo-controlled. Table 54.3 contains the results of 12 placebo-controlled studies of hormone replacement therapy that examined its effect on various aspects of sexuality.

In considering the effects of hormone replacement therapy on sexuality, it is important to understand that sex hormones in hormone replacement therapy products can be structurally the same or differ from those found naturally in the human female and that the way in which the hormones are delivered can vary from oral to transdermal to percutaneous to implant. Both the structure of the hormone and the mode of delivery can make a difference in the hormone's effects on the individual. Both oral delivery and nonhuman structure can contribute to an overproduction of a variety of proteins produced by the liver (L'Hermite, 1990; Lorrain *et al*., 1999). This effect on liver proteins is referred to as a first-pass effect and does not occur when nonoral modes of delivery are used. One of these liver proteins that is overproduced is sex hormone-binding globulin, which preferentially binds testosterone (Johnson and Everitt, 1995). Thus, Mathur and his colleagues (1985) have reported that oral use of conjugated equine estrogens results in a significant decline in bioavailable testosterone.

A. Vaginal Dryness, Atrophy, and Pain with Coitus

Five placebo-controlled studies examined vaginal dryness and found a significant decrease using a variety of estrogen products (see Table 54.3). Three other studies examined vaginal smears, measured change in atrophic vaginitis, or asked about inadequate lubrication. While atrophic vaginitis and

TABLE 54.2 Research Examining Sexual Variables at Two or More Menopausal Phases[a]

Study	Subjects	Phases	Method	No. of sex measures	Results for sex measures compared at ≥2 phases
Bachmann *et al.* (1985)	$n=22$, M age=55.3, no HRT, 20 had sex partners	Post-, pre- (retrospective)	Sexual behavior inventroy (E2, A, FSH, LH)	?	Sexual interest, ↓ 54%; frequency coitus, ↓; vaginal dryness, ↑ 40% (no stat. eval.)
Bachmann and Leiblum (1991)	$n=59$ (ages 60–70), M age = 63.9 No HRT, had sex partners	Post-, pre- (retrospective)	Sexual history questionnaire, interview (T, free T, E2, LH)	?	Sexual desire, ↓, $P<0.0001$
Bottiglioni and De Aloysio (1982)	$n=347$ (ages 51–65)	Early post-, late post-, pre-(retrospective)	Interview and "semicomputerized questionnaire" completed after the interview	5	Change sexual drive, ↓; Coital frequency, ↓; orgasmic response, ↓; satisfaction sexual intercourse, ↓ (%s only, no stat. eval.)
Dennerstein *et al.* (1994)[b]	$n=1103$ (ages 45–55), intact, no HRT	Pre-, peri-, post-	Telephone survey	3	Change sexual interest, ↓, $P<0.00001$; sex/previous year, ↓, $P<0.02$; pain with coitus, ↑, $P<0.0001$
Hagstad (1988)	$n=1188$ (ages 40–66), intact, no HRT	Pre-, peri-, post-	Postal questionnaire	2	Vaginal dryness: peri-/post-, ↑, $P<0.05$; vaginal discomfort: pre-/post-, ↑, $P<0.001$
Hallström (1973, 1979)	$n=800$ (ages 38–54), M age=47.2, intact, no HRT, had sex partners	Pre-, peri-, early post-, late post-	1- to 2-Hour standardized interview	24	Sexual interest, ↓, $P<0.05$ change/sexual interest, ↓, $P<0.05$; coital frequency, ↓, $P<0.001$ change/coital frequency, ↓, $P<0.01$; capacity orgasm, ns; change/capacity orgasm, ↓, $P<0.02$
Holte (1991)	$n=1566$ (ages 45–55), M age=50.7, intact, no HRT	Pre-, peri-, post-	Postal questionnaire	1	Vaginal dryness: frequently, ↑, $P<0.00001$ occasionally ↑, $P<0.00001$
Holte (1992)	$n=59$ (ages 47–55), M age=51.1, intact, no HRT	Pre-, post- (longitudinal)	3- to 4-Hour semistructured interview with fixed response categories	3	Vaginal dryness: yes/no, ↑, $P<0.05$; frequency, ↑, $P<0.05$; distress, ↑, $P<0.07$
Huerta *et al.* (1995a)	$n=222$ (ages 36–61), M age=47.7, intact, no HRT	Pre-, early post-, late post-	Questionnaire, Likkert scale ratings	13	Loss of sexual interest, $P=0.01$
Huerta *et al.* (1995b)	$n=151$ (ages 36–70), M age=49.3, intact, no HRT	Pre-, early post-, late post-	Questionnaire, Likkert scale ratings	13	Loss of sexual interest: severe, $P<0.005$; decreased, $P<0.005$
Hunter *et al.* (1986)	$n=474$ (ages 45–54), intact, no HRT	Pre-, peri-, post-	Postal questionnaire	3	Loss of sexual interest, ↓, $P<?$; vaginal dryness, ↑, $P<?$; dissatisfaction sexual relationship, ns
Kinsey *et al.* (1953)	— (ages 33–56), intact, had orgasm in last 2 years	Post-, pre- (retrospective)	Interview	3	Sexual response: ($n=31$) ↑ 13%, ↓ 48%; frequency total outlet: ($n=127$) ↑ 5%, ↓ 53%; median frequency total outlet: ($n=475$) ↓ (no stat. eval.)
Koster and Garde (1993)	$n=474$ (all aged 51)	Pre-, peri-, early post-	Postal questionnaire	2	Frequency of sexual desire: less, > monthly, ns; change in sexual desire: less, more, ns

continues

TABLE 54.2 *Continued*

Study	Subjects	Phases	Method	No. of sex measures	Results for sex measures compared at ≥2 phases
McCoy *et al.* (1985)	$n=43$ (ages 41–56), M age=49.1 intact, no HRT, had sex partners	Early peri-, late peri-	Daily calender records of sexual intercourse (E2, T)	1	No. of days with coitus: 4-week period, ↓, $P<0.05$ 10-week period, ↓, $P<0.03$
McCoy and Davidson (1985)	$n=16$ (ages 47–56), intact, no HRT, had sex pertners	Peri-, early post- (longitudinal)	Daily calender records of sexual intercourse, sex questionnaire (E2,T)	14	Sex thoughts, fant., ↓, $P<0.001$; vaginal lubrication, ↓, $P<0.01$; No. of days with coitus, ↓, $P<0.05$; dyspareunia, ns; orgasm frequency, ns; satis. partner as lover, ↓, $P<0.05$
Tungphaisal *et al.* (1991)	$n=100$ (ages 40–62), M age=56.8, intact, no HRT	Post-, pre- (retrospective)	Sex questionnaire, medical history, physical, pelvic exams (FSH, LH, E2,T)	?	Sexual desire, ↓ 95%; sexual activity, ↓ 94%; orgasm, ↓ 100%; masturbation, ↓ 100% (no stat. eval.)

[a] Reprinted from McCoy (1998), with permission of Elsevier Science. HRT, hormone replacement therapy; T, testosterone; M age, mean age; ns, not significant.
[b] There was no statistical evaluation of these data by Dennerstein *et al.* Chi-square analyses were computed by the author using the reported frequency data.

vaginal smears improved with hormone replacement therapy. Myers *et al.*, (1990) found no change in reported inadequate lubrication with naturally menopausal women. One study (Coope *et al.*, 1975) used a combination of an oral, structurally nonhuman estrogen and progestagen and obtained no change in dryness. The failure of this study to find a significant change in vaginal dryness may be due to the anti-estrogenic properties of progestagens.

Of six studies examining pain with sexual intercourse, only Coope *et al.* (1975) reported any positive change and in only 6 of 30 women subjects. The failure to find significant change probably has to do with the relatively short length of these studies. Semmens and his colleagues (1985) did a 2-year study of 23 postmenopausal women (ages 51–70), giving women conjugated equine estrogen and using direct observation of vaginal fluid, vaginal pH, vaginal blood flow, and transvaginal potential difference. There was significant improvement in these variables at 1 month, with the exception of transvaginal potential difference, which showed significant improvement by 6 months. Both vaginal blood flow and transvaginal potential difference showed further significant increases at 12 months and vaginal blood flow continued to increase. Thus, this work suggests that estrogens appear to correct vaginal atrophy serious enough to cause pain, but more than 3 to 6 months time may be required before women report significant improvement.

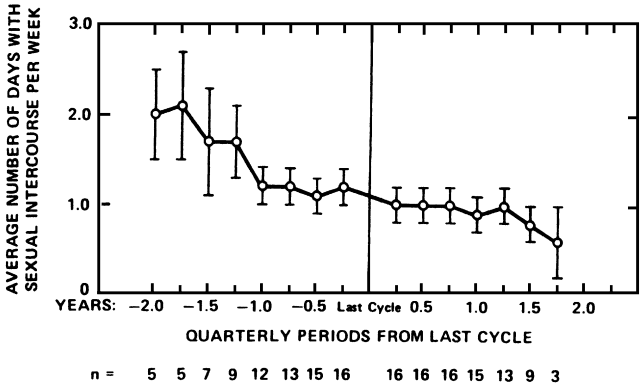

FIG. 54.2. Average number of days with sexual intercourse (± SE) by quarterly (13 week) periods before and following the last menstrual cycle. [From McCoy and Davidson, 1985 with permission from Elsevier Science.]

B. Sexual Interest

Table 54.3 describes 12 placebo-controlled studies that have examined hormone replacement therapy in relationship to sexuality. Eight of these placebo-controlled studies have involved surgically menopausal women, but only three studies have investigated the effects of androgens on sexual interest. Using methyl testosterone, an oral, structurally nonhuman androgen, Greenblatt and his colleagues (1950) found a 42% increase in sexual interest for a mixed group of ovariectomized and naturally menopausal women. Using an injection of testosterone enanthate alone or combined with estradiol, Sherwin *et al.* (1985) reported a significant increase over placebo in sexual interest, sexual arousal, and sexual fantasy in ovariectomized, hysterectomized women. Using the same dose of methyl testosterone as Greenblatt *et al.* (1950) combined with conjugated equine estrogen, Myers *et al.* (1990) found no effect of the combination on sexual interest in a group of nine naturally and one surgically menopausal women compared with 10 naturally menopausal women using a placebo. The only significant finding for sexuality in this study was that the group getting methyl testosterone and conjugated equine estrogen reported significantly more pleasure in masturbation than women given a placebo. Because the assay used in this study was not sensitive to methyl testosterone the assay for testosterone revealed that giving methyl testosterone to these women had significantly lowered the endogenous production of testosterone compared with baseline levels.

Bakke (1965) gave 27 hysterectomized women a birth control pill containing the usual oral, structurally nonhuman

TABLE 54.3. The Effect of HRT over Placebo on Sexual Interest, Vaginal Dryness, Frequency of Coitus, and Pain with Coitus in Surgically and Naturally Menopausal Women

Research	Subjects	Hormones	Findings
Bakke (1965)	27 hyx CA M=52.3±1.6	**I**, Mestranol 0.075 mg (E); **II**, same + norethynodrel 5 mg (E + P) (Enovid); **III**, placebo (2 months each)	Interest E + P, ↑, 22%; E, ↑, 12.5%; placebo, ↑ 0%
Campbell (1976) Campbell, and Whitehead (1977)	a. 64 Late peri-/post-menopausal	**I**, conjugated equine estrogens 1.25 mg (CEE) (3 weeks, 1 week off); **II**, placebo (same) (2 months, each)	Interest: CEE, no change; dryness: CEE, ↓, $P<0.01$; pain: CEE, no change; f coitus: CEE, no change
	b. 56 Late peri-/post-menopausal	Same (for 6 month each)	Interest: CEE, no change; dryness: CEE, ↓, $P<0.001$; pain: CEE, no change; f coitus: CEE, no change
Coope *et al.* (1975)	30 (7 hyx, 4 ovx/ hyx, 10 post-, 9 peri-) CA 40–61; M=52	**I**, Conjugated equine estrogens 1.25 mg (CEE) (Premarin); **II**, placebo (3 months each)	Interest: CEE, no change, ↓ in 4 Ss; dryness: CEE, ↓ in 6 Ss, vaginal cytology, ↑, $P<0.01$; pain: CEE, ↓ in 6 Ss
Dennerstein *et al.* (1980)	28 ovx/hyx CA M=46.2±8.92	**I**, Ethinyl estradiol 50 μg (EE); **II**, levonogestrel 250 μg (P); **III**, EE + P (Nordiol); **IV**, placebo (3 months each)	Interest: EE, ↑, $P<0.01$; dryness: EE, ↓, $P<0.05$; f coitus: EE, EE + P, no change
Fedor-Freybergh (1977)	25 Postmenopausal CA 47–70; M=56.5	**I**, Estradiol-17β-valerianate 2 mg (E) (Progynon); **II**, placebo (3 months each)	Interest: E, change from absence to slight presence or presence
Greenblatt *et al.* (1950)	31 Postmenopausal (9 ovx) CA 23–62; M=43	**I**, Diethylstilbestrol 0.25 mg (DES); **II**, methyl testosterone 5 mg (mT); **III**, DES + mT; **IV**, placebo (1 month each)	Interest: mT, ↑, 42%; DES + mT, ↑ 23.5%; DES, ↑ 12.3%; placebo, ↑ 1.8%; dryness: vaginal smear, DES, ↑; mT + DES, ↑
Myers *et al.* (1990)	40 Postmenopausal (3 ovx) CA M=58.3	**I**, Conjugated equine estrogens 0.625 mg (CEE) (Premarin); **II**, CEE + medroxyprogesterone acetate (P) (Provera). **III**, CEE + methyl testosterone 5 mg (mT); **IV**, placebo (8 weeks each)	Interest: (and number of sexual thoughts) CEE, CEE + P, CEE + mT, no change; pleasure/masturbation: CEE + mT, ↑, $P<0.046$; dryness: CEE, CEE + P, CEE + mT, no change; pain: CEE, CEE + P, CEE + mT, no change; f coitus CEE, CEE + P, CEE + mT, no change
Nathorst-Böös *et al.*, (1993)	224 Postmenopausal CA 44–64; M=52	**I**, Estradiol 50μg (E) (Estraderm patch); **II**, placebo (3 months each)	Interest: E, ↑, $P<0.01$; dryness: E, ↓, $P<0.001$; pain: E, ↓, $P<0.001$
Paterson (1982)	20 hyx (8 ovx/hyx)	**I**, Sequential mestranol (E) + norethisterone (P) (Syntex Menophase); **II**, placebo (3 months each)	Interest: E + P, no change; dryness: E + P, no change; pain: E + P, no change
Sherwin *et al.* (1985)	43 ovx/hyx CA M=45.8±3.3	**I**, Estradiol valerate (E) (Delestrogen); **II**, testosterone enanthate (T) (Delatestryl). **III**, E + T (Climacteron), **IV**, placebo (3 months each), all by injection	Interest: T, ↑, $P<0.001$; E + T, ↑, $P<0.001$; same for sexual fantasy
Utian (1972)	50 ovx/hyx CA 45–55	**I**, Oestradiol valerate 4 mg (E) (6 months); **II**, conjugated equine estrogens 5 mg (CEE); **III**, placebo (II, III 3 months)	Interest: E, no change; CEE, no change; dryness: atrophic vaginitis, E, ↓, $P<0.001$; CEE; ↓, $P<0.001$

CA, cancer; hyx, hysterectomy; M, mean age; ovx, ovariectomy.

estrogen and a progestin and found that 22% of women reported increased sexual interest. Paterson (1982) used a sequential oral contraceptive type product with hysterectomized and ovariectomized, hysterectomized women and found no change in sexual interest. Dennerstein *et al*. (1980) used an oral contraceptive and found a significant increase over placebo in sexual interest in surgically menopausal women receiving a high dose of unopposed ethinyl estradiol. This very strong, structurally nonhuman estradiol commonly found in oral contraceptives is no longer used in hormone replacement therapy with postmenopausal women.

Interestingly, in a small group of naturally menopausal women, Fedor-Freybergh (1977) found a change from absence of sexual interest to slight presence using an oral but structurally human estradiol. In the largest of the placebo-controlled studies of hormone replacement therapy, Nathorst-Böös *et al.* (1993) also examined the effects of estradiol on sexual interest in naturally menopausal women. Not surprisingly, he found a significant increase in sexual interest over placebo with a transdermal estradiol patch.

None of five studies examining the effects of conjugated equine estrogens on sexual interest in either naturally or surgically menopausal women has found any change. This is not surprising given the fact that conjugated equine estrogen derived from pregnant mares' urine causes overproduction of sex hormone-binding globulin which selectively binds androgens and, to some extent, estrogens as well (Geola *et al*., 1980; Myers *et al*., 1990).

While the 12 studies in Table 54.3 are difficult to compare given their many differences, nonetheless these data suggest some tentative conclusions: (1) To improve sexual interest, fantasy, and arousal in surgically menopausal women, it is necessary to include testosterone in hormone replacement therapy. (2) If testosterone is administered to naturally menopausal women you may depress their own production of testosterone. (3) Naturally menopausal women may show an increase in sexual interest in response to estrogens if they are structurally human and administered transdermally; oral conjugated equine estrogen lowers bioavailable testosterone, is not effective in increasing sexual interest, and may decrease it.

C. Frequency of Sexual Intercourse

All eight studies in Table 54.2 that examined frequency of sexual intercourse found a decrease from early to later in the menopausal transition, but none of the four studies in Table 54.3 found that hormone replacement therapy increased the frequency of sexual intercourse or activity. Either frequency of sexual intercourse is very difficult to change with replacement hormones or future studies must follow subjects for considerably longer than 3 to 6 months in order to see a positive effect.

IV. Summary and Conclusions

Female sexuality is best conceptualized as having the three components of sexual attractiveness, interest, and responsiveness. Previous research makes it clear that female sexual interest and activity decline both with age and in relationship to the menopause. In one sense menopause and age are confounded because the number of years since menopause reflects both increasing age and increasing years with decreased hormone levels both centrally and peripherally. One effect occurring over time is atrophy of reproductive structures. Such atrophy due to hormone deficiency has the effect of increasing the time it takes to respond and reducing the intensity of the response to adequate sexual stimulation.

Research focusing on change with age has identified marital status, regular sexual activity, past sexuality, physical and mental health, and religious devoutness as important variables in relationship to sexual interest and activity. Thus, a married, mentally and physically healthy woman who has been sexually interested and active in the past, who has regular sexual activity with her functioning partner, and is not very religious, is more likely to maintain sexual activity with advancing age.

Studies concerned with the effects of menopause on sexuality have found rather consistent declines in sexual interest, vaginal lubrication, and frequency of sexual intercourse with mixed findings and fewer studies for orgasmic capacity and satisfaction with the partner. No good evidence exists on the effect of menopause on sexual attractiveness, but the obvious hypothesis is that the effect is a negative one.

Placebo-controlled studies of hormone replacement therapy and sexuality show a consistent positive effect of estrogen products on vaginal dryness and vaginal atrophy. Such studies also support the need for androgen replacement in surgically menopausal women in order to restore sexual interest and arousal. Our understanding of feminine sexual interest in naturally postmenopausal women is still incomplete. It is a reasonable conclusion that estrogen as well as androgens play major roles. A better understanding of sexual attractiveness or ''sexiness'' in cycling women, including the role of pheromones, would undoubtedly shed further light on this issue.

References

Andreyko, J. B., Monroe, S. E., Marshall, L. A., Fluker, M. R., Nerenberg, C. A., and Jaffe, R. B. (1992). Concordant suppression of serum immunoreactive luteinizing hormone (LH), α-subunit bioactive LH, and testosterone in postmenopausal women by a potent gonadotropin releasing hormone antagonist (Detirelix). *J. Clin. Endocrinol. Metab.* **74**, 399–405.

Bachmann, G. A., and Leiblum, S. R. (1991). Sexuality in sexagenarian women. *Maturitas* **13**, 43–50.

Bachmann, G. A., Leiblum, S. R., Kemmann, E., Colburn, D. W., Swartzman, L., and Sheldon, R. (1984). Sexual expression and its determinants in the post-menopausal woman. *Maturitas* **6**, 19–29.

Bachmann, G. A., Leiblum, S. R., Sandler, B., Ainsley, W., Narcessian, R., Shelden, R., and Hymans, H. N. (1985). Correlates of sexual desire in post-menopausal women. *Maturitas* **7**, 211–216.

Bakke, J. L. (1965). A double-blind study of progestin-estrogen combination in the management of the menopause. *Pac. Med. Surg*. **73**, 200–205

Beach, F. A. (1976). Sexual attractivity, proceptivity, and receptivity in female mammals. *Horm. Behav.* **7**, 105–138.

Bottiglioni, F., and De Aloysio, D. (1982). Female sexual activity as a function of climacteric conditions and age. *Maturitas* **4**, 27–32.

Bretschneider, J. G., and McCoy, N. L. (1988). Sexual interest and behavior in healthy 80- to 102-year-olds. *Arch. Sex. Behav.* **17**, 109–129.

Campbell, B. C., and Udry, J. R. (1994). Implication of hormonal influences on sexual behavior for demographic models of reproduction. *Ann. N.Y. Acad. Sci*. **709**, 117–127.

Campbell, S. (1976). Double blind psychometric studies on the effects of natural estrogens on post-menopausal women. *In* ''The Management of the Menopause and the Post-Menopausal Years'' (S. Campbell, ed.), pp. 149–158. MTP Press, Lancaster, UK.

Campbell, S., and Whitehead, M. (1977). Oestrogen therapy and the menopausal syndrome. *Clin. Obstet. Gynaecol*. **4**, 31–47.

Christenson, C. V., and Gagnon, J. H. (1965). Sexual behavior in a group of older women. *J. Gerontol.* **20**, 351–356.

Christenson, C. V., and Johnson, A. B. (1973). Sexual patterns in a group of older never-married women. *J. Geriatr. Psychiatry.* **6**, 80–98.

Coope, J., Thomson, J. M., and Poller, L. (1975). Effects of ''natural estrogens'' replacement therapy on menopausal symptoms and blood clotting. *Br. Med. J.* **4**, 139–143.

Costoff, A., and Mahesh, V. B. (1975). Primordial follicles with normal oocytes in the ovaries of postmenopausal women. *J. Am. Geriatr. Soc.* **23**, 193–196.

Cumming, D. C., Rebar, R. W., Hopper, B. R., and Yen, S. S. C. (1982). Evidence for an influence of the ovary on circulating dehydroepiandrosterone sulfate levels. *J. Clin. Endocrinol. Metab.* **54**, 1069–1071.

Cutler, W. B. (1999). Human sex-attractant pheromones: Discovery, research, development, and application in sex therapy. *Psychiat. Ann.* **29**, 54–59.

De Nicola, P., and Peruzza, M. (1974). Sex in the aged. *J. Am. Geriatr. Soc*. **22**, 380–382.

Dennerstein, L., Burrows, G. D., Wood, C., and Hyman, G. (1980). Hormones and sexuality: Effect of estrogen and progestogen. *Obstet. Gynecol*. **56**, 316–322.

Dennerstein, L., Smith, A. M. A., Morse, C. A., and Burger, H. G. (1994). Sexuality and the menopause. *Psychosom. Obstet. Gynecol*. **15**, 59–66.

Fedor-Freybergh, P. (1977). The influence of oestrogens on the wellbeing and mental performance in climacteric and postmenopausal women. *Acta Obstet. Gynecol. Scand.* **64** (Suppl.), 1–91.

Geola, F. L., Frumar, A. M., Tataryn, I. V., Lu, K. H., Hershman, J. M., Eggena, P., Sambhi, M. P., and Judd, H. L. (1980). Biological effects of various doses of conjugated equine estrogens in postmenopausal women. *J. Clin. Endocrinol. Metab.* **51**, 620–625.

George, L. K., and Weiler, S. J. (1981). Sexuality in middle and late life: The effects of age, cohort, and gender. *Arch. Gen. Psychiatry* **38**, 919–923.

Greenblatt, R. B., Barfield, W. E., Garner, J. F., Calk, G. L., and Harrod, J. P. (1950). Evaluation of an oestrogen, androgen, oestrogen-androgen combination, and a placebo in the treatment of the menopause. *J. Clin. Endocrinol. Metab.* **10**, 1547–1558.

Hagstad, A. (1988). Gynecology and sexuality in middle-aged women. *Women Health* **13**, 57–80.

Hallström, T. (1973). ''Mental Disorder and Sexuality in the Climacterium.'' Scandinavian University Books, Stockholm.

Hallström, T. (1979). Sexuality of women in middle age: The Göteborg Study. *J. Biosoc. Sci.* **6** (Suppl.), 165–175.

Holte, A. (1991). Prevalence of climacteric complaints in a representative sample of middle-aged women in Oslo, Norway. *J. Psychosom. Obstet. Gynaecol*. **12**, 303–315.

Holte, A. (1992). Influences of natural menopause on health complaints: A prospective study of healthy Norwegian women. *Maturitas* **14**, 127–141.

Huerta, R., Mena, A., Malacara, J. M., and DeLeon, J. D. (1995a). Symptoms at perimenopausal period: Its association with attitudes toward sexuality, life-style, family function and FSH levels. *Psychoneuroendocrinology* **20**, 135–167.

Huerta, R., Mena, A., Malacara, J. M., and DeLeon, J. D. (1995b). Symptoms at the menopausal and premenopausal years: Their relationship with insulin, glucose, cortisol, FSH, prolactin, obesity and attitudes towards sexuality. *Psychoneuroendocrinology* **20**, 851–864.

Hunter, M., Battersby, R., and Whitehead, M. (1986). Relationships between psychological symptoms, somatic complaints and menopausal status. *Maturitas* **8**, 217–228.

Johnson, M. H., and Everitt, B. J. (1995). ''Essential Reproduction,'' 4th ed. Blackwell Scientific, Oxford, UK.

Judd, H. L., Judd, G. E., Lucas, W. E., and Yen, S. S. C. (1974). Endocrine function of the post-menopausal ovary: Concentration of androgens and estrogens in ovarian and peripheral vein blood. *J. Clin. Endocrinol. Metab.* **39**, 1020–1024.

Kaplan, H. S. (1992). A neglected issue: The sexual side effects of current treatments for breast cancer. *J. Sex Marital Ther.* **18**, 3–19.

Kinsey, A. C., Pomeroy, W. B., Martin, C. E., and Gebhard, P. H. (1953). ''Sexual Behavior in the Human Female.'' Saunders, Philadelphia.

Koster, A., and Garde, K. (1993). Sexual desire and menopausal development: A prospective study of Danish women born in 1936. *Maturitas* **16**, 49–60.

Leiblum, S., Bachmann, G., Kemmann, E., Colburn, D., and Swartzman, L. (1983). Vaginal atrophy in the postmenopausal woman: The importance of sexual activity and hormones. *JAMA, J. Am. Med. Assoc.* **249**, 2195–2198.

L'Hermite, M. (1990). Risks of estrogens and progestogens. *Maturitas* **12**, 215–246.

Lindgren, R., Berg, G., Hammar, M., and Zuccon, E. (1993). Hormonal replacement therapy and sexuality in a population of Swedish postmenopausal women. *Acta Obstet. Gynecol. Scand.* **72**, 292–297.

Longcope, C., Jaffee, W., and Griffing, G. (1981). Production rates of androgens and oestrogens in post-menopausal women. *Maturitas* **3**, 215–223.

Lorrain, J., Lalumiere, G., Filion, A., and Caron, P. (1999). Use of transdermal estrogen gel in postmenopausal women. *Menopause Manage.* **8**, 11–15.

Masters, W. E., and Johnson, V. E. (1966). ''Human Sexual Response.'' Little Brown, Boston.

Mathur, R. S., Landgrebe, S. C., Moody, L. O., Semmens, J. P., and Williamson, H. O. (1985). The effect of estrogen treatment on plasma concentrations of steroid hormones, gonadotropins, prolactin and sex hormone-binding globulin in post-menopausal women. *Maturitas* **7**, 129–133.

McCoy, N. L. (1991). Menopause and sexuality. *In* ''The Menopause and Replacement Therapy: Facts and Controversies'' (R. Sitruk-Ware, and W. Utian, eds.), Clin. Gynecol. Ser., pp. 73–100. Dekker, New York.

McCoy, N. L. (1998). Methodological problems in the study of sexuality and the menopause. *Maturitas* **29**, 51–60.

McCoy, N. L., and Davidson, J. M. (1985). A longitudinal study of the effects of menopause on sexuality. *Maturitas* **7**, 203–210.

McCoy, N., Cutler, W., and Davidson, J. M. (1985). Relationships among sexual behavior, hot flashes, and hormone levels in perimenopausal women. *Arch. Sex. Behav.* **14**, 381–390.

McKinlay, S. M., Brambilla, D. J., and Posner, J. G. (1992). The normal menopause transition. *Maturitas* **14**, 103–115.

Metcalf, M. G., Donald, R. A., and Livesey, J. H. (1982). Pituitary-ovarian function before during and after the menopause: A longitudinal study. *Clin. Endocrinol.* (Oxford) **17**, 489–494.

Morris, N. M., Udry, R. J., Khan-Dawood, F., and Dawood, M. Y. (1987). Marital sex frequency and midcycle female testosterone. *Arch. Sex. Behav.* **16**, 27–37.

Myers, L. S., Dixen, J., Morrissette, D., Carmichael, M., and Davidson, J. M. (1990). Effects of estrogen, androgen, and progestin on sexual psychophysiology and behavior in postmenopausal women. *J. Clin. Endocrinol. Metab.* **70**, 1124–1131.

Nathorst-Böös, J., Wiklund, I., Mattsson, L.-A., Sandin, K., and von Schoultz, B. (1993). Is sexual life influenced by transdermal estrogen therapy? A double blind placebo controlled study in postmenopausal women. *Acta Obstet. Gynecol. Scand*. **72**, 656–660.

Newman, G., and Nichols, C. R. (1960). Sexual activities and attitudes in older persons. *JAMA, J. Am. Med. Assoc.* **173**, 33–35.

Orentreich, N., Brind, J. L., Rizer, R. L., and Vogelman, J. H. (1984). Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. *J. Clin. Endocrinol. Metab.* **59**, 551–555.

Paterson, M. E. L. (1982). A randomized, double-blind, crossover study of sex steroid administration in the surgical menopause. *Maturitas* **4**, 83–94.

Perper, T. (1985). "Sex Signals: The Biology of Love." ISI Press, Philadelphia.

Persky, H., O'Brien, C. P., and Khan, M. A. (1976). Reproductive hormone levels, sexual activity and moods during the menstrual cycle. *Psychosom. Med.* **38**, 62–63.

Persky, H., Lief, H. I., Strauss, D., Miller, W. R., and O'Brien, C. P. (1978). Plasma testosterone level and sexual behavior of couples. *Arch. Sex. Behav.* **7**, 157–173.

Persson, G. (1980). Sexuality in a 70 year old urban population. *J. Psychosom. Res.* **24**, 335–342.

Pfeiffer, E. and Davis, G. C. (1972). Determinants of sexual behavior in middle and old age. *J. Am. Geriatr. Soc.* **20**, 151–158.

Pfeiffer, E., Vervoerdt, A., and Wang, H.-S. (1968). Sexual behavior in aged men and women. I. Observations on 254 community volunteers. *Arch. Gen. Psychiatry.* **19**, 753–758.

Pfeiffer, E., Vervoerdt, A., and Davis, G. C. (1972). Sexual behavior in middle life. *Am. J. Psychiatry.* **128**, 1262–1267.

Richardson, S. J. and Nelson, J. F. (1990). Follicular depletion during menopausal transition. *Ann. N.Y. Acad. Sci.* **592**, 13–20.

Santoro, N., Rosenberg, J., Adel, T., and Skurnick, J. H. (1996). Characteristic of reproductive hormonal dynamics in the perimenopause. *J. Clin. Endocrinol. Metab.* **81**, 1495–1501.

Sarrel, P. M. (1982). Sex problems after menopause: A study of fifty married couples treated in a sex counseling programme. *Maturitas* **4**, 231–237.

Semmens, J. P., Tsai, C. C., Semmens, E. C., and Loadholt, C. B. (1985). Effects of estrogen therapy on vaginal physiology during menopause. *Obstet. Gynecol.* **66**, 15–18.

Sherwin, B. B. (1988). A comparative analysis of the role of androgen in human male and female sexual behavior: Behavioral specificity, critical thresholds, and sensitivity. *Psychobiology* **16**, 416–425.

Sherwin, B. B., and Gelfand, M. M. (1987). The role of androgen in the maintenance of sexual functioning in oophorectomized women. *Psychosom. Med.* **49**, 397–409.

Sherwin, B. B., Gelfand, M. M., and Brender, W. (1985). Androgen enhances motivation in females: A prospective, crossover study of sex steroid administration in the surgical menopause. *Psychosom. Med.* **47**, 339–351.

Sluijmer, A. V., Heineman, M. J., Koudstaal, J., Theunissen, P. H. M. H., de Jong, F. H., and Evers, J. L. H. (1998). Relationship between ovarian production of estrone, estradiol, testosterone, and androstenedione and the ovarian degree of stromal hyperplasia in postmenopausal women. *Menopause* **5**, 207–210.

Smith, K. E., and Judd, H. L. (1994). Menopause and postmenopause. *In* "Current Obstetric and Gynecologic Diagnosis and Treatment" (A. H. DeCherney and M. L. Pernoll, eds.), 8th ed., pp. 1030–1050. Appleton & Lange, Norwalk, CT.

Soules, M. R., Battaglia, D. E., and Klein, N. A. (1998). Inhibin and reproductive aging in women. *Maturitas* **30**, 193–204.

Stevens, A., and Lowe, J. S. (1992). "Histology," p. 334. Gower Medical, London.

Todarello, O., and Boscia, F. M. (1985). Sexuality in aging: A study of a group of 300 elderly men and women. *J. Endocrinol. Invest.* **8** (Suppl. 2), 123–130.

Treloar, A. E. (1981). Menstrual cyclicity and the premenopause. *Maturitas* **3**, 249–264.

Tungphaisal, S., Chandeying, V., Sutthijumroon, S., Krisanapan, O., and Udomratn, P. (1991). Postmenopausal sexuality in Thai women. *Asia-Oceania J. Obstet. Gynecol.* **17**, 143–146.

Udry, R. J., Morris, N. M., and Waller, L. (1973). Effect of contraceptive pills on sexual activity in the luteal phase of the human menstrual cycle. *Arch. Sex. Behav.* **2**, 205–214.

Utian, W. H. (1972). The true clinical features of postmenopause and oophorectomy, and their response to oestrogen therapy. *S. Afr. Med. J.* **46**, 732–737.

Vermeulen, A. (1976). The hormonal activity of the postmenopausal ovary. *J. Clin. Endocrinol. Metab.* **42**, 247–253.

Vermeulen, A. (1980). Sex hormone status of the postmenopausal woman. *Maturitas* **2**, 81–89.

Verwoerdt, A., Pfeiffer, E., and Wang, H.-S. (1969a). Sexual behavior in senescence: Changes in sexual activity and interest of aging men and women. *J. Geriatr. Psychiatry* **2**, 163–180.

Verwoerdt, A., Pfeiffer, E., and Wang, H.-S. (1969b). Sexual behavior in senescence. II. Patterns of sexual activity and interest. *Geriatrics* **24**, 137–154.

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Naomi E. Rance and Ty W. Abel

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Hypothalamic Neuropeptide Gene Expression in Postmenopausal Women

I. Introduction

Menopause marks the cessation of function in a dynamic physiological system regulating the cyclic release of pituitary and gonadal hormones. Menopause, with a mean onset age of 51, occurs relatively early in a woman's life span. Thus, the average woman will spend approximately one-third of her life in the postmenopausal phase. At the time of the last menstrual cycle, the profound loss of ovarian cyclicity stands in sharp contrast to the continued functioning of all the other organ systems. In addition, there are millions of young women who experience premature ovarian failure or surgical removal of the ovaries. Many of these women are offered hormone replacement therapy, yet there is little information on the effects of hormone withdrawal and replacement on the human brain.

The loss of ovarian estrogen secretion has been linked to many of the symptoms of menopause including vasomotor flushes (hot flashes), mood changes and an increased risk for osteoporosis and cardiovascular disease (Gambrell, 1986; Speroff, 1993). Interestingly, outcomes such as osteoporosis and cardiovascular disease seem to represent accelerated aging changes, suggesting an interaction between gonadal steroids and somatic aging. In contrast, hot flashes and mood changes likely represent direct effects of hormone withdrawal on central nervous system mechanisms regulating body temperature and mood (Casper and Yen, 1985; Fink *et al*., 1998). Treatment with exogenous ovarian hormones is now standard practice in the management of the symptoms and risk factors associated with estrogen deficiency. Hormone replacement therapy eliminates hot flashes in postmenopausal women while reducing the risk of age- and hormone-related degenerative conditions. Furthermore, hormone replacement therapy enhances mood and improves some aspects of cognitive function in estrogen-deficient women (Sherwin, 1991, 1996). Finally, some evidence suggests that hormone replacement therapy might delay the onset of and/or ameliorate symptoms in Alzheimer's disease (Paganini-Hill, 1996; Henderson, 1997). These findings have stimulated interest in identifying the sites and mechanisms of steroid hormone action in the primate central nervous system.

The hypothalamus is a regulator as well as a major target of gonadal steroid hormones. Clearly, many of the targets of estrogen in the hypothalamus are involved in the regulation of reproduction. However, it is likely that hypothalamic neurons also mediate the effects of gonadal hormones on other parts of the nervous system. Estrogen withdrawal-induced vasomotor flushes, for example, reflect activation of the autonomic nervous system secondary to disrupted hypothalamic thermoregulatory mechanisms. In addition, ovarian steroid receptors in the hypothalamus could mediate hormonal effects on mood through connections with the limbic system (Sherwin, 1996), or via descending input to the midbrain and interactions with the mesolimbic dopamine system (Nauta and Haymaker, 1969; Spanagel *et al*., 1992). Two subtypes of estrogen receptors (ERs) have now been identified, ERα (Greene *et al*., 1986) and ERβ (Kuiper *et al*., 1996). In addition to actions via the classical intranuclear receptors, steroid hormones may have direct membrane effects (Nabekura *et al*., 1986).

II. Control of the Reproductive Cycle through Reciprocal Interactions between Ovarian Secretions, Pituitary Gonadotrophs, and the GnRH Pulse Generator in the Medial Basal Hypothalamus

The reproductive axis is a dynamic, highly regulated control system with the timing of the menstrual cycle relying upon reciprocal interactions among the ovary, pituitary, and brain. Gonadotropin-releasing hormone (GnRH) is synthesized by neurons in the hypothalamus and released into the hypophyseal portal circulation. GnRH is transported to the anterior pituitary gland where it stimulates the production and release of the gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). LH and FSH reach the ovary via the systemic circulation and regulate folliculogenesis, ovulation, and the secretion of steroid and protein hormones. Two types

of ovarian secretions control gonadotropin secretion: ovarian steroids such as estrogen and progesterone, and the glycoprotein hormones including inhibin and activin. Inhibin is a secretory product of both the male and the female gonads that is capable of suppressing FSH secretion directly at the level of the pituitary gland (Burger, 1989).

Normal reproductive function relies on the pulsatile release of GnRH into the hypophyseal portal circulation. The frequency and amplitude of GnRH pulses vary over the menstrual cycle and these patterns have physiological significance. Constant exposure to GnRH or its analogs, for example, desensitizes the pituitary, resulting in little or no LH secretion and a hypogonadal state (Belchetz *et al*., 1978; Nakai *et al*., 1978). Furthermore, alterations in the pulsatile pattern of LH secretion may have deleterious effects upon ovarian follicle development (Pohl *et al*., 1983). The neural circuits subserving pulsatile GnRH secretion in the primate are located within the medial basal hypothalamus (Knobil, 1990). Destruction of the MBH, for example, results in the cessation of LH and FSH secretion (Plant *et al*., 1978). Gonadotropin secretion is stimulated in these animals, however, by the administration of exogenous GnRH, indicating intact pituitary function (Nakai *et al*., 1978). Interestingly, complete removal of all afferent input to the medial basal hypothalamus does not interfere with the negative feedback effects of estrogen on LH secretion (Krey *et al*., 1975). In fact, many animals subjected to this treatment display relatively normal reproductive function, including normal pulsatile LH secretion and spontaneous ovulation (Krey *et al*., 1975). These findings suggest that the neural substrate responsible for the rhythmic release of GnRH (the hypothetical ''GnRH pulse generator'') resides within the medial basal hypothalamus.

Electrophysiological correlates of the GnRH pulse generator have been demonstrated in the medial basal hypothalamus (Knobil, 1990). There is synchronized, multiunit activity in the medial basal hypothalamus that is coincident with pulses of LH in the peripheral circulation (Silverman *et al*., 1986). These multiunit volleys are affected by many of the variables that alter pulsatile LH secretion. The frequency of synchronized multiunit activity varies, for example, over the menstrual cycle (O'Byrne and Knobil, 1993). In addition, removal of the ovaries leads to a greatly prolonged duration and an increase in the maximal frequency of synchronized neuronal activity in the medial basal hypothalamus, an effect rapidly reversed by estrogen replacement (O'Byrne *et al*., 1993). The neurochemical identity of hypothalamic neurons that contribute to the multiunit activity of the GnRH-pulse generator has not been established. Part of the activity may originate within the intrinsic rhythmic activity of the GnRH neurons (Wetsel *et al*., 1992), but contributions by other neuronal populations seems highly likely.

The timing of the menstrual cycle is critically dependent upon the pattern of estrogen secretion from the dominant ovarian follicle. Throughout most of the menstrual cycle, estrogen restrains the level of LH secretion from the anterior pituitary gland (negative feedback). At mid-cycle, however, rapidly increasing estrogen levels result in a surge of LH (positive feedback), culminating in ovulation of the dominant follicle. The feedback effects of estrogen on LH secretion are complex and occur at the level of both the pituitary and the hypothalamus (Ferin *et al*., 1984). GnRH neurons do not express estrogen receptors (Shivers *et al*., 1983). Steroid receptors, however, are present in many other hypothalamic neurons that express a variety of neurotransmitters or neuropeptides. Thus, estrogen may modify GnRH secretion through genomic effects via one or more interneurons that synapse on GnRH neurons (Herbison, 1998). The intricate interplay of transmitter systems allows for modification of the function of the reproductive axis in response to various physiological and pathological states.

III. The Perimenopausal Period Is Characterized by an Accelerated Loss of Ovarian Follicles and a Selective Rise in FSH Secretion

Ultimately, menopause is a consequence of ovarian failure. The human ovary contains a finite number of germ cells *in utero*. In the first four decades after birth, there is a exponential decline of primordial follicles (Block, 1952). At approximately 38 years of age, follicular loss accelerates (Richardson *et al*., 1987). Menopause finally occurs when insufficient follicles are available to maintain ovarian hormone secretion. The cause of follicular decline, especially the accelerated phase, remains to be elucidated.

Alterations in gonadotropin secretion occur prior to menopause. One of the earliest and most consistent changes in hormone secretion in the perimenopausal period is a selective increase in plasma FSH (the monotropic FSH rise) (Reyes *et al*., 1977; Ebbiary *et al*., 1994). This rise in FSH secretion has been postulated to be responsible for the accelerated follicular decline by promoting increased numbers of follicles into the growing pool (Nelson *et al*., 1995). Once in the growing pool, follicles must either ovulate or become atretic. Whether the early, monotropic FSH rise is due to ovarian or hypothalamic factors is a current subject of investigation and controversy (Nelson *et al*., 1995; Wise *et al*., 1997; Soules *et al*., 1998). In favor of the ovarian hypothesis is the recent demonstration that elevated FSH secretion in older premenopausal women is correlated with a significant decrease in inhibin B in peripheral blood (Klein *et al*., 1996). These data suggest that the lifelong decline in follicles ultimately reaches a threshold, resulting in decreased ovarian inhibin secretion and, as a result, increased FSH secretion (Soules *et al*., 1998).

Alternatively, studies in experimental animals have demonstrated that hypothalamic mechanisms may produce a selective rise in FSH secretion. In particular, slowing of the GnRH pulse frequency results in an elevation in FSH secretion without increasing LH secretion (Wise *et al*., 1979; Wildt *et al*., 1981). In support of this theory is a recent study showing a reduction in LH pulse frequency (a reflection of GnRH pulse frequency) in older vs younger premenopausal women (Matt *et al*., 1998). Ovarian function is also directly sensitive to alterations in the patterns of pulsatile gonadotropin secretion (Pohl *et al*., 1983), thereby providing a second explanation for how hypothalamic factors could promote ovarian failure (Finch *et al*., 1984). Unlike the ovary, however, the human

hypothalamus and pituitary are still capable of functioning in postmenopausal women, as evidenced by the ability of exogenous ovarian hormones to stimulate an LH surge (Odell and Swerdloff, 1968).

IV. The Postmenopausal State Is Characterized by Profound Estrogen Deficiency and Gonadotropin Hypersecretion

Regardless of the cause, the hormonal milieu of postmenopausal women is characterized by a profound estrogen deficiency. The loss of ovarian follicles results in levels of estrogen and progesterone that are comparable to the levels in women who have undergone therapeutic oophorectomy (Wallach *et al*., 1970; Monroe *et al*., 1972; Chakravarti *et al*., 1977). Hypersecretion of pituitary gonadotropins occurs secondary to the loss of ovarian hormones, and continues for decades. Indeed, the mean gonadotropin concentration, LH pulse frequency, and pulse amplitude are not significantly different between young oophorectomized and older postmenopausal women (Alexander *et al*., 1990). In advanced age, the levels of LH and FSH may become attenuated (Rossmanith *et al*., 1991). Alternatively, the hypersecretion of gonadotropins may continue undiminished in some women at 90–100 years of age (Scaglia *et al*., 1976).

Determining the precise mechanism for gonadotropin hypersecretion in the postmenopausal period has been hampered by the difficulty of measuring alterations in hypothalamic GnRH secretion (Crowley *et al*., 1985). Clearly, the invasive techniques employed to investigate hypothalamic reproductive control mechanisms in laboratory animals cannot be used in human subjects. GnRH is not detectable in the peripheral circulation due to a short half-life and the relative confinement of the peptide to the hypophyseal portal circulation. Studies in humans, therefore, have relied heavily on pharmacological manipulations, using agonists and antagonists of neurotransmitters and neuropeptides that are putatively involved in the regulation of GnRH secretion. Following the administration of these agents, pulsatile LH release is measured in the peripheral circulation. Because pulses of LH in peripheral plasma are linked to pulsatile secretion of GnRH into the portal system (Clarke and Cummins, 1982), alterations in hypothalamic GnRH secretion are inferred from changes in peripheral LH pulses. However, a change in the amplitude of LH secretion may reflect alterations at either the hypothalamic or pituitary level. Furthermore, if pituitary sensitivity is altered, a pulse of GnRH may not always translate into a pulse of LH. Thus, although much has been learned using this indirect approach, uncertainty as to the sites and mechanisms of actions of various pharmacological agents limits the conclusions that can be made about the role of central neuropeptides and neurotransmitters in human reproduction.

A recent approach to studying hypothalamic regulatory mechanisms in the human is to quantify relative levels of messenger RNAs (mRNAs). The technique of *in situ* hybridization histochemistry is particularly well suited for the investigation of human autopsy material due to the relative stability of mRNA species with prolonged postmortem intervals (Johnson *et al*., 1986). In addition, alterations in gene expression have been correlated with changes in neuronal activity (Comb *et al*., 1987; Young and Zoeller, 1987; Blum *et al*., 1987; Uhl and Nishimori, 1990; Petersen *et al*., 1991). Therefore, a change in neuropeptide gene expression may provide an indirect assessment of neuronal activity in the postmortem human brain. A further level of anatomical resolution is provided by the localization of mRNA species within tissue sections.

V. Anatomy of GnRH Neurons in the Primate Hypothalamus and Basal Forebrain

During prenatal development, GnRH neurons originate outside the brain in the olfactory placode and then migrate along the terminal nerve to enter the forebrain (Schwanzel-Fukuda and Pfaff, 1989; Wray *et al*., 1989; Schwanzel-Fukuda *et al*., 1996). Two waves of GnRH neurons migrate from the olfactory placode at different times in embryogenesis in monkeys (Quanbeck *et al*., 1997). In adult monkeys, GnRH neurons are diffusely distributed (Barry *et al*., 1985). One subpopulation of GnRH neurons resides within the medial basal hypothalamus (Silverman *et al*., 1982; Goldsmith and Song, 1987), in the region of the GnRH pulse generator (Silverman *et al*., 1986). These neurons project to the median eminence where the peptide is released in the primary capillary plexus of the portal system (Silverman *et al*., 1977,1982; Goldsmith and Song, 1987). Extrahypothalamic GnRH projections have also been identified. For example, GnRH neurons send projections to the midbrain, amygdala, hippocampus, and olfactory structures (Barry *et al*., 1985). These pathways suggest that GnRH may function in the brain as a neuromodulator as well as a hypophysiotropic hormone. Indeed, GnRH has been shown to promote sexual behavior, including lordosis, in female rodents (Moss and McCann, 1973; Pfaff, 1973).

In the human brain, *in situ* hybridization studies have identified a diffuse distribution of GnRH neurons with three morphological subtypes (Rance *et al*., 1994). A group of small oval neurons with high levels of gene expression (type I) were present within the medial basal hypothalamus, the putative control center for reproduction in the primate (Fig. 55.1A). A second population of small, round to oval, lightly labeled GnRH neurons (type II) were identified within the septal-preoptic region, the bed nucleus of the stria terminalis–amygdala continuum, and the ventral globus pallidus (Fig. 55.1B). Finally, a third population of large, round GnRH neurons, intermediate in labeling intensity (type III), were located in the magnocellular basal forebrain complex, ventral globus pallidus, and putamen (Fig. 55.1C). The presence of these subpopulations of GnRH neurons in the primate central nervous system has recently been confirmed using immunocytochemical methods (Quanbeck *et al*., 1997). Interestingly, the type II and type III neurons migrate from the olfactory placode earlier in embryogenesis than the type I GnRH neurons. The limited immunoreactivity of the type II and type III neurons to most GnRH antisera (Quanbeck *et al*., 1997) explains the failure of the previous

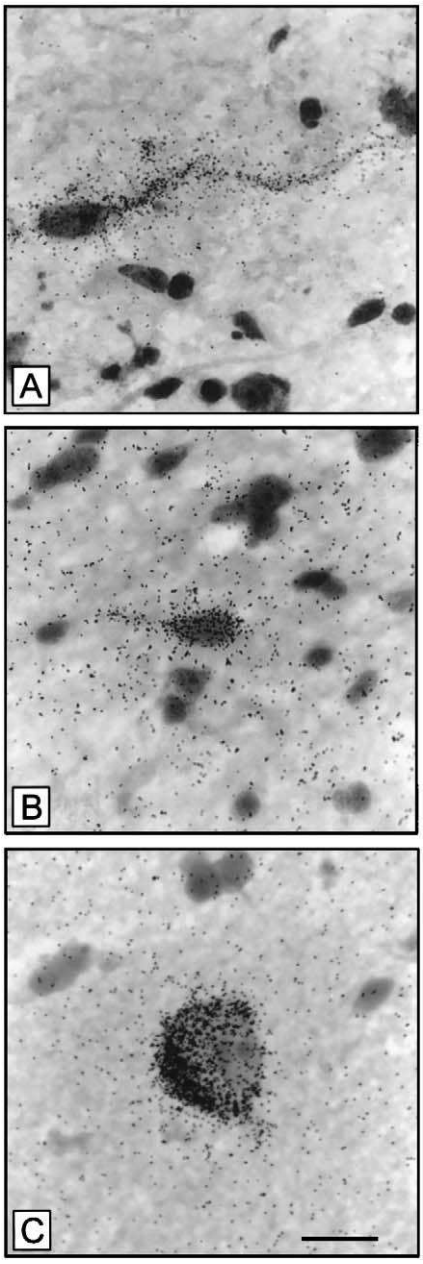

FIG. 55.1. Photomicrographs of three subtypes of neurons expressing GnRH mRNA in the human hypothalamus and basal forebrain. The silver grains mark the location of mRNA and the sections are counterstained with toluidine blue. (A) Small, elongated type I neuron in the medial basal hypothalamus. (B) Small, oval type II neuron in the septal-preoptic region. (C) Large, type III GnRH neuron in the putamen. The section in (A) was developed after 1 week exposure, compared to 3 months exposure for (B) and (C). The cell bodies of the type I neurons after 3 months of exposure are completely obscured because of the intense labeling. (Modified with permission from Rance *et al*., 1994), © Wiley-Liss, Inc., a subsidiary of John Wiley and Sons, Inc.)

immunohistochemical studies to identify these neurons in the human brain (Stopa *et al*., 1991). The location of these neurons in many extrahypothalamic regions implies that the function of GnRH neurons is not limited to reproductive processes.

VI. Gene Expression Is Increased in a Subpopulation of GnRH Neurons in the Medial Basal Hypothalamus of Postmenopausal Women

The presence of morphological subgroups of GnRH neurons suggests that different functional subpopulations of GnRH neurons exist within the human brain. Additional support for this hypothesis was obtained in a study comparing GnRH gene expression in pre- and postmenopausal women (Rance and Uswandi, 1996). In particular, the gene expression of the heavily labeled type I neurons increased in the medial basal hypothalamus of postmenopausal women (Fig. 55.2). In contrast, there was no change in gene expression in small, lightly-labeled subpopulation of type II GnRH neurons in the septal-preoptic area (Fig. 55.2). Thus, gene expression is regulated differentially among the two morphological subtypes of GnRH neurons in the human hypothalamus. More importantly, this study provided the first demonstration of an increase in GnRH gene expression in the postmenopausal human hypothalamus. Based on animal studies (Sagrillo *et al*., 1996; Herbison, 1998) we proposed that the increase in GnRH gene expression in postmenopausal women is secondary to estrogen withdrawal. This hypothesis is supported by a recent demonstration that estrogen replacement decreases GnRH gene

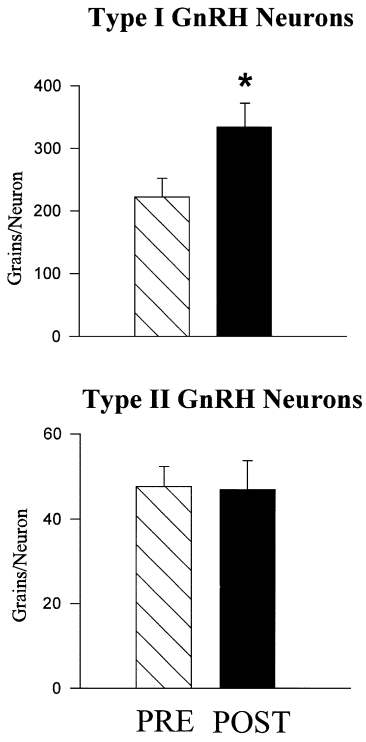

FIG. 55.2. Number of grains per neuron associated with type I (top) and type II (bottom) GnRH neurons in the hypothalamus of premenopausal and postmenopausal women. * $P<0.05$. GnRH gene expression is increased in the heavily labeled neurons (type I) in the medial basal hypothalamus but not the lightly labeled (type II) neurons in the septal-preoptic area. (Modified with permission from Rance and Uswandi, 1996, © The Endocrine Society.)

expression in the medial basal hypothalamus of ovariectomized monkeys (El Majdoubi *et al*., 1998).

The increase in GnRH gene expression in the medial basal hypothalamus of postmenopausal women provides evidence that increased GnRH secretion contributes to the elevated levels of circulating gonadotropins in these women. The hypothalamic content of the GnRH peptide, however, decreases in postmenopausal and oophorectomized young women (Parker and Porter, 1984). Although these data may appear contradictory, it is possible that the decline in peptide content reflects decreased storage of hormone due to increased release of GnRH peptide into the portal system. An analogous situation is the decrease in GnRH concentration in several discrete areas of rat hypothalamus during the proestrus surge of LH (Wise *et al*., 1981). Estrogen and inhibin withdrawal may also have direct effects on the anterior pituitary gland, contributing to the hypersecretion of gonadotropins in postmenopausal women. The loss of ovarian inhibin would directly increase FSH secretion from the anterior pituitary gland. In addition, the anterior pituitary gland in postmenopausal women is highly responsive to GnRH stimulation (Hanker *et al*., 1981). Finally, there are biochemical changes in the pituitary gland that may prolong the half-life of LH in peripheral blood (Sharpless *et al*., 1999).

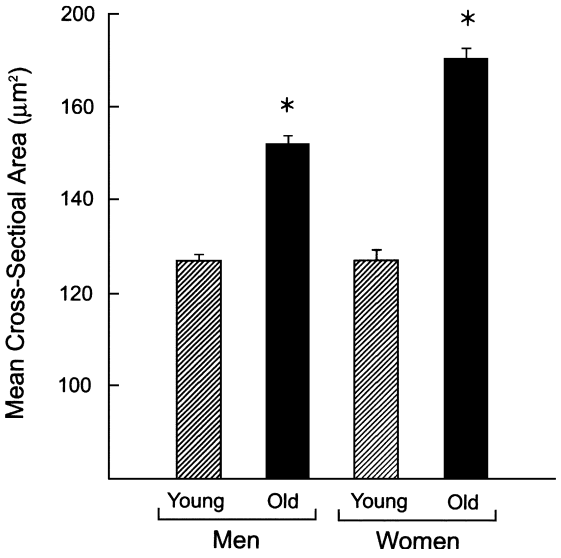

FIG. 55.3. Mean profile areas of neurons in the infundibular nucleus of men and women. * Significantly different from all other groups with $P<0.05$. (From Rance *et al*., 1993, with permission from Elsevier Science.)

VII. Postmenopausal Hypertrophy of Neurons Expressing Estrogen Receptor mRNA in the Human Infundibular Nucleus

More than 30 years ago, Sheehan and Kovacs (1966) described neuronal hypertrophy in the hypothalamus of postmenopausal women. Using semiquantitative methods, these investigators observed a pronounced enlargement of neurons in a subregion of the infundibular nucleus that they named the subventricular nucleus. The enlargement of these neurons was associated with morphologic features of hypertrophy including increased Nissl substance and enlarged nuclei and nucleoli. Based on similar findings in other hypogonadal conditions, the authors proposed that postmenopausal neuronal hypertrophy resulted from the complete loss of ovarian estrogen (Sheehan, 1967). The significance of these findings was not clear at the time, however, because relatively little was known about the role of the infundibular nucleus in reproductive regulation.

The observation of neuronal hypertrophy in the infundibular nucleus of postmenopausal women was subsequently confirmed using quantitative computer microscopy (Rance *et al*., 1990). These studies showed that the mean profile area of Nissl-stained neurons was increased by 30% in postmenopausal women. Neuronal hypertrophy also occurs in the infundibular nucleus of older men, but to a modest extent compared to that seen in postmenopausal women (Fig. 55.3) (Rance *et al*., 1993). These findings correlate well with the differences in gonadal function between the two groups. Gonadal steroids decline to castrate levels in older women (Baird and Guevara, 1969), compared to only a mild decrease in the circulating levels of testosterone in older men (Pirke and Doerr, 1970; Royer *et al*., 1984; Tenover *et al*., 1987).

The association of neuronal hypertrophy with estrogen withdrawal and gonadotropin hypersecretion in postmenopausal women suggests that the hypertrophied neurons participate in the hypothalamic circuitry regulating estrogen negative feedback. The identification of ERα mRNA within the hypertrophied neurons provided strong support for this hypothesis (Rance *et al*., 1990). Indeed, the presence of ER mRNA provides a selective marker for the subpopulation of hypertrophied infundibular neurons. The hypertrophied neurons, however, did not express GnRH mRNA (Rance *et al*., 1990). This finding was not surprising in light of previous studies showing that estrogen receptors are not present within GnRH neurons in the hypothalamus of laboratory rats (Shivers *et al*., 1983).

VIII. Hypertrophy and Increased Gene Expression of Neurons Expressing Substance P, Neurokinin B, and Estrogen Receptor mRNA in the Infundibular Nucleus of Postmenopausal Women

To determine the neuropeptide/neurotransmitter identity of the hypertrophied neurons, hypothalamic sections from postmenopausal women were screened with a panel of more than 10 oligonucleotide probes (Rance and Young, 1991). The oligonucleotide probes were targeted to peptides that have been shown to be present in the arcuate nucleus (the homolog of the primate infundibular nucleus) of laboratory rats or implicated in the hypothalamic control of reproduction. These studies revealed that the hypertrophied neurons expressed Substance P (SP) and neurokinin B (NKB) gene transcripts (Fig. 55.4). SP and NKB are both members of the tachykinin peptide family and share the same C-terminal sequence (Maggio, 1988). The SP, NKB, and ERα probes labeled a majority

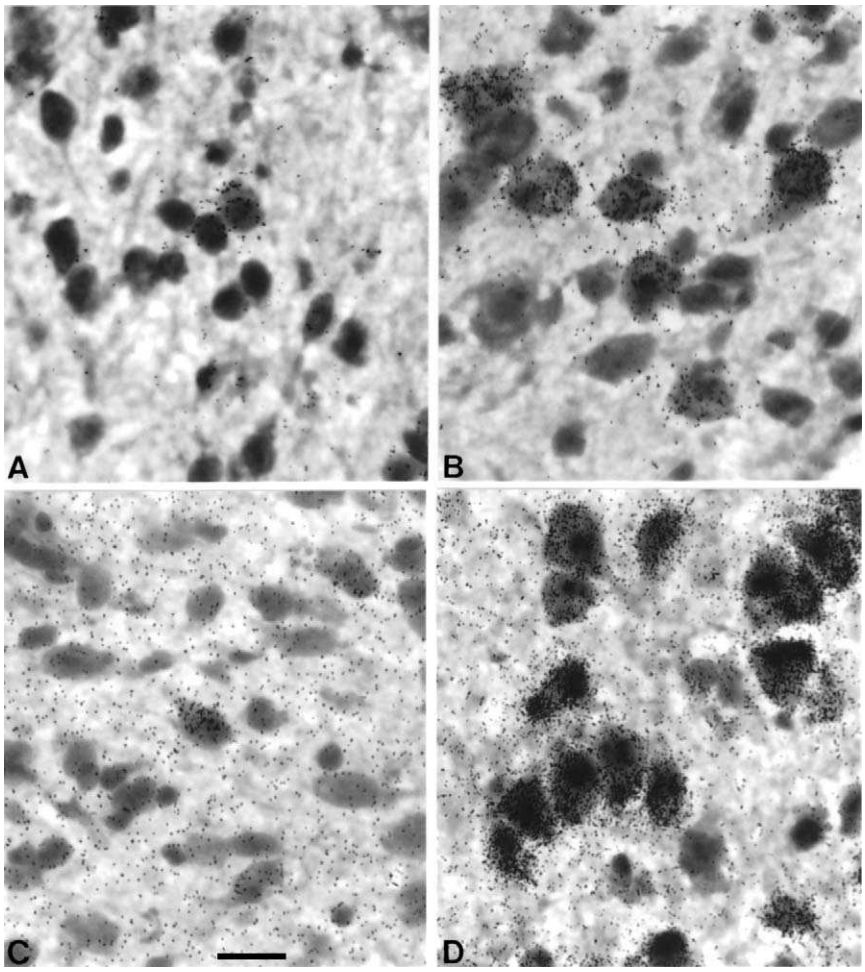

FIG. 55.4. Photomicrographs of neurons in the infundibular nucleus of a premenopausal (A,C) and a postmenopausal (B,D) woman labeled by *in situ* hybridization using probes complementary to substance P (A,B) or neurokinin B (C,D) mRNAs. The silver grains mark the location of the mRNAs and the sections are counterstained with toluidine blue. In postmenopausal women, Substance P and neurokinin B neurons are larger, more numerous, and display increased numbers of autoradiographic grains. (From Rance and Young, 1991, with permission. © The Endocrine Society.)

of hypertrophied neurons in adjacent sections, providing indirect evidence for the colocalization of these mRNAs (Rance and Young, 1991). Neurons expressing SP and NKB gene transcripts nearly doubled in mean profile area in the postmenopausal group and was accompanied by an increase in the number of autoradiographic grains per cell. The most prominent finding, however, was a marked increase in the number of neurons expressing both SP and NKB gene transcripts (Fig. 55.4). The dramatic increase in tachykinin gene expression in the hypertrophied neurons provided further evidence that the activity of these neurons increases in postmenopausal women.

The hypertrophy of ERα, SP, and NKB mRNA-containing neurons in postmenopausal women occurred in association with ovarian failure, estrogen withdrawal, increased hypothalamic GnRH gene expression, and gonadotropin hypersecretion. These findings suggest that the hypertrophied neurons participate in a hypothalamic circuit mediating estrogen negative feedback on LH release (Rance and Young, 1991). This hypothesis is further supported by animal studies suggesting a role for SP in the regulation of LH secretion. Tachykinin neurons in the rat arcuate nucleus contain receptors for gonadal steroids (Akesson and Micevych, 1988; Ciofi *et al*., 1994) and are modulated by ovarian steroids (Antonowicz *et al*., 1982; Tsuruo *et al*., 1984; Jarry *et al*., 1988; Brown *et al*., 1990; Akesson *et al*., 1991). In addition, tachykinin levels in the median eminence, as well as the number and size of arcuate tachykinin-immunoreactive neurons vary over the rat estrous cycle (Antonowicz *et al*., 1982; Tsuruo *et al*., 1984; Micevych *et al*., 1988). Furthermore, tachykinin immunoreactive neurons in the arcuate nucleus project to and synapse on LHRH neurons in the rat hypothalamus (Tsuruo *et al*., 1991).

Both inhibitory (Kerdelhué *et al*., 1997) and stimulatory (Arisawa *et al*., 1990) effects of SP on LH secretion have been described. The positive effects have been demonstrated by the stimulation of LH secretion into peripheral blood by intracerebroventricular injection of SP (Vijayan and McCann, 1979; Arisawa *et al*., 1990) and the inhibitory effect by injection of SP antiserum (Dees *et al*., 1985; Arisawa *et al*., 1990).

Moreover, intracerebroventricular injection of an SP antagonist inhibits LH secretion in castrated male rats (Dees *et al*., 1985). In an *in vitro* perfusion system, SP stimulates LH release from the rat hypothalamus and pituitary in a dose-dependent manner (Ohtsuka *et al*., 1987). More importantly, SP induces LHRH release from the isolated medial basal hypothalamus (Ohtsuka *et al*., 1987). These data suggest that the increased activity of the hypertrophied tachykinin neurons in postmenopausal women may contribute to the gonadotropin hypersecretion observed after the menopause. In contrast to the data on SP, virtually no information is available to ascertain if NKB plays a role in reproductive regulation.

IX. Long-Term Gonadectomy Results in Increased Neurokinin B Gene Expression in the Arcuate Nucleus of Both Male and Female Rats

The finding of ERα mRNA within the hypertrophied neurons in postmenopausal women provides strong support for the hypothesis that the changes in morphology and tachykinin gene expression are secondary to ovarian failure. The observations comparing pre- and postmenopausal women are confounded, however, by the presence of two variables, age and gonadal status. Although it would be of great interest to determine if ovariectomy of young women produces similar changes in the human hypothalamus, these tissues are not readily available. Therefore, the rat was used as a model to determine if withdrawal of gonadal steroids could produce alterations in neuronal morphology or tachykinin gene expression in the arcuate nucleus, the homolog of the human infundibular nucleus.

Studies of the rat arcuate nucleus provide convincing evidence that gonadal steroids modulate NKB gene expression. Long-term gonadectomy resulted in increased numbers of arcuate neurons expressing NKB gene transcripts in both males and females (Rance and Bruce, 1994; Danzer *et al*., 1999). No change in cell size was observed in female rats (Rance and Bruce, 1994). In male rats, however, the profile area of NKB neurons was increased by gonadectomy (Danzer *et al*., 1999). Furthermore, testosterone or estrogen replacement in orchidectomized male rats reduced NKB gene expression to levels observed in intact animals (Danzer *et al*., 1999). Finally, it has recently been shown that hormone replacement therapy dramatically decreases NKB gene expression in the ovariectomized long-tailed macaque monkey (described in section XII).

Interestingly, the changes in cell size after long-term gonadectomy of male rats are not confined to arcuate NKB neurons. Long-term orchidectomy also results in an increase in the mean somatic profile area of arcuate neuroendocrine neurons. These neurons are characterized by projections to the primary capillary plexus of the medium eminence. The increase in cell size after orchidectomy was accompanied by a significant growth in the dendritic tree and an increase in dendritic spines (Danzer *et al*., 1998). These data suggest that the withdrawal of gonadal steroids ultimately results in a substantial remodeling of hypothalamic neuronal circuits in the arcuate nucleus.

X. Opioid Peptides Provide an Inhibitory Influence on the Regulation of Gonadotropin Secretion in the Macaque Monkey

Endogenous opioids have an important role in the regulation of gonadotropin secretion in humans (Yen *et al*., 1985; Gindoff and Ferin, 1987; Howlett and Rees, 1987; Genazzani and Petraglia, 1989). Morphine, an opioid agonist, has a powerful inhibitory influence on LH secretion (Gindoff and Ferin, 1987). Conversely, naloxone, an opioid antagonist, increases both the frequency and amplitude of LH pulses in peripheral plasma (Ropert *et al*., 1981). Naloxone also stimulates GnRH release from an *in vitro* preparation of the human fetal hypothalamus (Rasmussen *et al*., 1983). Studies in nonhuman primates provide compelling evidence that the inhibitory effects of endogenous opioids occur at the level of the hypothalamus. Opioid antagonists increase the levels of GnRH secretion into the hypophyseal portal blood of monkeys (Pau *et al*., 1996). Furthermore, the GnRH pulse generator in the primate medial basal hypothalamus is inhibited by morphine, an effect that is blocked by naloxone (Kesner *et al*., 1986).

The efficacy of the opioid antagonists to induce LH secretion depends on the ovarian steroid milieu. In young women, naloxone stimulation of LH secretion is absent in the low-estrogen environment of the early follicular phase (Quigley and Yen, 1980) and following oophorectomy (Shoupe *et al*., 1985). Similarly, naloxone fails to increase LH secretion in estrogen-deficient postmenopausal women (Reid *et al*., 1983; Melis *et al*., 1984; Casper and Alapin-Rubillovitz, 1985; Dawood *et al*., 1986; Cagnacci *et al*., 1991). Hormone replacement therapy in oophorectomized or postmenopausal women reestablishes the LH response to naloxone (Melis *et al*., 1984; Casper and Alapin-Rubillovitz, 1985; Shoupe *et al*., 1985; Dawood *et al*., 1986; Cagnacci *et al*., 1991). These data suggest that the endogenous opioid inhibition of LH secretion (''opioid tone'') is reduced when estrogen levels are low.

There is considerable evidence that β-endorphin, one of the endogenous opioid peptides, participates in the control of reproduction in the primate. β-Endorphin neurons are located within the primate medial basal hypothalamus in the region of the GnRH pulse generator. β-Endorphin neurons synapse on GnRH neurons in the monkey hypothalamus (Thind and Goldsmith, 1988) and secretion of β-endorphin into the hypophyseal portal blood of macaque monkeys varies over the menstrual cycle (Wehrenberg *et al*., 1982). Furthermore, ovariectomy reduces the secretion of β-endorphin in portal blood, and this effect is reversed by estrogen replacement (Wardlaw *et al*., 1982b).

β-Endorphin neurons are also sensitive to the effects of aging, as demonstrated by the consistent age-associated decrease in the levels of β-endorphin peptide or precursor mRNA in the hypothalamus of both male and female rodents (Gambert *et al*., 1980; Barden *et al*., 1981; Forman *et al*., 1981; Missale *et al*., 1983; Rogers *et al*., 1985; Nelson *et al*., 1988; Lloyd *et al*., 1991; Gruenewald and Matsumoto, 1991). Therefore, both aging and changes in the levels of ovarian steroids have been linked to alterations in hypothalamic β-endorphin neurons in experimental animals.

XI. Menopause Is Associated with a Decline in the Number of Neurons Expressing Proopiomelanocortin mRNA in the Human Infundibular Nucleus

We recently showed that menopause is associated with a change in the hypothalamic expression of proopiomelanocortin mRNA, the precursor mRNA for β-endorphin. *In situ* hybridization and computer-assisted microscopy were used to investigate two anatomical sites, the infundibular nucleus and the retrochiasmatic area. In contrast to the elevation in tachykinin and GnRH gene expression in postmenopausal women, the number of infundibular neurons expressing proopiomelanocortin mRNA was reduced. This finding was region-specific, as no change was detected in the number of proopiomelanocortin neurons in the retrochiasmatic area (Fig. 55.5).

The decrease in proopiomelanocortin gene expression in postmenopausal women is consistent with the decreased ability of naloxone to elicit LH secretion in this group of women (Reid *et al*., 1983). Thus, a decline in the inhibitory input by proopiomelanocortin neurons could contribute to the elevation in GnRH gene expression and gonadotropin hypersecretion of menopause (Rance and Uswandi, 1996). In addition, β-endorphin and other products of the posttranslational processing of proopiomelanocortin have been implicated in the modulation of virtually every hypothalamic function, including reproduction, temperature regulation, nociception, reinforcement, feeding and energy balance, stress responses, and immune function (Grossman, 1983; Koob and Bloom, 1983; Reid, 1983; Ferin *et al*., 1984; Khachaturian *et al*., 1985; Mountjoy and Wong, 1997; Lipton *et al*., 1998). Endogenous opioids have also been implicated in the ovarian steroid modulation of mood (Reid, 1983) and in the mechanism of postmenopausal flushes (Casper and Yen, 1985). Therefore, an alteration of proopiomelanocortin neuronal function could have a considerable impact on the physiology of postmenopausal women.

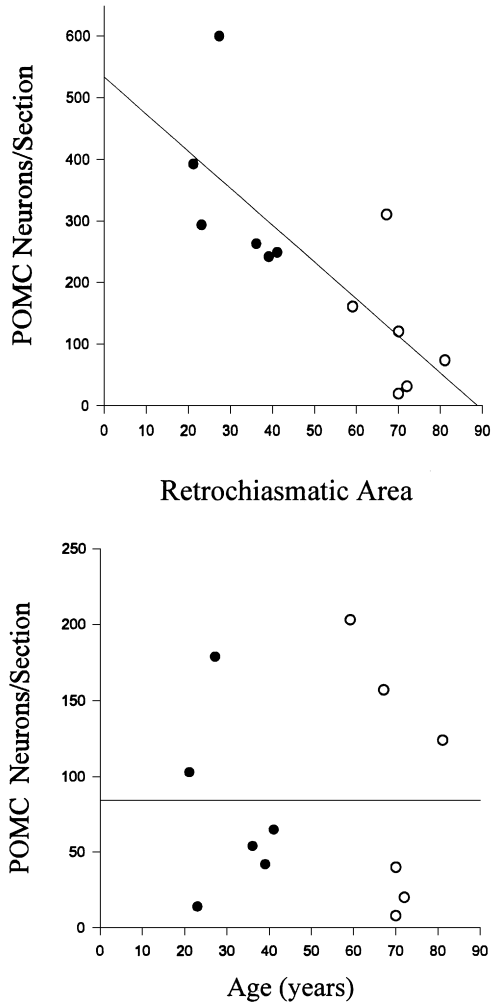

FIG. 55.5. Scatterplots and regression lines showing the relationship between age and number of neurons containing proopiomelanocortin mRNA in the infundibular nucleus (top) and retrochiasmatic area (bottom) of premenopausal (closed circles) and postmenopausal (open circles) subjects. Each data point represents the mean of one subject. There is a significant inverse relationship between subject age and number of proopiomelanocortin neurons in the infundibular nucleus ($r=-0.78$, $P=0.003$). In contrast, there is no correlation between age and the number of labeled neurons in the retrochiasmatic area. (From Abel and Rance, 1999, with permission from Elsevier Science.)

XII. Effects of Hormone Replacement Therapy on Hypothalamic Neuropeptide Gene Expression in a Primate Model of Menopause

In a study of proopiomelanocortin neurons in the infundibular nucleus of pre- and postmenopausal women (section XI), a strong inverse correlation was found between the number of neurons expressing proopiomelanocortin gene transcripts and subject age (Fig. 55.5) (Abel and Rance, 1999). These findings suggest that the decline in proopiomelanocortin gene expression is secondary to aging rather than loss of ovarian hormones. This interpretation is complicated, however, by studies in experimental animals showing modulation of β-endorphin neurons by gonadal steroids (Wardlaw *et al*., 1982a; Chowen-Breed *et al*., 1989; Adams *et al*., 1991). Therefore, a contribution from ovarian failure to changes in proopiomelanocortin gene expression in postmenopausal women could not be excluded. These questions were further explored using young, ovariectomized long-tailed macaque monkeys as a primate model of menopause (Abel *et al*., 1999). This animal model was used to test the effects of hormone replacement on hypothalamic neuropeptide gene expression.

Young, ovariectomized monkeys represent an excellent model of postmenopausal women for several reasons. The patterns of gonadotropin and ovarian steroid secretion over the monkey menstrual cycle are nearly identical to those of humans (MacDonald, 1971; Jewett and Dukelow, 1972; Williams and Hodgen, 1982; Robinson and Goy, 1986). Monkeys also exhibit ovarian failure with increasing age which culminates in menopause (Hodgen *et al*., 1977). In addition, ovariectomy in young monkeys results in an endocrine profile characterized by markedly reduced ovarian steroid levels with concomitant gonadotropin hypersecretion (Monroe *et al*., 1972) similar to the endocrine profile of menopause. Furthermore, evidence suggests that castration in young monkeys is

followed by thermoregulatory instability that shares many features of the postmenopausal flush (Jelinek *et al*., 1984; Dierschke, 1985). These data suggest that the ovariectomized young monkey is an excellent model for determining the relative contributions of gonadal steroid withdrawal and aging to postmenopausal alterations in hypothalamic gene expression.

Young monkeys were ovariectomized and divided into three groups: ovariectomized without hormone replacement therapy, ovariectomized plus estrogen, and ovariectomized plus estrogen and progesterone. Hormone replacement therapy was administered for a period of 30 months in doses designed to mimic current therapeutic regimens. *In situ* hybridization was used to label neurons expressing either NKB or proopiomelanocortin mRNAs. In the infundibular nucleus of ovariectomized monkeys without hormone replacement therapy, there were large neurons expressing NKB gene transcripts. Hormone replacement therapy, however, markedly suppressed NKB gene expression (Fig. 55.6). Indeed, no NKB neurons were visualized in the infundibular nucleus of any of the monkeys receiving hormone replacement therapy. In contrast, administration of hormone replacement therapy had no effect on several parameters of the proopiomelanocortin neurons, including the number of neurons expressing proopiomelanocortin gene transcripts, the size or shape of these neurons, or the number of autoradiographic grains per cell (Abel *et al*., 1999).

The contrasting effects of hormone replacement therapy on neuropeptide mRNAs in the macaque monkey model suggest that the changes in proopiomelanocortin and NKB gene expression in postmenopausal women may be mediated by different variables. Clearly, NKB gene expression in the primate hypothalamus is exquisitely sensitive to alterations in the steroid milieu. These data provide strong support for the hypothesis that increased NKB gene expression occurs in the hypothalamus of postmenopausal women secondary to the estrogen withdrawal of menopause. In contrast to the effects of estrogen on NKB gene expression, there was no effect of hormone replacement therapy on the proopiomelanocortin system of neurons. Thus, factors other than ovarian estrogen withdrawal may be more important in influencing this peptide system in postmenopausal women. The close correlation between the decline in proopiomelanocortin gene expression and subject age suggests that aging may be a more important factor (Fig. 55.5).

The ovariectomized, long-tailed macaque monkey was also used to determine whether long-term hormone replacement therapy produces signs of estrogen toxicity in the primate hypothalamus (Abel *et al*., 1999). The toxic effects of long-term, continuous estrogen in the arcuate nucleus of female rodents have been well-described (Finch *et al*., 1984; Desjardins *et al*., 1995), but it was not known if a similar syndrome occurs in primates. Two parameters that have been shown to be sensitive and specific markers of estrogen toxicity in the rat were examined: proopiomelanocortin neurons and microglial cells (Brawer *et al*., 1983; Desjardins *et al*., 1995). In the infundibular nucleus of ovariectomized monkeys receiving hormone replacement therapy no alterations were identified in the system of neurons expressing proopiomelanocortin mRNA or in microglial cells. Thus, we found no evidence for a neuropathological effect of sustained, unopposed estrogen in the primate hypothalamus (Abel *et al*., 1999). These findings are of considerable importance given the millions of women who are currently receiving continuous hormone replacement therapy.

XIII. Summary

Menopause is characterized by ovarian failure, gonadotropin hypersecretion, and dramatic changes in morphology and neuropeptide gene expression in medial basal hypothalamus. The

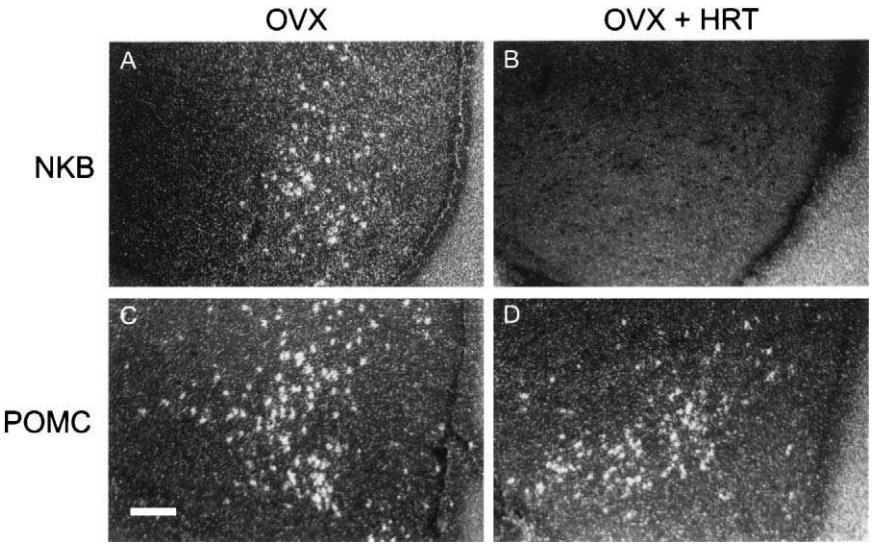

FIG. 55.6. Darkfield photomicrographs of the infundibular nucleus of ovariectomized monkeys either untreated (A and C) or receiving hormone replacement therapy (B and D). The sections have been hybridized to radiolabeled oligonucleotide probes complementary to NKB (A and B) or proopiomelanocortin (POMC; C and D) mRNAs. The clusters of silver grains show the location of labeled neurons. Administration of hormone replacement therapy (HRT) resulted in complete suppression of NKB gene expression. In contrast, proopiomelanocortin gene expression was unaffected. (From Abel *et al*., 1999, with permission. © The Endocrine Society.)

most pronounced change is the increased NKB and SP gene expression in hypertrophied infundibular neurons that coexpress ERα mRNA. GnRH gene expression also increases, but in a separate subpopulation of neurons within the medial basal hypothalamus. In contrast, the number of neurons expressing proopiomelanocortin gene transcripts is decreased within the infundibular nucleus.

Changes in gene expression have been interpreted as reflecting alterations in neuronal activity and subsequent neurosecretion. In each of the hypothalamic systems that are altered in postmenopausal women, there is evidence to support this interpretation. For example, the increase in hypothalamic tachykinin gene expression is accompanied by neuronal hypertrophy, a classic morphological sign of increased activity. In addition, the rise in GnRH gene expression is accompanied by a hypersecretion of LH and FSH from the anterior pituitary gland. Finally, pharmacological studies have demonstrated a decrease in the inhibitory influence of endogenous opioid peptides on LH release in the postmenopausal human.

The rise in tachykinin gene expression in postmenopausal women is most likely due to the estrogen-deficient state. This conclusion is based on the increase in NKB gene expression after gonadectomy of male and female rats and the suppression of NKB gene expression after hormone replacement therapy in the ovariectomized monkey. There are also considerable data in experimental animals to support the concept that removal of estrogen negative feedback results in increased hypothalamic GnRH gene expression and secretion of GnRH into hypophyseal portal blood (Sagrillo *et al*., 1996; Herbison, 1998). The mechanism for the decline in proopiomelanocortin gene expression appears more elusive, as both aging and steroid hormones have been shown to regulate this peptide in experimental animals. Hormone replacement therapy did not alter proopiomelanocortin gene expression in the ovariectomized long-tailed macaque monkey, however, suggesting that age may be a more important factor. Thus, the changes in gene expression in postmenopausal women may be both a reflection of the GnRH axis operating in a steroid-free environment and the superimposed effects of hypothalamic aging.

What is the physiological relevance of the changes in hypothalamic gene expression? The simplest explanation is that the increase in GnRH gene expression and the gonadotropin hypersecretion of menopause are secondary to an increased stimulation by tachykinin peptides, combined with a decline in the inhibitory influence of opioid peptides. This conjecture fits well into our current understanding of hypothalamic physiology in which the various patterns of GnRH secretion depend upon the coordinated participation of multiple afferent systems (Kalra *et al*., 1997). From this perspective, it also appears likely that other neuropeptide systems will be altered in postmenopausal women, although perhaps none more robustly than the tachykinin system. In addition to reproductive physiology, however, hypothalamic alterations in postmenopausal women could be related to the various symptoms associated with menopause such as hot flushes, mood changes, and subtle alterations in cognitive function. Hot flushes in particular are related to the hypothalamic control of thermoregulation, and timed with the pulsatile release of LH. Thus, understanding the hypothalamic changes in menopause will shed light not only on regulation of gonadotropin secretion and hypothalamic aging in the human, but may also have practical implications for the health of postmenopausal women.

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References

Abel, T. W., and Rance, N. E. (1999). Proopiomelanocortin gene expression is decreased in the infundibular nucleus of postmenopausal women. *Mol. Brain Res*. **69**, 202–208.

Abel, T. W., Voytko, M. L., and Rance, N. E. (1999). The effects of hormone replacement therapy on hypothalamic neuropeptide gene expression in a primate model of menopause. *J. Clin. Endocrinol. Metab.* **84**, 2111–2118.

Adams, L. A., Vician, L., Clifton, D. K., and Steiner, R. A. (1991). Testosterone regulates pro-opiomelanocortin gene expression in the primate brain. *Endocrinology (Baltimore)* **128**, 1881–1886.

Akesson, T. R., and Micevych, P. E. (1988). Estrogen concentration by substance P-immunoreactive neurons in the medial basal hypothalamus of the female rat. *J. Neurosci. Res*. **19**, 412–419.

Akesson, T. R., Sternini, C., and Micevych, P. E. (1991). Continuous estrogen decreases neurokinin B expression in the rat arcuate nucleus. *Mol. Cell. Neurosci*. **2**, 299–304.

Alexander, S. E., Aksel, S., Hazelton, J. M., Yeoman, R. R., and Gilmore, S. M. (1990). The effect of aging on hypothalamic function in oophorectomized women. *Am. J. Obstet. Gynecol*. **162**, 446–449.

Antonowicz, U., Jakubowska-Naziemblo, B., Cannon, D., and Powell, D. (1982). Immunoreactive substance P content in median eminence and pituitary gland during oestrus, dioestrus and after anterior hypothalamic deafferentation. *Endokrinologie* **79**, 25–34.

Arisawa, M., De Palatis, L., Ho, R., Snyder, G. D., Yu, W. H., Pan, G., and McCann, S. M. (1990). Stimulatory role of substance P on gonadotropin release in ovariectomized rats. *Neuroendocrinology* **51**, 523–529.

Baird, D. T., and Guevara, A. (1969). Concentration of unconjugated estrone and estradiol in peripheral plasma in nonpregnant women throughout the menstrual cycle, castrate and postmenopausal women and in men. *J. Clin. Endocrinol. Metab.* **29**, 149–156.

Barden, N., Dupont, A., Labrie, F., Mérand, Y., Rouleau, D., Vaudry, H., and Boissier, J. R. (1981). Age-dependent changes in the β-endorphin content of discrete rat brain nuclei. *Brain Res*. **208**, 209–212.

Barry, J., Hoffman, G. E., and Wray, S. (1985). LHRH-containing systems. *In* "Handbook of Chemical Neuroanatomy" (A. Björklund and T. Hökfelt, eds.), Vol. 4, Part I, pp. 166–215. Elsevier, Amsterdam.

Belchetz, P. E., Plant, T. M., Nakai, Y., Keogh, E. J., and Knobil, E. (1978). Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. *Science* **202**, 631–633.

Block, E. (1952). Quantitative morphological investigations of the follicular system in women. *Acta Anat*. **14**, 108–123.

Blum, M., McEwen, B. S., and Roberts, J. L. (1987). Transcriptional analysis of tyrosine hydroxylase gene expression in the tuberoinfundibular dopaminergic neurons of the rat arcuate nucleus after estrogen treatment. *J. Biol. Chem*. **262**, 817–821.

Brawer, J., Schipper, H., and Robaire, B. (1983). Effects of long term androgen and estradiol exposure on the hypothalamus. *Endocrinology (Baltimore)* **112**, 194–199.

Brown, E. R., Harlan, R. E., and Krause, J. E. (1990). Gonadal steroid regulation of substance P (SP) and SP-encoding messenger ribonucleic acids in the rat anterior pituitary and hypothalamus. *Endocrinology (Baltimore)* **126**, 330–340.

Burger, H. G. (1989). Inhibin, a member of a new peptide family. *Reprod. Fertil. Dev.* **1**, 1–13.

Cagnacci, A., Melis, G. B., Soldani, R., Bonuccelli, U., Piccini, P., Napolitano, A., Muratorio, A., and Fioretti, P. (1991). Altered neuroendocrine regulation of luteinizing hormone secretion in postmenopausal women with Parkinson's disease. *Neuroendocrinology* **53**, 549–555.

Casper, R. F., and Alapin-Rubillovitz, S. (1985). Progestins increase endogenous opioid peptide activity in postmenopausal women. *J. Clin. Endocrinol. Metab.* **60**, 34–36.

Casper, R. F., and Yen, S. S. C. (1985). Neuroendocrinology of menopausal flushes: An hypothesis of flush mechanism. *Clin. Endocrinol. (Oxford)* **22**, 293–312.

Chakravarti, S., Collins, W. P., Newton, J. R., Oram, D. H., and Studd, J. W. W. (1977). Endocrine changes and symptomatology after oophorectomy in premenopausal women. *Br. J. Obstet. Gynaecol.* **84**, 769–775.

Chowen-Breed, J., Fraser, H. M., Vician, L., Damassa, D. A., Clifton, D. K., and Steiner, R. A. (1989). Testosterone regulation of proopiomelanocortin messenger ribonucleic acid in the arcuate nucleus of the male rat. *Endocrinology (Baltimore)* **124**, 1697–1702.

Ciofi, P., Krause, J. E., Prins, G. S., and Mazzuca, M. (1994). Presence of nuclear androgen receptor-like immunoreactivity in neurokinin B-containing neurons of the hypothalamic arcuate nucleus of the adult male rat. *Neurosci. Lett.* **182**, 193–196.

Clarke, I. J., and Cummins, J. T. (1982). The temporal relationship between gonadotropin releasing hormone (GnRH) and luteininzing hormone (LH) secretion in ovariectomized ewes. *Endocrinology (Baltimore)* **111**, 1737–1739.

Comb, M., Hyman, S. E., and Goodman, H. M. (1987). Mechanisms of trans-synaptic regulation of gene expression. *Trends Neurosci.* **10**, 473–478.

Crowley, W. F., Jr., Filicori, M., Spratt, D. I., and Santoro, N. F. (1985). The physiology of gonadotropin-releasing hormone (GnRH) secretion in men and women. *Recent Prog. Horm. Res.* **41**, 473–531.

Danzer, S. C., McMullen, N. T., and Rance, N. E. (1998). Dendritic growth of arcuate neuroendocrine neurons following orchidectomy in adult rats. *J. Comp. Neurol.* **390**, 234–246.

Danzer, S. C., Price, R. O., McMullen, N. T., and Rance, N. E. (1999). Sex steroid modulation of neurokinin B gene expression in the arcuate nucleus of adult male rats. *Mol. Brain Res.* **66**, 200–204.

Dawood, M. Y., Khan-Dawood, F. S., and Ramos, J. (1986). The effect of estrogen-progestin treatment on opioid control of gonadotropin and prolactin secretion in postmenopausal women. *Am. J. Obstet. Gynecol.* **155**, 1246–1251.

Dees, W. L., Skelley, C. W., and Kozlowski, G. P. (1985). Central effects of an antagonist and an antiserum to substance P on serum gonadotropin and prolactin secretion. *Life Sci.* **37**, 1627–1631.

Desjardins, G. C., Beaudet, A., Meaney, M. J., and Brawer, J. R. (1995). Estrogen-induced hypothalamic beta-endorphin neuron loss: A possible model of hypothalamic aging. *Exp. Gerontol.* **30**, 253–267.

Dierschke, D. J. (1985). Temperature changes suggestive of hot flushes in rhesus monkeys: Preliminary observations. *J. Med. Primatol.* **14**, 271–280.

Ebbiary, N. A. A., Lenton, E. A., and Cooke, I. D. (1994). Hypothalamic-pituitary ageing: Progressive increase in FSH and LH concentrations throughout the reproductive life in regularly menstruating women. *Clin. Endocrinol. (Oxford)* **41**, 199–206.

El Majdoubi, M., Sahu, A., and Plant, T. M. (1998). Effect of estrogen on hypothalamic transforming growth factor alpha and gonadotropin-releasing hormone gene expression in the female rhesus monkey. *Neuroendocrinology* **67**, 228–235.

Ferin, M., Van Vugt, D., and Wardlaw, S. (1984). The hypothalamic control of the menstrual cycle and the role of endogenous opioid peptides. *Recent Prog. Horm. Res.* **40**, 441–485.

Finch, C. E., Felicio, L. S., Mobbs, C. V., and Nelson, J. F. (1984). Ovarian and steroidal influences on neuroendocrine aging processes in female rodents. *Endocr. Rev.* **5**, 467–497.

Fink, G., Sumner, B. E. H., McQueen, J. K., Wilson, H., and Rosie, R. (1998). Sex steroid control of mood, mental state and memory. *Clin. Exp. Pharmacol. Physiol.* **25**, 764–775.

Forman, L. J., Sonntag, W. E., Van Vugt, D. A., and Meites, J. (1981). Immunoreactive β-endorphin in the plasma, pituitary and hypothalamus of young and old male rats. *Neurobiol. Aging* **2**, 281–284.

Gambert, S. R., Garthwaite, T. L., Pontzer, C. H., and Hagen, T. C. (1980). Age-related changes in central nervous system beta-endorphin and ACTH. *Neuroendocrinology* **31**, 252–255.

Gambrell, R. D., Jr. (1986). The menopause. *Invest. Radiol.* **21**, 369–378.

Genazzani, A. R., and Petraglia, F. (1989). Opioid control of luteinizing hormone secretion in humans. *J. Steroid Biochem.* **33**, 751–755.

Gindoff, P. R., and Ferin, M. (1987). Brain opioid peptides and menstrual cyclicity. *Semin. Reprod. Endocrinol.* **5**, 125–133.

Goldsmith, P. C., and Song, T. (1987). The gonadotropin-releasing hormone containing ventral hypothalamic tract in the fetal rhesus monkey (*Macaca mulatta*). *J. Comp. Neurol.* **257**, 130–139.

Greene, G. L., Gilna, P., Waterfield, M., Baker, A., Hort, Y., and Shine, J. (1986). Sequence and expression of human estrogen receptor complementary DNA. *Science* **231**, 1150–1154.

Grossman, A. (1983). Brain opiates and neuroendocrine function. *Clin. Endocrinol. Metab.* **12**, 725–746.

Gruenewald, D. A., and Matsumoto, A. M. (1991). Age-related decrease in proopiomelanocortin gene expression in the arcuate nucleus of the male rat brain. *Neurobiol. Aging* **12**, 113–121.

Hanker, J. P., Ende, U., Bohnet, H. G., and Schneider, H. P. G. (1981). Intermittent stimulation with LH-RH in postmenopausal hypergonadotropism. *Horm. Metab. Res.* **13**, 696–699.

Henderson, V. W. (1997). The epidemiology of estrogen replacement therapy and Alzheimer's disease. *Neurology* **48** (Suppl. 7), S27–S35.

Herbison, A. E. (1998). Multimodal influence of estrogen upon gonadotropin-releasing hormone neurons. *Endocr. Rev.* **19**, 302–330.

Hodgen, G. D., Goodman, A. L., O'Connor, A., and Johnson, D. K. (1977). Menopause in rhesus monkeys: Model for study of disorders in the human climacteric. *Am. J. Obstet. Gynecol.* **127**, 581–584.

Howlett, T. A., and Rees, L. H. (1987). Endogenous opioid peptides and human reproduction. *In* "Oxford Reviews of Reproductive Biology" (J. R. Clarke, ed.), pp. 260–293. Clarendon Press, Oxford.

Jarry, H., Perschl, A., Meissner, H., and Wuttke, W. (1988). In vivo release rates of substance P in the preoptic/anterior hypothalamic area of ovariectomized and ovariectomized estrogen-primed rats: Correlation with luteinizing hormone and prolactin levels. *Neurosci. Lett.* **88**, 189–194.

Jelinek, J., Kappen, A., Schönbaum, E., and Lomax, P. (1984). A primate model of human postmenopausal hot flushes. *J. Clin. Endocrinol. Metab.* **59**, 1224–1228.

Jewett, D. A., and Dukelow, W. R. (1972). Cyclicity and gestation length of *Macaca fascicularis. Primates* **13**, 327–330.

Johnson, S. A., Morgan, D. G., and Finch, C. E. (1986). Extensive postmortem stability of RNA from rat and human brain. *J. Neurosci. Res*, **16**, 267–280.

Kalra, S. P., Horvath, T., Naftolin, F., Xu, B., Pu, S., and Kalra, P. S. (1997). The interactive language of the hypothalamus for the gonadotropin releasing hormone (GNRH) system. *J. Neuroendocrinol.* **9**, 569–576.

Kerdelhué, B., Gordon, K., Williams, R., Lenoir, V., Fardin, V., Chevalier, P., Garret, C., Duval, P., Kolm, P., Hodgen, G., Jones, H., and Jones, G. S. (1997). Stimulatory effect of a specific substance P antagonist (RPR 100893) of the human NK_1 receptor on the estradiol- induced LH and FSH surges in the ovariectomized cynomolgus monkey. *J. Neurosci. Res.* **50**, 94–103.

Kesner, J. S., Kaufman, J. M., Wilson, R. C., Kuroda, G., and Knobil, E. (1986). The effect of morphine on the electrophysiological activity of the hypothalamic luteinizing hormone-releasing hormone pulse generator in the rhesus monkey. *Neuroendocrinology* **43**, 686–688.

Khachaturian, H., Lewis, M. E., Tsou, K., and Watson, S. J. (1985). β-endorphin, α-MSH, ACTH, and related peptides. *In* "Handbook of Chemical Neuroanatomy" (A. Björklund and T. Hökfelt, eds.), Vol. 4, Part I, pp. 216–255. Elsevier, Amsterdam.

Klein, N. A., Illingworth, P. J., Groome, N. P., McNeilly, A. S., Battaglia, D. E., and Soules, M. R. (1996). Decreased inhibin B secretion is associated with the monotropic FSH rise in older, ovulatory women: A study of serum and follicular fluid levels of dimeric inhibin A and B in spontaneous menstrual cycles. *J. Clin. Endocrinol. Metab.* **81**, 2742–2745.

Knobil, E. (1990). The GnRH pulse generator. *Am. J. Obstet. Gynecol.* **163**, 1721–1727.

Koob, G. F., and Bloom, F. E. (1983). Behavioural effects of opioid peptides. *Br. Med. Bull.* **39**, 89–94.

Krey, L. C., Butler, W. R., and Knobil, E. (1975). Surgical disconnection of the medial basal hypothalamus and pituitary function in the rhesus monkey. I. Gonadotropin secretion. *Endocrinology (Baltimore)* **96**, 1073–1087.

Kuiper, G. G. J. M., Enmark, E., Pelto-Huikko, M., Nilsson, S., and Gustafsson, J.-A. (1996). Cloning of a novel estrogen receptor expressed in rat prostate and ovary. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 5925–5930.

Lipton, J. M., Catania, A., and Delgado, R. (1998). Peptide modulation of inflammatory processes within the brain. *Neuroimmunomodulation* **5**, 178–183.

Lloyd, J. M., Scarbrough, K., Weiland, N. G., and Wise, P. M. (1991). Age-related changes in proopiomelanocortin (POMC) gene expression in the periarcuate region of ovariectomized rats. *Endocrinology (Baltimore)* **129**, 1896–1902.

MacDonald, G. J. (1971). Reproductive patterns of three species of macaques. *Fertil. Steril.* **22**, 373–377.

Maggio, J. E. (1988). Tachykinins. *Annu. Rev. Neurosci.* **11**, 13–28.

Matt, D. W., Kauma, S. W., Pincus, S. M., Veldhuis, J. D., and Evans, W. S. (1998). Characteristics of luteinizing hormone secretion in younger versus older premenopausal women. *Am. J. Obstet. Gynecol.* **178**, 504–510.

Melis, G. B., Paoletti, A. M., Gambacciani, M., Mais, V., and Fioretti, P. (1984). Evidence that estrogens inhibit LH secretion through opioids in postmenopausal women using naloxone. *Neuroendocrinology* **39**, 60–63.

Micevych, P. E., Matt, D. W., and Go, V. L. W. (1988). Concentrations of cholecystokinin, substance P, and bombesin in discrete regions of male and female rat brain: Sex differences and estrogen effects. *Exp. Neurol.* **100**, 416–425.

Missale, C., Govoni, S., Croce, L., Bosio, A., Spano, P. F., and Trabucchi, M. (1983). Changes of β-endorphin and met-enkephalin content in the hypothalamus-pituitary axis induced by aging. *J. Neurochem.* **40**, 20–24.

Monroe, S. E., Jaffe, R. B., and Midgley, A. R., Jr. (1972). Regulation of human gonadotropins. XIII. Changes in serum gonadotropins in menstruating women in response to oophorectomy. *J. Clin. Endocrinol. Metab.* **34**, 420–422.

Moss, R. L., and McCann, S. M. (1973). Induction of mating behavior in rats by luteinizing hormone-releasing factor. *Science* **181**, 177–179.

Mountjoy, K. G., and Wong, J. (1997). Obesity, diabetes and functions for proopiomelanocortin-derived peptides. *Mol. Cell. Endocrinol.* **128**, 171–177.

Nabekura, J., Oomura, Y., Minami, T., Mizuno, Y., and Fukuda, A. (1986). Mechanism of the rapid effect of 17β-estradiol on medial amygdala neurons. *Science* **233**, 226–228.

Nakai, Y., Plant, T. M., Hess, D. L., Keogh, E. J., and Knobil, E. (1978). On the sites of the negative and positive feedback actions of estradiol in the control of gonadotropin secretion in the rhesus monkey. *Endocrinology (Baltimore)* **102**, 1008–1014.

Nauta, W. J. H., and Haymaker, W. (1969). Hypothalamic nuclei and fiber connections. *In* "The Hypothalamus" (W. Haymaker, E. Anderson, and W. J. H. Nauta, eds.), pp. 136–209. Thomas, Springfield, IL.

Nelson, J. F., Bender, M., and Schachter, B. S. (1988). Age-related changes in proopiomelanocortin messenger ribonucleic acid levels in hypothalamus and pituitary of female C57BL/6J mice. *Endocrinology (Baltimore)* **123**, 340–344.

Nelson, J. F., Karelus, K., Bergman, M. D., and Felicio, L. S. (1995). Neuroendocrine involvement in aging: Evidence from studies of reproductive aging and caloric restriction. *Neurobiol. Aging* **16**, 837–843.

O'Byrne, K. T., and Knobil, E. (1993). Electrophysiological approaches to gonadotrophin releasing hormone pulse generator activity in the rhesus monkey. *Hum. Reprod.* **8** (Suppl. 2), 37–40.

O'Byrne, K. T., Chen, M.-D., Nishihara, M., Williams, C. L., Thalabard, J.-C., Hotchkiss, J., and Knobil, E. (1993). Ovarian control of gonadotropin hormone-releasing hormone pulse generator activity in the rhesus monkey: Duration of the associated hypothalamic signal. *Neuroendocrinology* **57**, 588–592.

Odell, W. D., and Swerdloff, R. S. (1968). Progestogen-induced luteinizing and follicle-stimulating hormone surge in postmenopausal women: A simulated ovulatory peak. *Proc. Natl. Acad. Sci. U.S.A.* **61**, 529–536.

Ohtsuka, S., Miyake, A., Nishizaki, T., Tasaka, K., Aono, T., and Tanizawa, O. (1987). Substance P stimulates gonadotropin-releasing hormone release from rat hypothalamus in vitro with involvement of oestrogen. *Acta Endocrinol. (Copenhagen)* **115**, 247–252.

Paganini-Hill, A. (1996). Oestrogen replacement therapy and Alzheimer's disease. *Br. J. Obstet. Gynaecol.* **103** (Suppl. 13), 80–86.

Parker, C. R., Jr., and Porter, J. C. (1984). Luteinizing hormone-releasing hormone and thyrotropin-releasing hormone in the hypothalamus of women: Effects of age and reproductive status. *J. Clin. Endocrinol. Metab.* **58**, 488–491.

Pau, K.-Y. F., Berria, M., Hess, D. L., and Spies, H. G. (1996). Opiatergic influence on gonadotropin-releasing hormone and luteinizing hormone release during the macaque menstrual cycle. *Biol. Reprod.* **55**, 478–484.

Petersen, S. L., McCrone, S., Keller, M., and Gardner, E. (1991). Rapid increase in LHRH mRNA levels following NMDA. *Endocrinology (Baltimore)* **129**, 1679–1681.

Pfaff, D. W. (1973). Luteinizing hormone-releasing factor potentiates lordosis behavior in hypophysectomized ovariectomized female rats. *Science* **182**, 1148–1149.

Pirke, K. M., and Doerr, P. (1970). Age related changes in free plasma testosterone, dihydrotestosterone and oestradiol. *Acta Endocrinol. (Copenhagen)* **80**, 171–178.

Plant, T. M., Krey, L. C., Moossy, J., McCormack, J. T., Hess, D. L., and Knobil, E. (1978). The arcuate nucleus and the control of gonadotropin and prolactin secretion in the female rhesus monkey (*Macaca mulatta*). *Endocrinology* **102**, 52–62.

Pohl, C. R., Richardson, D. W., Hutchison, J. S., Germak, J. A., and Knobil, E. (1983). Hypophysiotropic signal frequency and the functioning of the pituitary-ovarian system in the rhesus monkey. *Endocrinology (Baltimore)* **112**, 2076–2080.

Quanbeck, C., Sherwood, N. M., Millar, R. P., and Terasawa, E. (1997). Two populations of luteinizing hormone-releasing hormone neurons in the forebrain of the rhesus macaque during embryonic development. *J. Comp. Neurol.* **380**, 293–309.

Quigley, M. E., and Yen, S. S. C. (1980). The role of endogenous opiates on LH secretion during the menstrual cycle. *J. Clin. Endocrinol. Metab.* **51**, 179–181.

Rance, N. E., and Bruce, T. R. (1994). Neurokinin B gene expression is increased in the arcuate nucleus of ovariectomized rats. *Neuroendocrinology* **60**, 337–345.

Rance, N. E., and Uswandi, S. V. (1996). Gonadotropin-releasing hormone gene expression is increased in the medial basal hypothalamus of postmenopausal women. *J. Clin. Endocrinol. Metab.* **81**, 3540–3546.

Rance, N. E., and Young, W. S., III (1991). Hypertrophy and increased gene expression of neurons containing neurokinin-B and substance-P messenger ribonucleic acids in the hypothalami of postmenopausal women. *Endocrinology (Baltimore)* **128**, 2239–2247.

Rance, N. E., McMullen, N. T., Smialek, J. E., Price, D. L., and Young, W. S., III (1990). Postmenopausal hypertrophy of neurons expressing

the estrogen receptor gene in the human hypothalamus. *J. Clin. Endocrinol. Metab.* **71**, 79–85.

Rance, N. E., Uswandi, S. V., and McMullen, N. T. (1993). Neuronal hypertrophy in the hypothalamus of older men. *Neurobiol. Aging* **14**, 337–342.

Rance, N. E., Young, W. S., III, and McMullen, N. T. (1994). Topography of neurons expressing luteinizing hormone-releasing hormone gene transcripts in the human hypothalamus and basal forebrain. *J. Comp. Neurol*. **339**, 573–586.

Rasmussen, D. D., Liu, J. H., Wolf, P. L., and Yen, S. S. C. (1983). Endogenous opioid regulation of gonadotropin-releasing hormone release from the human fetal hypothalamus *in vitro*. *J. Clin. Endocrinol. Metab.* **57**, 881–884.

Reid, R. L. (1983). Endogenous opioid activity and the premenstrual syndrome. *Lancet* **2**, 786.

Reid, R. L., Quigley, M. E., and Yen, S. S. C. (1983). The disappearance of opioidergic regulation of gonadotropin secretion in postmenopausal women. *J. Clin. Endocrinol. Metab.* **57**, 1107–1110.

Reyes, F. I., Winter, J. S. D., and Faiman, C. (1977). Pituitary-ovarian relationships preceding the menopause. I. A cross-sectional study of serum follice-stimulating hormone, luteinizing hormone, prolactin, estradiol, and progesterone levels. *Am. J. Obstet. Gynecol*. **129**, 557–564.

Richardson, S. J., Senikas, V., and Nelson, J. F. (1987). Follicular depletion during the menopausal transition: Evidence for acclelerated loss and ultimate exhaustion. *J. Clin. Endocrinol. Metab.* **65**, 1231–1237.

Robinson, J. A., and Goy, R. W. (1986). Steroid hormones and the ovarian cycle. *In* ''Comparative Primate Biology'' (R. W. Dukelow and J. Erwin, eds.), Vol. 3, pp. 63–91. Liss, New York.

Rogers, J., Shoemaker, W. J., Morgan, D. G., and Finch, C. E. (1985). Senescent change in tissue weight and immunoreactive β-endorphin, enkephalin and vasopressin in eight regions of C57BL/6J mouse brain and pituitary. *Neurobiol. Aging* **6**, 1–9.

Ropert, J. F., Quigley, M. E., and Yen, S. S. C. (1981). Endogenous opiates modulate pulsatile luteinizing hormone release in humans. *J. Clin. Endocrinol. Metab.* **52**, 583–585.

Rossmanith, W. G., Scherbaum, W. A., and Lauritzen, C. (1991). Gonadotropin secretion during aging in postmenopausal women. *Neuroendocrinology* **54**, 211–218.

Royer, G. L., Jr., Seckman, C. E., Schwartz, J. H., Bennett, K. P., and Hendrix, J. W. (1984). Relationship between age and levels of total, free, and bound testosterone in healthy subjects. *Curr. Ther. Res*. **35**, 345–353.

Sagrillo, C. A., Grattan, D. R., McCarthy, M. M., and Selmanoff, M. K. (1996). Hormonal and neurotransmitter regulation of GnRH gene expression and related reproductive behaviors. *Behav. Genet*. **26**, 241–277.

Scaglia, H., Medina, M., Pinto-Ferreira, A. L., Vázques, G., Gual, C., and Pérez-Palacios, G. (1976). Pituitary LH and FSH secretion and responsiveness in women of old age. *Acta Endocrinol. (Copenhagen)* **81**, 673–679.

Schwanzel-Fukuda, M., and Pfaff, D. W. (1989). Origin of luteinizing hormone-releasing hormone neurons. *Nature (London)* **338**, 161–164.

Schwanzel-Fukuda, M., Crossin, K. L., Pfaff, D. W., Bouloux, P. M. G., Hardelin, J.-P., and Petit, C. (1996). Migration of luteinizing hormone-releasing hormone (LHRH) neurons in early human embryos. *J. Comp. Neurol*. **366**, 547–557.

Sharpless, J. L., Supko, J. G., Martin, K. A., and Hall, J. E. (1999). Disappearance of endogenous luteinizing hormone is prolonged in postmenopausal women. *J. Clin. Endocrinol. Metab.* **84**, 688–694.

Sheehan, H. L. (1967). Variations in the subventricular nucleus. *J. Pathol. Bacteriol*. **94**, 409–416.

Sheehan, H. L., and Kovács, K. (1966). The subventricular nucleus of the human hypothalamus. *Brain* **89**, 589–614.

Sherwin, B. B. (1991). The impact of different doses of estrogen and progestin on mood and sexual behavior in postmenopausal women. *J. Clin. Endocrinol. Metab.* **72**, 336–343.

Sherwin, B. B. (1996). Hormones, mood, and cognitive functioning in postmenopausal women. *Obstet. Gynecol*. **87**, 20S–26S.

Shivers, B. D., Harlan, R. E., Morrell, J. I., and Pfaff, D. W. (1983). Absence of oestradiol concentration in cell nuclei of LHRH-immunoreactive neurones. *Nature (London)* **304**, 345–347.

Shoupe, D., Montz, F. J., and Lobo, R. A. (1985). The effects of estrogen and progestin on endogenous opioid activity in oophorectomized women. *J. Clin. Endocrinol. Metab.* **60**, 178–183.

Silverman, A. J., Antunes, J. L., Ferin, M., and Zimmerman, E. A. (1977). The distribution of luteinizing hormone-releasing hormone (LHRH) in the hypothalamus of the rhesus monkey. Light microscopic studies using immunoperoxidase technique. *Endocrinology (Baltimore)* **101**, 134–142.

Silverman, A. J., Antunes, J. L., Abrams, G. M., Nilaver, G., Thau, R., Robinson, J. A., Ferin, M., and Krey, L. C. (1982). The luteinizing hormone-releasing hormone pathways in rhesus (*Macaca mulatta*) and pigtailed (*Macaca nemestrina*) monkeys: New observations on thick, unembedded sections. *J. Comp. Neurol*. **211**, 309–317.

Silverman, A.-J., Wilson, R., Kesner, J. S., and Knobil, E. (1986). Hypothalamic localization of multiunit electrical activity associated with pulsatile LH release in the rhesus monkey. *Neuroendocrinology* **44**, 168–171.

Soules, M. R., Battaglia, D. E., and Klein, N. A. (1998). Inhibin and reproductive aging in women. *Maturitas* **30**, 193–204.

Spanagel, R., Herz, A., and Shippenberg, T. S. (1992). Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proc. Natl. Acad. Sci. U.S.A*. **89**, 2046–2050.

Speroff, L. (1993). Menopause and hormone replacement therapy. *Clin. Geriatr. Med*. **9**, 33–54.

Stopa, E. G., Koh, E. T., Svendsen, C. N., Rogers, W. T., Schwaber, J. S., and King, J. C. (1991). Computer-assisted mapping of immunoreactive mammalian gonadotropin-releasing hormone in adult human basal forebrain and amygdala. *Endocrinology (Baltimore)* **128**, 3199–3207.

Tenover, J. S., Matsumoto, A. M., Plymate, S. R., and Bremner, W. J. (1987). The effects of aging in normal men on bioavailable testosterone and luteinizing hormone secretion: Response to clomiphene citrate. *J. Clin. Endocrinol. Metab.* **65**, 1118–1126.

Thind, K. K., and Goldsmith, P. C. (1988). Infundibular gonadotropin-releasing hormone neurons are inhibited by direct opioid and autoregulatory synapses in juvenile monkeys. *Neuroendocrinology* **47**, 203–216.

Tsuruo, Y., Hisano, S., Okamura, Y., Tsukamoto, N., and Daikoku, S. (1984). Hypothalamic substance P-containing neurons, sex-dependent topographical differences and ultrastructural transformations associated with stages of the estrous cycle. *Brain Res*. **305**, 331–341.

Tsuruo, Y., Kawano, H., Hisano, S., Kagotani, Y., Daikoku, S., Zhang, T., and Yanaihara, N. (1991). Substance P-containing neurons innervating LHRH-containing neurons in the septo-preoptic area of rats. *Neuroendocrinology* **53**, 236–245.

Uhl, G. R., and Nishimori, T. (1990). Neuropeptide gene expression and neural activity: assessing a working hypothesis in nucleus caudalis and dorsal horn neurons expressing preproenkephalin and preprodynorphin. *Cell. Mol. Neurobiol*. **10**, 73–98.

Vijayan, E., and McCann, S. M. (1979). *In vivo* and *in vitro* effects of substance P and neurotensin on gonadotropin and prolactin release. *Endocrinology (Baltimore)* **105**, 64–68.

Wallach, E. E., Root, A. W., and Garcia, C.-R. (1970). Serum gonadotropin responses to estrogen and progestogen in recently castrated human females. *J. Clin. Endocrinol. Metab.* **31**, 376–381.

Wardlaw, S. L., Thoron, L., and Frantz, A. G. (1982a). Effects of sex steroids on brain β-endorphin. *Brain Res*. **245**, 327–331.

Wardlaw, S. L., Wehrenberg, W. B., Ferin, M., Antunes, J. L., and Frantz, A. G. (1982b). Effect of sex steroids on β-endorphin in hypophyseal portal blood. *J. Clin. Endocrinol. Metab.* **55**, 877–881.

Wehrenberg, W. B., Wardlaw, S. L., Frantz, A. G., and Ferin, M. (1982). β-endorphin in hypophyseal portal blood: Variations throughout the menstrual cycle. *Endocrinology (Baltimore)* **111**, 879–881.

Wetsel, W. C., Valença, M. M., Merchenthaler, I., Liposits, Z., López, F. J., Weiner, R. I., Mellon, P. L., and Negro-Vilar, A. (1992). Intrinsic

pulsatile secretory activity of immortalized luteinizing hormone-releasing hormone-secreting neurons. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 4149–4153.

Wildt, L., Häusler, A., Marshall, G., Hutchison, J. S., Plant, T. M., Belchetz, P. E., and Knobil, E. (1981). Frequency and amplitude of gonadotropin-releasing hormone stimulation and gonadotropin secretion in the rhesus monkey. *Endocrinology (Baltimore)* **109**, 376–385.

Williams, R. F., and Hodgen, G. D. (1982). The reproductive cycle in female macaques. *Am. J. Primatol*. **1** (Suppl.), 181–192.

Wise, P. M., Rance, N., Barr, G. D., and Barraclough, C. A. (1979). Further evidence that luteinizing hormone-releasing hormone also is follicle-stimulating hormone-releasing hormone. *Endocrinology (Baltimore)* **104**, 940–947.

Wise, P. M., Rance, N., Selmanoff, M., and Barraclough, C. A. (1981). Changes in radioimmunoassayable luteinizing hormone-releasing hormone in discrete brain areas of the rat at various times on proestrus, diestrous day 1, and after phenobarbital administration. *Endocrinology (Baltimore)* **108**, 2179–2185.

Wise, P. M., Kashon, M. L., Krajnak, K. M., Rosewell, K. L., Cai, A., Scarbrough, K., Harney, J. P., McShane, T., Lloyd, J. M., and Weiland, N. G. (1997). Aging of the female reproductive system: A window into brain aging. *Recent. Prog. Horm. Res.* **52**, 279–305.

Wray, S., Grant, P., and Gainer, H. (1989). Evidence that cells expressing luteinizing hormone-releasing hormone mRNA in the mouse are derived from progenitor cells in the olfactory placode. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8132–8136.

Yen, S. S. C., Quigley, M. E., Reid, R. L., Ropert, J. F., and Cetel, N. S. (1985). Neuroendocrinology of opioid peptides and their role in the control of gonadotropin and prolactin secretion. *Am. J. Obstet. Gynecol.* **152**, 485–493.

Young, W. S., III, and Zoeller, R. T. (1987). Neuroendocrine gene expression in the hypothalamus: *In situ* hybridization histochemical studies. *Cell. Mol. Neurobiol*. **7**, 353–366.

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Neuroendocrine Aspects of Female Reproductive Aging

I. Introduction

The female reproductive system has been a well studied model in research on aging of neuroendocrine systems for several reasons. First, it illustrates how changes in complex positive and negative interactions between the hypothalamus, pituitary, and peripheral endocrine targets each contribute and ultimately lead to permanent age-related changes. Second, age-related infertility occurs relatively early during the life span of many mammalian species. Therefore, we anticipate that this system will provide insights into the aging process in the absence of confounding pathological changes that complicate many studies in aging. Third, since the average life span of women has increased dramatically during the past century, an increasing fraction of women will live a larger proportion of their lives in the postmenopausal state. Hence, understanding the regulation of the menopausal transition and the repercussions of the menopause will aid in the treatment of health care needs of a significant portion of the elderly population. Fourth, it has become increasingly clear that estrogen affects far more than just reproductive functions and ''classic'' reproductive target organs. Indeed, estrogen influences bone and mineral metabolism, blood pressure and cardiovascular function, memory and cognition, circadian rhythmicity and the incidence and progression of age-related neurodegenerative diseases, such as Alzheimer's disease. Thus, the cessation of menses and resulting hypoestrogenicity impacts multiple and diverse physiological systems and a better understanding of the factors that lead to age-related infertility becomes more and more important.

For many years, it has been accepted that the menopause resulted simply from an exhaustion of the pool of follicles in the ovary (vom Saal *et al*., 1994) and that hypothalamic/pituitary alterations occurred in response to deteriorating ovarian function. However, recent findings suggest that both the brain and the ovaries are involved in female reproductive senescence. Although ovarian follicles are virtually depleted in postmenopausal women (Block, 1952; Costoff and Mahesh, 1975), several lines of evidence have led investigators to believe that the brain contributes to the sequence of events that lead to reproductive decline. Thus, subtle changes in the temporal pattern and synchrony of neural and endocrine signals, which are detectable in both women (Matt *et al*., 1998) and animal models (Wise *et al*., 1997) prior to the cessation of reproductive cycles, may contribute to the accelerated loss of follicles that occurs during the middle-age transition to acyclicity and infertility.

Most of the studies on the role of the neuroendocrine axis in the transition to infertility have been performed in laboratory animal models since many of the approaches are invasive and/or require euthanizing the animal. By definition, rodents do not undergo the ''meno''-pause, since they never experience menses during their reproductive life span. Nevertheless, rodents have been excellent experimental models and have provided important insights into factors that regulate other stages of reproductive life such as pre- and perinatal development, puberty, and maintenance of regular cycles in the adult. In some cases, studies using indirect methods suggest that parallel changes occur in humans and laboratory rodents during normal or pathological aging. Therefore, it is likely that changes in the hypothalamic–anterior pituitary axis occur during middle age in women and contribute to the cascade of events that lead to the menopause. Furthermore, we believe that information gained from studies performed in laboratory animal models regarding mechanisms that regulate the transition to acyclicity and infertility can be extrapolated to humans and will allow us to formulate concepts that may be generalized to human reproductive aging.

Some of the earliest evidence suggesting that the hypothalamus plays a role in reproductive aging came from two experimental approaches. First, transplantation of ovaries of old animals to the kidney capsule of young ovariectomized females hosts, revealed that follicular development and ovulation occurred under the influence of neuroendocrine signals of the young host (Peng and Huang, 1972; Aschheim, 1983). This shows that depletion of ovarian oocytes is not the cause of the acyclic state Second, administration of drugs that restored the level of activity of monoaminergic neurotransmitters,

progesterone treatment or electrochemical stimulation of the preoptic area of old rats (Everett, 1940, 1943, 1980; Clemens *et al*., 1969; Quadri *et al*., 1973; Huang *et al*., 1976; Clemens and Bennett, 1977; Cooper, 1977; Cooper and Walker, 1979; Everett and Tyrey, 1982) resulted in restoration of estrous cyclicity. These results implicate changing hypothalamic function as a crucial element in reproductive decline. More recent studies that have focused their attention on the middle-age transition period, suggest that hypothalamic changes, although subtle, may contribute to the onset of irregular cycles that ultimately lead to acyclicity (for review, see Wise *et al*., 1997).

II. Changes in the Pattern of Gonadotropin Secretion Occur during Middle Age

The patterns of secretion of both luteinizing hormone (LH) and follicle stimulating hormone (FSH) are altered during middle age and may reflect changes in hypothalamo-pituitary function. Both the preovulatory surge of LH, as well as the pulsatile secretion of LH, are altered during middle age. The preovulatory LH surge is delayed and attenuated in middle-aged rats prior to overt changes in the length or regularity of the LH surge (Cooper *et al*., 1980; Wise, 1982b; Nass *et al*., 1984). In fact, Nass *et al*. (1984) found that alterations in the preovulatory LH surge occurred in only some of the middle-aged rat and that this was an excellent predictor of which animals were likely to begin the transition to irregular cycles within the next few cycles (Fig. 56.1). Changes in pulsatile LH secretion are apparent in middle-aged laboratory animals and in women (Fig. 56.2). We (Scarbrough and Wise, 1990) found that the interpulse interval and average duration of individual pulses increased with age and with reproductive decline and the amplitude of LH pulses decreased. Similar changes have been reported recently in middle-aged women prior to any change in menstrual cycle length. Matt *et al*. (1998) demonstrated a significant increase in the interpulse interval and the duration of individual LH pulses in regularly cycling middle-aged women during the mid to late follicular phase of the menstrual cycle. Interestingly, other investigators have found either an increase in LH pulse frequency or no change during the perimenopausal period (Reame *et al*., 1996). Thus, it appears that changes in the characteristics of pulsatile gonadotropin secretion may depend upon the time of the menstrual cycle studied, whether subjects have already exhibited changes in menstrual cycle length or the method used to analyze LH pulses. Together, these data strongly suggest that subtle changes in the integrity of the GnRH pulse generator occur early, prior to the transition from regular to irregular cycles, and may be a component of the cascade of events that contributes to reproductive aging.

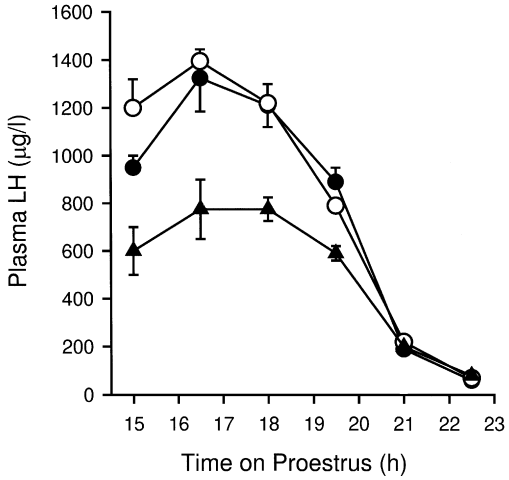

FIG. 56.1. Plasma LH concentrations in young and middle-aged rats on proestrus. Middle-aged rats that became irregularly cycling within 1–2 months after the blood samples were collected (triangles) showed significantly attenuated LH surges compared to young (solid circles) or middle-aged rats that continued to cycle (open circles). Each data point represents the mean ± SE. From Nass *et al*. (1984).

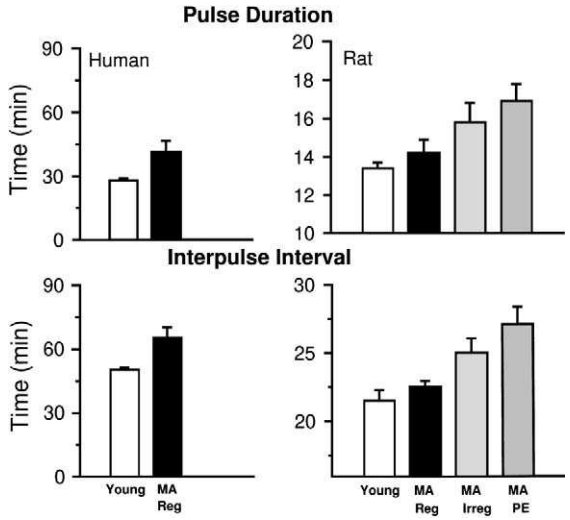

FIG. 56.2. (Left) LH pulse duration and interpulse interval obtained from 8 hr of sampling during the mid to late follicular phase of menstrual cycle in young and middle-aged women. Each data point represents the mean ± SE. Modified from Matt *et al*. (1998). (Right) LH pulse duration and interpulse interval of ovariectomized young and middle-aged rats at various stages of reproductive senescence obtained from 3 hr of sampling.). Each data point represents the mean ± SE. Modified from Scarbrough and Wise (1990).

Increases in plasma FSH have been reported in both middle-aged rats and women; however, whether these alterations can be explained by changes in hypothalamic influences or by changes in ovarian inhibin secretion are not clear. Using a newly developed assay that differentiates between inhibin A and B and the nonbiologically active forms, Klein *et al*. (1996) reported a significant selective decline in plasma inhibin B in middle-aged women who exhibited a selective increase in plasma FSH (Fig. 56.3). These data are strikingly similar to the selective increase in FSH reported in middle-aged cycling rats that were detectable when inhibin-like activity in the ovarian vein decreased (DePaolo, 1987) (Fig. 56.3). Both of these groups of investigators argued that the rise in FSH resulted only from changes in ovarian function. However, it is important to bear in mind that the ratio of FSH:LH secreted is influenced by the pattern of pulsatile GnRH secretion. FSH

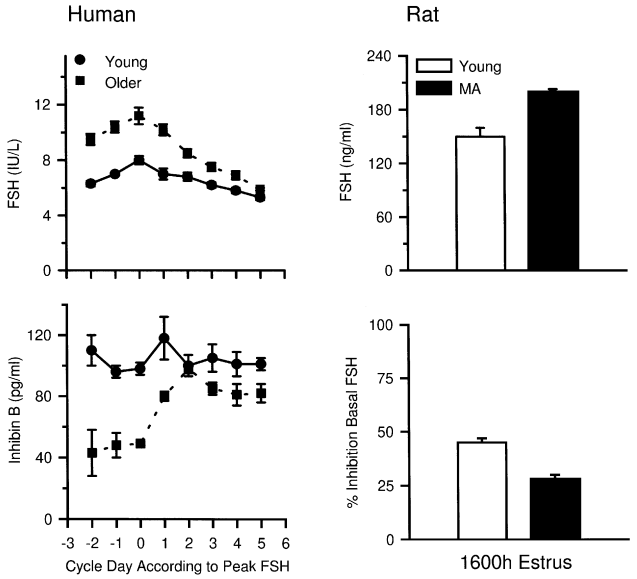

FIG. 56.3. (Left) Daily FSH (top) and inhibin B (bottom) in older and younger women. Each data point represents the mean ± SE. Modified from Klein *et al*. (1996). (Right) Plasma FSH and FSH suppressing activity in ovarian vein serum in 3- and 7-month-old rats during the periovulatory period. Each data point represents the mean ± SE. Modified from DePaolo (1987).

secretion is favored by a slowing in the frequency of GnRH pulses (Knobil, 1980), which is thought to occur with age. Thus, the selective and monotropic rise in FSH during the early stages of the menopausal transition may result from changes at both the level of the ovary and hypothalamus.

III. Age-Related Changes in GnRH Neurons

It has been difficult to evaluate intrinsic age-related changes in GnRH neurons because there are surprisingly few GnRH neurons and they are diffusely distributed. In rats, GnRH cell bodies are scattered throughout the medial preoptic area, organum vasculosum of the lamina terminalis and diagonal band of Broca and axons from the majority of these neurons terminate in the lateral regions of the external lamina of the median eminence, where GnRH is released into the hypophysial portal plexus (Silverman, 1994). Furthermore, some GnRH neurons, which may not be anatomically or morphologically discrete subpopulations, appear to have functions that are not directly involved in gonadotropin secretion (Jennes *et al*., 1997). In humans, there appear to be at least two anatomically distinct populations of GnRH neurons, one in the preoptic region and a second in the tuberoinfundibular region of the hypothalamus (Rance and Uswandi, 1996). Whether these populations reflect distinct functional populations is not clear. For all of these reasons, it has been difficult to quantify GnRH release patterns over extended periods of time in individual animals under controlled experimental condition; although a few investigators have successfully achieved this technically difficult feat in several animal models (Levine and Ramirez, 1982; Levine and Duffy, 1988; Rubin and Bridges, 1989). As an alternate strategy, investigators have utilized semiquantitative *in situ* hybridization to assess gene expression in individual cells (Rubin *et al*., 1997) and dual label immunocytochemistry (Lloyd *et al*., 1994; Rubin *et al*., 1995) to identify activated GnRH neurons. Using *in situ* hybridization, Rubin and colleagues (1997) recently showed that the diurnal pattern of GnRH gene expression is altered in middle-aged steroid-treated rats (Fig. 56.4). In young rats, GnRH mRNA levels in the preoptic region decreased from morning to afternoon and then were restored during the evening. In contrast, in middle-aged rats, there was no change through the day. Assessment of the activation of GnRH neurons using the expression of Fos within the nuclei of GnRH neurons as an index of neuronal activation demonstrates that although GnRH peptide may not change, the extent of their activation is attenuated during middle age (Lloyd *et al*., 1994; Rubin *et al*., 1995) (Fig. 56.5). In young animals, Fos is expressed in GnRH neurons coincident with both proestrous and steroid-induced LH surges (Lee *et al*., 1990, 1992). In contrast, in middle-aged proestrous rats that continue to display regular cycles, the

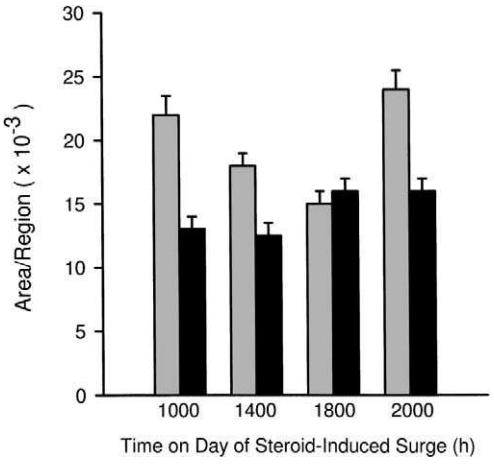

FIG. 56.4. GnRH mRNA in anatomically matched sections through the rostral preoptic area on the day of a steroid-induced LH surge in young (stippled bars) and middle-aged (black bars) rats. Mean hybridization area/region provides an estimate of the summed number of GnRH mRNA-positive neurons. From Rubin *et al*. (1997).

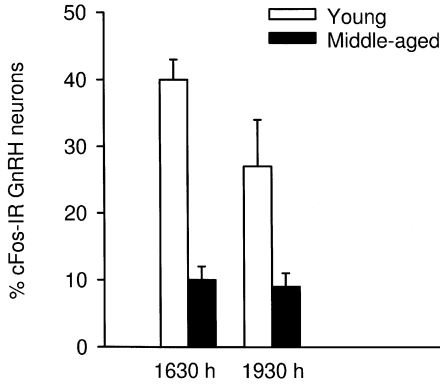

FIG. 56.5. Percentage of GnRH neurons expressing Fos during the proestrous LH surge in young and middle-aged rats. From Lloyd *et al*. (1994).

intensity of Fos staining in GnRH neurons is lower, the percentage of Fos-expressing GnRH neurons is dramatically lower around the time of peak LH release, the neuroanatomical distribution of activated GnRH neurons is different and the extent of activation no longer correlates with serum LH levels. This suggests an age-related desynchronization of the mechanisms involved in generating the proestrous LH surge.

Rubin and Bridges (1989) reported alterations in GnRH release from the mediobasal hypothalamus of steroid-primed middle-aged rats, as detected by perfusion of the preoptic area using push–pull cannula methods. These functional changes become apparent prior to any detectable change in the morphology or distribution of GnRH neurons of aging male rats (Witkin, 1987), any age-related differences in the number of GnRH mRNA containing cells (Rubin *et al*., 1997), or the distribution of GnRH-immunoreactive forms expressed in GnRH neurons (Hoffman and Finch, 1986). Thus, functional changes in GnRH neurons appear to occur prior to changes in the ability to maintain regular estrous cyclicity and are a more sensitive measure of the status of GnRH neuronal activity than morphological criteria.

Very few studies have been performed in postmenopausal women, and none have examined directly GnRH neuronal changes during the perimenopausal transition. Parker and Porter (1984) demonstrated that GnRH levels in the medial basal hypothalamus were lower in postmenopausal women than in young women. In contrast, Rance and Uswandi (1996) found that GnRH mRNA levels in the tuberoinfundibular region were elevated in postmenopausal women; however, levels in the preoptic region were not influenced by aging. As mentioned previously, the functional significance of these anatomically discrete populations of GnRH neurons is not clear; however, it appears that the GnRH containing neurons whose cell bodies are located in the medial basal hypothalamus respond to the hypoestrogenic state. Obviously, many more studies must be done before we know whether changes in mRNA are translated into alterations in GnRH peptide.

IV. Age-Related Changes in Afferent Inputs to GnRH Neurons

A plethora of neurotransmitters and neuropeptides are thought to regulate or modulate GnRH synthesis and/or release. Excellent reviews provide a rich historical appreciation of the experimental approaches that have been used to delineate the roles of each of these (Barraclough *et al*., 1984; Kalra, 1986; Kalra and Crowley, 1992; Kordon *et al*., 1994; Brann and Mahesh, 1994, 1997). We have limited our discussion to only those that thus far have been studied rather extensively with regard to reproductive aging. Specifically we will focus on norepinephrine, excitatory amino acids, neuropeptide Y (NPY), and endogenous opioid peptides.

Generally, studies that examined the neuronal circuitry that regulates GnRH neurons have focused on two principal pathways: (1) brain stem circuitry, primarily monoaminergic neurons in the medulla and pons, and (2) local circuit integration by way of cells in the hypothalamus and in immediate proximity to the GnRH neurons. The importance of neuronal input into GnRH neurons cannot be underestimated. They not only provide information from the environment and integrate reproductive function with other neuroendocrine systems, but they are the pathway that communicates information on steroidal milieu since GnRH neurons did not appear to express estrogen receptors (Shivers *et al*., 1983; Herbison and Theodosis, 1992). More recent studies, however, suggest that GnRH neurons may express low levels of estrogen receptor (Butler *et al*., 1999; Skynner *et al*., 1999).

A. The Role of Excitatory and Inhibitory Inputs into GnRH Neurons in Young Animals

A great deal of evidence indicates that noradrenergic neurons, originating predominantly from the A1 and A2 regions of the brain stem (Gitler and Barraclough, 1988), exert powerful and essential stimulatory effects on GnRH neurons that lead to LH release. Therefore, numerous studies have focused on these neurons to assess whether changes in this population of cells can explain changes in the pattern of gonadotropin secretion with age. Destruction of norepinephrine neurons or local blockade of α-adrenergic receptors decreases pulsatile release of LH (Weick, 1978; Hancke and Wuttke, 1979). Furthermore, endogenous GnRH secretion correlates with changes in pulsatile norepinephrine release, suggesting that norepinephrine plays a role in driving GnRH rhythms (Terasawa *et al*., 1988). Expression of GnRH mRNA is positively regulated by central noradrenergic neurotransmission, suggesting that norepinephrine also regulates GnRH synthesis (He *et al*., 1993; Kim *et al*., 1994). The generation of the LH surge also depends on the functional integrity of brain stem noradrenergic neurons since inhibition of norepinephrine synthesis or blockade of α-adrenergic receptors prevents the LH surge and subsequent ovulation (Kalra *et al*., 1972; Kalra and McCann, 1974; Clifton and Sawyer, 1979; Adler *et al*., 1983; Coen and Coombs, 1983). Further support for a role of norepinephrine in triggering the LH surge is the evidence that norepinephrine turnover rates (Rance *et al*., 1981; Wise *et al*., 1981) and release, as measured by push–pull perfusion (Mohankumar *et al*., 1994), increase in discrete regions of the hypothalamus that regulate GnRH synthesis and/or release at the time of the preovulatory and steroid-induced LH surge. Together, these diverse lines of evidence suggest that norepinephrine plays an important stimulatory role in GnRH neuronal activity, GnRH secretion, and ultimately LH secretion.

Norepinephrine/GnRH interactions may be mediated by both direct and indirect pathways. Noradrenergic nerve terminals have been found to end in close proximity to GnRH-containing neurons in the medial septum-diagonal band of Broca complex and medial preoptic area (Jennes *et al*., 1982; Leranth *et al*., 1988). It appears that α_{1B}-adrenergic receptors exist on GnRH neurons (Hosny and Jennes, 1998); therefore, it is likely that norepinephrine stimulates GnRH synthesis and/or release directly. On the other hand, noradrenergic synapses are also observed on GABAergic neurons in the medial preoptic area (Leranth *et al*., 1988), thus raising the possibility that norepinephrine works by multiple mechanisms to regulate GnRH release.

A lion's share of GnRH regulation appears to be accomplished by local circuitry. Anatomical studies have demonstrated that GnRH neurons are extensively innervated by

neurons whose cell bodies reside within the preoptic area, septum, and arcuate nucleus. The major neurotransmitters and neuropeptides in this category that have been examined in young animals and are thought to be relevant to aging are excitatory amino acid-containing neurons that are presumably scattered in the vicinity of GnRH cell bodies and NPY- and β-endorphin-containing neurons whose cell bodies reside in the arcuate nucleus.

Excitatory amino acid neurotransmission in the brain is carried out primarily by the two acidic amino acid neurotransmitters, glutamate and aspartate. Two main types of receptors mediate the effects of the excitatory amino acids: the metabotropic receptor family and the ionotropic receptor family (for review, see Brann, 1995). The ionotropic family of receptors includes the AMPA-, kainate-, and NMDA-preferring receptors. All three ionotropic receptor subtypes appear to be involved in the regulation of LH secretion. Excitatory amino acids induce a rapid increase in GnRH and/or LH release (Ondo *et al*., 1988; Bourguignon *et al*., 1989; Donoso *et al*., 1990; Lopez *et al*., 1992; Arias *et al*., 1993) and enhance GnRH mRNA and protein expression (Lee *et al*., 1993; Gore and Roberts, 1994). Conversely, studies utilizing the pharmacological blockade of NMDA, AMPA, and kainate receptors have shown that the excitatory amino acids play a role in both pulsatile LH release as well as the generation of the LH surge (Brann and Mahesh, 1991a,b; Brann *et al*., 1993; Luderer *et al*., 1993; Ping *et al*., 1994b). Further evidence that their release is an important physiological trigger of GnRH secretion is suggested by the findings that increased glutamate and aspartate release in the preoptic region, as measured with push–pull perfusion or *in vivo* microdialysis, slightly precedes and/or parallels the LH surge (Demling *et al*., 1985; Jarry *et al*., 1992, 1995; Ping *et al*., 1994a).

The excitatory amino acids may act on GnRH neurons, in part, via interactions with noradrenergic or nitric oxide neurotransmission (Rettori *et al*., 1993; Suh *et al*., 1994; Bhat *et al*., 1995). In support of direct action of excitatory amino acids on GnRH neurons, Eyigor and Jennes (1996) reported that kainic acid-2 (KA2)-receptor immunoreactivity is present in the cell bodies of many GnRH neurons and in their axon terminals in the median eminence.

NPY, a 36 amino acid peptide, acts at the level of hypothalamus as well as the anterior pituitary and has a clear role in facilitating GnRH release (Crowley and Kalra, 1987; Sabatino *et al*., 1989; Besecke *et al*., 1994) and potentiating the responsiveness of the gonadotroph to GnRH (Crowley *et al*., 1987; Bauer-Dantoin *et al*., 1991; 1992a). NPY innervation of the hypothalamus and preoptic area originates from two cell populations, one in the arcuate nucleus and the other in the brain stem where it is colocalized with norepinephrine (Sawchenko *et al*., 1985). Although NPY has been shown to influence pulsatile LH release (Kalra and Crowley, 1984), most of the attention has focused on its involvement in the generation of the LH surge. Evidence supporting a stimulatory action for NPY at the level of the hypothalamus in the culmination of the LH surge includes the findings that immunoneutralization of NPY (Wehrenberg *et al*., 1989), as well as blockade of NPY synthesis with antisense oligonucleotides (Kalra *et al*., 1995), prevent the LH surge in steroid-treated rats. Moreover, NPY gene expression and release are elevated prior to the proestrous and steroid-induced LH surge (Crowley *et al*., 1985; Bauer-Dantoin *et al*., 1992b; Watanobe and Takebe, 1992; Sahu *et al*., 1994). It is not clear if NPY exerts its effects through a direct effect on GnRH neurons or indirectly by altering the activity of other neuronal systems that in turn regulate GnRH release. However, NPY-containing terminals are found in close apposition to GnRH neurons in the medial preoptic area (Tsuruo *et al*., 1990).

A considerable body of evidence demonstrates that endogenous opioid peptides constitute an important inhibitory component of the neural circuitry that regulates LH secretion. Activation of opioid receptors with β-endorphin, dynorphin, and met-enkephalin analogs inhibits LH secretion (Bruni *et al*., 1977; Cicero *et al*., 1979; Kinoshita *et al*., 1980, 1982; Leadem and Kalra, 1985). More importantly, the demonstration that the general opioid antagonist, naloxone, enhances LH secretion on all days of the cycle (Gabriel *et al*., 1983; Piva *et al*., 1985) and advances the timing of the preovulatory LH surge (Allen and Kalra, 1986) has led to the hypothesis that endogenous opioid peptides normally exert an inhibitory tone on LH secretion and that a significant decrease in this inhibitory tone occurs to allow the expression of the neural trigger of the LH surge. Furthermore, steroids may exert their negative and positive feedback on GnRH through opiate peptides (Gabriel *et al*., 1983; Wise *et al*., 1990; Petersen *et al*., 1993).

The finding that the opioid receptor mRNAs (i.e., μ, δ, and κ) are all detectable within preoptic area cells but not on GnRH neurons, as assessed by dual label *in situ* hybridization, raises the question whether opioid peptides directly affect GnRH synthesis and/or release (Sanella and Petersen, 1997) However, it should be noted that Eckersell and Micevych (1998) recently demonstrated that both μ- and δ-opioid receptor immunoreactivity colocalized with GnRH fibers throughout the hypothalamus. Certainly, substantial evidence exists for both norepinephrine and excitatory amino acids as mediators of opioid peptide influences on GnRH secretion (Kalra, 1981; Kalra and Simpkins, 1981; Van Vugt *et al*., 1981; Adler and Crowley, 1984; Akabori and Barraclough, 1986; Miller and Gibson, 1994; Zhen and Gallo, 1995). Thus, endogenous opioid peptides most likely exert both indirect and direct actions on GnRH neurons.

B. Age-Related Changes in the Neurotransmitter Activity May Influence Patterns of GnRH and LH Secretion

In general, much less work has been done in aging animals. Thus, our knowledge relative to what changes take place and when they occur relative to reproductive senescence is still only rudimentary. In particular, few studies have focused on the middle-age transitional period and how these alterations may relate to changes in the pattern of GnRH synthesis and secretion, the ability to induce LH surges, and the maintenance of regular reproductive cycles. Nevertheless, several lines of evidence suggest that changes in pattern of stimulatory and/or inhibitory modulators of GnRH neuronal activity may play an important role during middle age. Over 20 years ago, Simpkins and colleagues (1977) proposed that alterations in catecholamine activity in the hypothalamus of old male rats may account for changes in gonadotropin secretion. We later

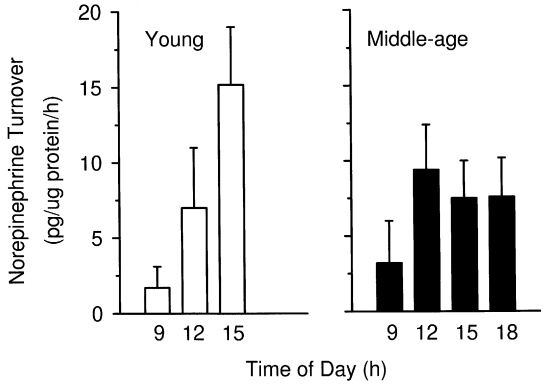

FIG. 56.6. norepinephrine turnover rates in the suprachiasmatic nucleus of young and middle-aged rats on proestrus. Turnover rates increased at the time of the LH surge in young rats, but failed to exhibit any change during the afternoon in middle-aged rats. Modified from Wise (1982a).

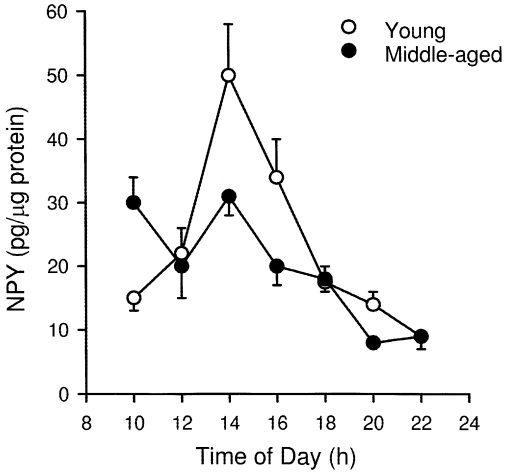

FIG. 56.7. NPY levels in the median eminence of young and middle-aged rats on proestrus. Each data point represents the mean ± SE. From Sahu and Kalra (1998).

reported changes in the rhythm of norepinephrine turnover rates in specific hypothalamic nuclei on proestrus (Wise, 1982a) and in estradiol-treated ovariectomized (Wise, 1984) middle-aged rats (Fig. 56.6). Interestingly, we found that the initial changes in rhythmicity were confined to the anterior hypothalamic regions that included the preoptic area. This region of the hypothalamus is thought to be directly innervated by neurons emanating from the suprachiasmatic nuclei that drive daily rhythms in multiple neurotransmitters (van der Beek *et al*., 1993, 1997). Similar age-related changes in norepinephrine release in the preoptic region have been confirmed using push–pull cannulae (Mohankumar *et al*., 1994). It is important to emphasize that at these early stages of aging alterations in the rhythmicity of neurotransmitter activity occur in the absence of overall changes in the average level of activity or concentrations of the neurotransmitter. At more advanced ages, changes in the average concentrations of norepinephrine (Walker *et al*., 1980; Simpkins, 1984) and dopamine (Simpkins *et al*., 1977; Estes and Simpkins, 1980; Gudelsky *et al*., 1981; Sarkar *et al*., 1982; Reymond *et al*., 1984; Simpkins, 1984) have been reported in males and females.

Several studies have shown that both glutamate content in many brain regions (Banay-Schwartz *et al*., 1989) and the responsiveness of GnRH neurons to glutamate are altered with age (Arias *et al*., 1996; Zuo *et al*., 1996). This decline in responsiveness becomes more pronounced with increased age such that hypothalami of 18-month-old rats release only 25% of the amount of GnRH seen in young adult animals in response to NMDA. These results are supported by *in vivo* studies which show dramatic increases in NMDA-mediated LH release after intrahypothalamic injections of NMDA in young rats but not in 18-month-old animals (Arias *et al*., 1996). Interestingly, male rats did not exhibit the same age-related changes (Bonavera *et al*., 1998).

NPY peptide and mRNA levels have been measured in young and middle-aged proestrous rats. Sahu and Kalra (1998) found that in young rats, NPY levels increased just prior to the LH surge and then decreased progressively during the surge. In contrast, in middle-aged rats, NPY levels did not change concomitant with an attentuate LH surge. The lack of changes in peptide were accompanied by similar age-related absence in a diurnal rhythm of hypothalamic NPY mRNA (Fig. 56.7).

Investigators have tested whether changes in opioid peptides occur with age since these peptides exert strong inhibitory actions on GnRH and are modulated by estradiol. Most studies have been performed in males. However, a few have focused on changes in the levels of proopiomelanocortin mRNA levels (Wise *et al*., 1990; Miller *et al*., 1995) and levels of β-endorphin, a major peptide that for which this mRNA codes, in females. We (Weiland *et al*., 1992) reported that proopiomelanocortin mRNA levels exhibited a daily rhythm in estradiol-treated ovariectomized young rats and that, by middle age, this rhythm was undetectable and the overall average mRNA level was suppressed (Fig. 56.8). No further decrease in mRNA

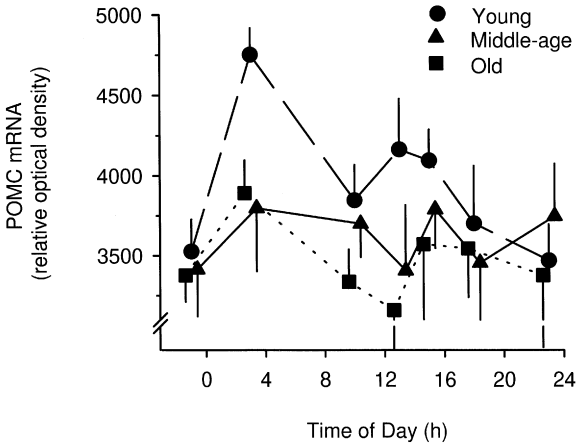

FIG. 56.8. Diurnal rhythm in proopiomelanocortin mRNA levels in the arcuate nuclei of young, middle-aged, and old ovariectomized, estradiol-treated rats. Proopiomelanocortin gene expression exhibits a diurnal rhythm in young rats; however, no rhythm is detectable in middle-aged or old rats. From Weiland *et al*. (1992).

levels occurred in older rats. Nelson and colleagues (1988; Karelus and Nelson, 1992) reported similar decreases in average level of proopiomelanocortin mRNA and an inability of estradiol to influence gene expression in aging mice.

C. Changes in Rhythmicity of Neurotransmitter Input into GnRH Neurons: A Potential Role for the Suprachiasmatic Nucleus

During the past 10 years, we have examined several aspects of some of the neurotransmitters that are thought to modulate GnRH release: turnover rates, neurotransmitter receptor densities, and gene expression. We have found a common theme that appears frequently, which is that the daily rhythmicity in the activity of many neurotransmitters (Wise, 1982a, 1984; Cohen and Wise, 1988), density of their receptors (Weiland and Wise, 1990), or the level of gene expression (Weiland *et al*., 1992) dampens or becomes completely undetectable with age in hypothalamic regions involved in regulating GnRH synthesis or secretion. Interestingly, we observed alterations when animals were middle-aged, as they were entering the transition to irregular cycles. These changes are subtle and may not be detectable if end points are measured at only one or two times of day.

Disruption of the coordination of multiple neural signals that is required for the proper precise timing of GnRH release, may ultimately lead to deterioration in the ability of rats to maintain regular estrous cycles. Everett and colleagues (1949) clearly established over 50 years ago that small changes in the temporal integrity of neurochemical events become greatly magnified in terms of the ability to maintain regular estrous cycles. This effect is different from the effects of timing on any other neuroendocrine system, which can be shifted by several hours without any major compounding impact on the peripheral endocrine rhythms that they drive. For example, desynchronization of neurochemical messages does not cause the CRH/ACTH/glucocorticoid rhythm to skip an entire day.

It is possible that fundamental changes at the level of the "biological clock" or the coupling to its outputs may cause increasing temporal desynchronization of neurotransmitter rhythms that are critical for stable, precise, and regular reproductive cycles. The suprachiasmatic nucleus of the hypothalamus is the master circadian pacemaker, or biological clock, in mammals (Moore-Ede *et al*., 1982; Turek, 1985; Turek and Van Cauter, 1994). These bilateral nuclei exhibit endogenous circadian rhythmicity: they continue to exhibit circadian electrophysiological activity and neuropeptide secretion patterns when maintained *in vitro* unlike any other region of the brain. Efferent connections to various regions of the brain communicate temporal information and drive outputs resulting in a pervasive circadian rhythmicity in most physiological functions. When lesioned, virtually all circadian rhythms (e.g., drinking, rest/activity, endocrine, temperature, metabolic) are abolished. We speculated that delay in the neural circadian pacemaker may lead to desynchronization in the timing of neurotransmitter signals that must be coordinated to trigger and LH surge or to maintain LH pulses of normal duration, amplitude, and frequency. Increased variability of diurnal hormone release, may, in turn, lead to estrous cycles of irregular and unpredictable length, and ultimately to acyclicity. Support for this hypothesis comes from studies that demonstrate that transplantation of fetal suprachiasmatic nucleus into the third ventricle of middle-aged animals restored the light-induced pattern of Fos immunoreactivity to that which was temporally and anatomically similar to that of young animals (Cai *et al*., 1997). Second, suppression of a key neuropeptide in the suprachiasmatic nucleus which communicates with GnRH neurons can mimic the effects of age on the estradiol-induced surges of LH (Harney *et al*., 1996). Recent evidence from our laboratory (Krajnak *et al*., 1998) suggests that the integrity of the cellular pacemakers that make up the biological clock may not age simultaneously since the diurnal rhythm of some neuropeptides of the clock remain intact even into old age; while others suffer from the effects of age relatively early during the life span (Fig. 56.9). These data would suggest that the cellular communication between cells of the suprachiasmatic nucleus and their ability to coordinate outputs of the clock may be the underlying cause of desynchronization of neurotransmitter signals to GnRH neurons.

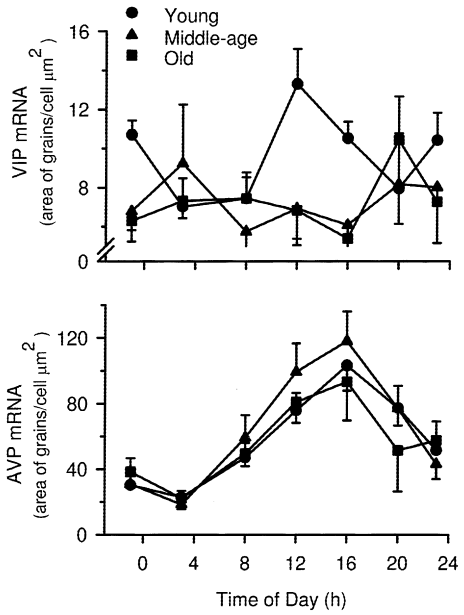

FIG. 56.9. Diurnal rhythm in vasoactive intestinal peptide (VIP) (top) and arginine-vasopressin (AVP) (bottom) mRNA levels in young and middle-aged female rats. The rhythm in VIP mRNA is present in young, but absent in middle-aged rats. In contrast, the rhythm in AVP mRNA levels remains unchanged with age. From Krajnak *et al*. (1998).

V. Summary

Accumulating evidence strongly supports the concept that the brain contributes to the transition from regular reproductive cycles to irregular cycles during aging in the female. Certainly, many more studies must be performed to thoroughly understand the role of each in the process that lead to reproductive senescence. However, compelling evidence from both human studies and those performed in animal models provide support for the idea that the brain, pituitary, and ovary work as a

precisely integrated system in adulthood and that desynchronization of signals among these elements of the reproductive axis lead to deterioration in the carefully timed cycle. Our goal must be to better understand the constellation of factors that interact to maintain regular reproductive cyclicity and how this dynamic balance changes with age. Only then will we understand the complex fabric of the functioning system and the multiple triggers that lead to reproductive decline. Ultimately we will be able to treat postmenopausal women more effectively during this period in their lives.

References

Adler, B. A., and Crowley, W. R. (1984). Modulation of luteinizing hormone release and catecholamine activity by opiates in the female rat. *Neuroendocrinology* **38**, 248–253.

Adler, B. A., Johnson, M. D., Lynch, C. O., and Crowley, W. R. (1983). Evidence that norepinephrine and epinephrine systems mediate the stimulatory effects of ovarian hormones on luteinizing hormone and luteinizing hormone-releasing hormone. *Endocrinology (Baltimore)* **113**, 1431–1438.

Akabori, A., and Barraclough, C. A. (1986). Gonadotropin responses to naloxone may depend upon spontaneous activity in noradrenergic neurons at the time of treatment. *Brain Res.* **362**, 55–62.

Allen, L. G., and Kalra, S. P. (1986). Evidence that a decrease in opioid tone may evoke preovulatory luteinizing hormone release in the rat. *Endocrinology (Baltimore)* **118**, 2375–2381.

Arias, P., Jarry, H., Leonhardt, S., Monguilevsky, J., and Wuttke, W. (1993). Estradiol modulates the LH release response to *N*-methyl-D-aspartate in adult female rats: Studies on hypothalamic luteinizing hormone-releasing hormone and neurotransmitter release. *Brain Res.* **248**, 177–179.

Arias, P., Carbone, S., Szwarcfarb, B., Feleder, C., Rodriguez, M., Scacchi, P., and Moguilevsky, J. A. (1996). Effects of aging on *N*-methyl-D-aspartate (NMDA)-induced GnRH and LH release in female rats. *Brain Res.* **740**, 234–238.

Aschheim, P. (1983). Relation of neuroendocrine system to reproductive decline in female rats. *In* ''Neuroendocrinology of Aging'' (J. Meites, ed.), pp. 73–101. Plenum, New York.

Banay-Schwartz, M., Lajtha, A., and Palkovits, M. (1989). Changes with aging in the levels of amino acids in rat CNS structural elements. I. Glutamate and related amino acids. *Neurochem. Res.* **14**, 555–562.

Barraclough, C. A., Wise P. M., and Selmanoff, M. K. (1984). A role for hypothalamic catecholamines in the regulation of gonadotropin secretion. *Recent Prog. Horm. Res.* **40**, 487–529.

Bauer-Dantoin, A. C., McDonald, J. K., and Levine, J. E. (1991). Neuropeptide Y potentiates luteinizing hormone (LH)-releasing hormone-stimulated LH surges in pentobarbital-blocked proestrous rats. *Endocrinology (Baltimore)* **129**, 402–408.

Bauer-Dantoin, A. C., McDonald, J. K., and Levine, J. E. (1992a). Neuropeptide Y potentiates luteinizing hormone (LH)-releasing hormone-induced LH secretion only under conditions leading to preovulatory LH surges. *Endocrinology (Baltimore)* **131**, 2946–2952.

Bauer-Dantoin, A. C., Urban, J. H., and Levine, J. E. (1992b). Neuropeptide Y gene expression in the arcuate nucleus is increased during preovulatory luteinizing hormone surges. *Endocrinology (Baltimore)* **131**, 2953–2958.

Besecke, L. M., Wolfe, A. M., Pierce, M. E., Takahashi, J. S., and Levine, J. E. (1994). Neuropetide Y stimulates luteinizing hormone-releasing hormone release from superfused hypothalamic GT1-7cells. *Endocrinology (Baltimore)* **135**, 1621–1627.

Bhat, G. K., Mahesh, V. B., Lamar, C. A., Ping, L., Aguan, K., and Brann, D. W. (1995). Histochemical localization of nitric oxide neurons in the hypothalamus: Association with gonadotropin-releasing hormone neurons and co-localization with *N*-methyl-D-aspartate receptors. *Neuroendocrinology* **62**, 178–186.

Block, E. (1952). Quantitative morphological investigations of the follicular system in women. Variations at different ages. *Acta Anat.* **14**, 108–123.

Bonavera, J. J., Swerdloff, R. S., Sinha Hikim, A. P., Lue, Y. H., and Wang, C. (1998). Aging results in attenuated gonadotropin releasing hormone-luteinizing hormone axis responsiveness to glutamate receptor agonist *N*-methyl-D-aspartate. *J. Neuroendocrinol.* **10**, 93–99.

Bourguignon, J.-P., Gerard, A., and Franchimont, P. (1989). Direct activation of gonadotropin-releasing hormone secretion through different receptors to neuroexcitatory amino acids. *Neuroendocrinology* **49**, 402–408.

Brann, D. W. (1995). Glutamate: A major excitatory transmitter in neuroendocrine regulation. *Neuroendocrinology* **61**, 213–225.

Brann, D. W., and Mahesh, V. B. (1991a). Endogenous excitatory amino acid involvement in the preovulatory and steroid-induced surge of gonadotropins in the female rat. *Endocrinology (Baltimore)* **128**, 1541–1547.

Brann, D. W., and Mahesh, V. B. (1991b). Endogenous excitatory amino acid regulation of the progesterone-induced LH and FSH surge in estrogen-primed ovariectomized rats. *Neuroendocrinology* **53**, 107–110.

Brann, D. W., and Mahesh, V. B. (1994). Excitatory amino acids: Function and significance in reproduction and neuroendocrine regulation. *Front. Neuroendocrinol.* **15**, 3–49.

Brann, D. W., and Mahesh, V. B. (1997). Excitatory amino acids: Evidence for a role in the control of reproduction and anterior pituitary hormone secretion. *Endocr. Rev.* **18**, 678–700.

Brann, D W., Ping, L., and Mahesh, V. B. (1993). Possible role of non-NMDA receptor-mediated neurotransmission in steroid-induced and preovulatory gonadotropin surges in the rat. *Mol. Cell Neurosci.* **4**, 1–6.

Bruni, J. F., Van Vugt, D., Marshall, S., and Meites, J. (1977). Effects of naloxone, morphine and methionine enkephalin on serum prolactin, luteinizing hormone, follicle stimulating hormone, thyroid stimulating hormone and growth hormone. *Life Sci.* **21**, 461–466.

Butler, J. A., Sjoberg, M., and Coen, C. W. (1999). Evidence for oestrogen receptor-α immunoreactivity in gonadotropin-releasing hormone-expressing neurones. *J. Neuroendocrinol.* **11**, 331–335.

Cai, A., Lehman, M. N., Lloyd, J. M., and Wise, P. M. (1997). Transplantation of fetal suprachiasmatic nuclei into middle-aged rats restores diurnal fos expression in the host. *Am. J. Physiol.* **272**, R422–R428.

Cicero, T. J, Schainker, B. A., and Meyer, E. R. (1979). Endogenous opioids participate in the regulation of the hypothalamic-pituitary-luteinizing hormone axis and testosterone's negative feedback control of luteinizing hormone. *Endocrinology (Baltimore)* **104**, 1286–1291.

Clemens, J. A., and Bennett, D. R. (1977). Do aging changes in the preoptic area contribute to loss of cyclic endocrine function? *J. Gerontol.* **32**, 19–24.

Clemens, J. A., Amenomori, Y., Jenkins, T., and Meites, J. (1969). Effects of hypothalamic stimulation, hormones, and drugs on ovarian function in old female rats. *Proc. Soc. Exp. Biol. Med.* **132**, 561–563.

Clifton, D. K., and Sawyer, C. H. (1979). LH release and ovulation in the rat following depletion of hypothalamic norepinephrine: Chronic vs. accute effects. *Neuroendocrinology* **28**, 442–449.

Coen, C. W., and Coombs, M. C. (1983). Effects of manipulating catecholamines on the incidence of the preovulatory surge of luteinizing hormone and ovulation in the rat: Evidence for a necessary involvement of hypothalamic adrenaline in the normal or 'midnight' surge. *Neuroscience* **10**, 187–206.

Cohen, I. R., and Wise, P. M. (1988). Age-related changes in the diurnal rhythm of serotonin turnover in microdissected brain areas of estradiol-treated ovariectomized rats. *Endocrinology (Baltimore)* **122**, 2626–2633.

Cooper, R. L. (1977). Reinstatement of ovarian cycles in aged female rats fed L-tyrosine supplemented diets. *Gerontologist* **17**, 49.

Cooper, R. L., and Walker, R. F. (1979). Potential therapeutic consequences of age-dependent changes in brain physiology. *Interdiscip. Top. Gerontol.* **15**, 54–76.

Cooper, R. L., Conn, P. M., and Walker, R. F. (1980). Characterization of the LH surge in middle-aged female rats. *Biol. Reprod.* **23**, 611–615.

Costoff, A., and Mahesh, V. B. (1975). Primordial follicles with normal oocytes in the ovaries of postmenopausal women. *J. Am. Geriatr. Soc.* **23**, 193–196.

Crowley, W. R., and Kalra, S. P. (1987). Neuropeptide Y stimulates the release of luteinizing hormone-releasing hormone from medial basal hypothalamus in vitro: Modulation by ovarian hormones. *Neuroendocrinology* **46**, 97–103.

Crowley, W. R., Tessel R. E., O'Donohue, T. L., Adler, B. A., and Kalra, S. P. (1985). Effects of ovarian hormones on the concentrations of immunoreactive neuropeptide Y in discrete brain regions of the female rat: Correlation with serum luteinizing hormone (LH) and median eminence LH-releasing hormone. *Endocrinology (Baltimore)* **117**, 1151–1155.

Crowley, W. R., Hassid, A., and Kalra, S. P. (1987). Neuropeptide Y enhances the release of luteinizing hormone (LH) induced by LH-releasing hormone. *Neuroendocrinology* **120**, 941–945.

Demling, J., Fuchs, E., Baumert, M., and Wuttke, W. (1985). Preoptic catecholamine, GABA, and glutamate release in ovariectomized and ovariectomized estrogen-primed rats utilizing a push-pull cannula technique. *Neuroendocrinology* **41**, 212–218.

DePaolo, L. V. (1987). Age-associated increases in serum follicle-stimulating hormone levels on estrus are accompanied by a reduction in the ovarian secretion of inhibin. *Exp. Aging Res.* **13**, 3–7.

Donoso, A. O., Lopez, F. J., and Negro-Vilar, A. (1990). Glutamate receptors of the non-*N*-methyl-D-aspartic acid type mediate the increase in luteinizing hormone-releasing hormone release by excitatory amino acids in vitro. *Endocrinology (Baltimore)* **126**, 414–420.

Eckersell, C. B., and Micevych, P. E. (1998). Colocalization of immunoreactivity for mu- and delta-opioid receptors with cholecystokinin, LHRH and tyrosine hydroxylase in the hypothalamus and limbic system of female rats. *Soc. Neurosci. Abstr.* **28**, 632.

Estes, K. S., and Simpkins, J. W. (1980). Age-related alterations in catecholamine concentrations in discrete preoptic area and hypothalamic regions in the male rat. *Brain Res.* **194**, 556–560.

Everett, J. W. (1940). The restoration of ovulatory cycles and corpus luteum formation in persistent estrous rats by progesterone. *Endocrinology (Baltimore)* **27**, 681–686.

Everett, J. W. (1943). Further studies on the relationship of progesterone to ovulation and luteinization in the persistent-estrous rat. *Endocrinology (Baltimore)* **32**, 285–292.

Everett, J. W. (1980). Reinstatement of estrous cycles in middle-aged spontaneously persistent estrous rats: Importance of circulating prolactin and the resulting facilitative action of progesterone. *Endocrinology (Baltimore)* **106**, 1691–1696.

Everett, J. W., and Tyrey, L. (1982). Comparison of luteinizing hormone surge responses to ovarian steroids in cyclic and spontaneously persistent estrous rats of middle age. *Biol. Reprod.* **26**, 663–672.

Everett, J. W., Sawyer, C. H., and Markee, J. E. (1949). A neurogenic timing factor in control of the ovulatory discharge of luteinizing hormone in the cyclic rat. *Endocrinology (Baltimore)* **44**, 234–250.

Eyigor, O., and Jennes, L. (1996). Identification of glutamate receptor sybtype mRNAs in gonadotropin-releasing hormone neurons in rat brain. *Endocrine* **4**, 133–139.

Gabriel, S. M., Simpkins, J. W., and Kalra, S. P. (1983). Modulation of endogenous opioid influence on luteinizing hormone secretion by progesterone and estrogen. *Endocrinology (Baltimore)* **113**, 1806–1811.

Gitler, M. S., and Barraclough, C. A. (1988). Stimulation of the medullary A1 noradrenergic system augments luteinizing hormone release induced by medial preoptic nucleus stimulation. Evaluation of A1 projections to the hypothalamus and of drugs which affect norepinephrine synthesis and adrenoreceptors. *Neuroendocrinology* **48**, 351–359.

Gore, A. C., and Roberts, J. L. (1994). Regulation of gonadotropin-releasing hormone gene expression by the excitatory amino acids kainic acid and N-methyl-D,L-aspartate in the male rat. *Endocrinology (Baltimore)* **134**, 2026–2031.

Gudelsky, G. A., Nansel, D. D., and Porter, J. C., (1981). Dopaminergic control of prolactin secretion in the aging male rat. *Brain Res.* **204**, 446–450.

Hancke, J. L., and Wuttke, W. (1979). Effects of chemical lesion of the ventral noradrenergic bundle or of the medial preoptic area on preovulatory LH release in rats. *Exp. Brain Res.* **35**, 127–134.

Harney, J. P., Scarbrough, K., Rosewell, K. L., and Wise P. M. (1996). In vivo antisense antagonism of vasoactive intestinal peptide in the suprachiasmatic nucleus causes aging-like changes in the estradiol-induced LH and prolactin surge. *Endocrinology (Baltimore)* **137**, 3696–3701.

He, J. R., Molnar, J., and Barraclough, C. A. (1993). Morphine amplifies norepinephrine (norepinephrine)-induced LH release but blocks norepinephrine-stimulated increases in LHRH mRNA levels: Comparison of responses obtained in ovariectomized, estrogen-treated normal and androgen-sterilized rats. *Mol. Brain Res.* **20**, 71–78.

Herbison, A. E., and Theodosis, D. T. (1992). Localization of oestrogen receptors in preoptic neurons containing neurotensin but not tyrosine hydroxylase, cholecystokinin or luteinizing hormone-releasing hormone in the male and female rat. *Neuroscience* **50**, 283–298.

Hoffman, G. E., and Finch, C. E. (1986). LHRH neurons in the female C57BL/6J mouse brain during reproductive aging: No loss up to middle age. *Neurobiol. Aging* **7**, 45–48.

Hosny, S., and Jennes, L. (1998). Identification of alpha-1B adrenergic receptor protein in GnRH neurons of the female rat. *J. Neuroendocrinol.* **110**, 687–692.

Huang, H. H., Marshall, S., and Meites, J. (1976). Induction of estrous cycles in old non-cyclic rats by progesterone, ACTH, ether stress or L-dopa. *Neuroendocrinology* **20**, 21–34.

Jarry, H., Hirsch, B., Leonhardt, S., and Wuttke, W. (1992). Amino acid neurotransmitter release in the preoptic area of rats during the positive feedback actions of estradiol on LH release. *Neuroendocrinology* **56**, 133–140.

Jarry, H., Leonhardt, S., Schwarze, T., and Wuttke, W. (1995). Preoptic rather than mediobasal hypothalamic amino acid neurotransmitter release regulates GnRH secretion during the estrogen-induced LH surge in the ovariectomized rat. *Neuroendocrinology* **62**, 479–486.

Jennes, L., Beckman, W. C., Stumpf, W. E., and Grzanna, R. (1982). Anatomical relationships of serotoninergic and noradrenalinergic projections with the GnRH system in septum and hypothalamus. *Exp. Brain Res.* **46**, 331–338.

Jennes, L., Eyigor, O., Janovick, J. A., and Conn, P. M. (1997). Brain gonadotropin releasing hormone receptors: Localization and regulation. *Recent Prog. Horm. Res.* **52**, 475–491.

Kalra, S. P. (1981). Neural loci involved in naloxone-induced luteinizing hormone release: Effects of a norepinephrine synthesis inhibitor. *Endocrinology (Baltimore)* **109**, 1805–1810.

Kalra, S. P. (1986). Neural circuitry involved in the control of LHRH secretion: A model for preovulatory LH release. *Front. Neuroendocrinol.* **9**, 31–75.

Kalra, S. P., and Crowley, W. R. (1984). Norepinephrine-like effects of neuropeptide Y on LH release in the rat. *Life Sci.* **35**, 1173–1176.

Kalra, S. P., and Crowley, W. F. (1992). Neuropeptide Y: A novel neuroendocrine peptide in the control of pituitary hormone secretion, and its relation to luteinizing hormone. *Front. Neuroendocrinol.* **13**, 1–46.

Kalra, S. P., and McCann, S. M. (1974). Effects of drugs modifying catecholamine synthesis on plasma LH and ovulation in the rat. *Neuroendocrinology* **15**, 79–91.

Kalra, S. P., and Simpkins, J. W. (1981). Evidence for noradrenergic mediation of opioid effects on luteinizing hormone secretion. *Endocrinology (Baltimore)* **109**, 776–782.

Kalra, P. S., Kalra, S. P., Krulich, L., Fawcett, C. P., and McCann, S. M. (1972). Involvement of norepinephrine in transmission of the stimulatory influence of progesterone on gonadotropin release. *Endocrinology (Baltimore)* **90**, 1168–1176.

Kalra, P. S., Bonavera, J. J., and Kalra, S. P. (1995). Central administration of antisense oligodeoxynucleotides to NPY mRNA reveals the critical role of newly synthesized NPY in regulation of LHRH release. *Regul. Pept.* **59**, 215–220.

Karelus, K., and Nelson, J. F. (1992). Aging impairs estrogenic suppression of hypothalamic proopiomelanocortin messenger ribonucleic acid in the mouse. *Neuroendocrinology* **55**, 627–633.

Kim, K., Lee, B. J., Cho, B. N., Kang, S. S., Choi, W. S., Park, S. D., Lee, C. C., Cho, W K., and Wuttke, W. (1994). Blockade of noradrenergic neurotransmission with diethyldithiocarbamic acid decreases the mRNA level of gonadotropin-releasing hormone in the hypothalamus of ovariectomized, steroid-treated prepubertal rats. *Neuroendocrinology* **59**, 539–544.

Kinoshita, F., Nakai, Y., Katakami, H., Kato, Y., Yajima, H., and Imura, H. (1980). Effect of beta-endorphin on pulsatile luteinizing hormone release in conscious castrated rats. *Life Sci.* **27**, 843–846.

Kinoshita, F., Nakai, Y., Katakami, H., and Imura, H. (1982). Suppressive effect of dynorphin-(1–13) on luteinizing hormone release in conscious castrated rats. *Life Sci.* **30**, 1915–1919.

Klein, N. A., Illingworth, P. J., Groome, N. P., McNeilly, A. S., Battaglia, D. E., and Soules, M. R. (1996). Decreased inhibin B secretion is associated with the monotropic FSH rise in older, ovulatory women: A study of serum and follicular fluid levels of dimeric inhibin A and B in spontaneous menstrual cycles. *J. Clin. Endocrinol. Metab.* **81**, 2742–2745.

Knobil, E. (1980). The neuroendocrine control of the menstrual cycle. *Recent Prog. Horm. Res.* **36**, 53–88.

Kordon, C., Drouva, S. V., Martinez de la Escalera, G., and Weiner, R. I. (1994). Role of classic and peptide neuromediators in the neuroendocrine regulation of LH and prolactin, *In* ''The Physiology of Reproduction'' (E. Knobil and J. D. Neill, eds.), pp. 1621–1681. Raven Press, New York.

Krajnak, K., Kashon, M. L., Rosewell, K. L., and Wise, P. M. (1998). Aging alters the rhythmic expression of vasoactive intestinal polypeptide mRNA, but not arginine vasopressin mRNA in the suprachiasmatic neclei of female rats. *J. Neurosci.* **18**, 4767–4774.

Leadem, C. A., and Kalra, S. P. (1985). Effects of endogenous opioid peptides and opiates on luteinizing hormone and prolactin secretion in ovariectomized rats. *Neuroendocrinology* **41**, 342–352.

Lee, W.-S., Smith, M. S., and Hoffman, G. E. (1990). Progesterone enhances the surge of luteinizing hormone by increasing the activation of luteinizing hormone-releasing hormone neurons. *Endocrinology (Baltimore)* **127**, 2604–2606.

Lee, W.-S., Smith, M. S., and Hoffman, G. E. (1992). cFos activity identifies recruitment of luteinizing hormone-releasing hormone neurons during the ascending phase of the proestrous luteinizing hormone surge. *J. Neuroendocrinol.* **4**, 161.

Lee, W. S., Abbud, R., Hoffman, G. E., and Smith, M. S. (1993). Effects of *N*-methyl-D-aspartate receptor activation on cFos expression in luteinizing hormone-releasing hormone neurons in female rats. *Endocrinology (Baltimore)* **133**, 2248–2254.

Leranth, C., MacLusky, N. J., Shanabrough, M., and Naftolin, F. (1988). Catecholaminergic innervation of luteinizing hormone-releasing hormone and glutamic acid decarboxylase immunopositive neurons in the rat medial preoptic area. An electron-microscopic double immunostaining and degeneration study. *Neuroendocrinology* **48**, 591–602.

Levine, J. E., and Duffy, M. T. (1988). Simultaneous measurement of luteinizing hormone (LH)-releasing hormone, LH, and follicle-stimulating hormone release in intact and short-term castrate rats. *Endocrinology (Baltimore)* **122**, 2211–2221.

Levine, J. E., and Ramirez, V. D. (1982). Luteinizing hormone-releasing hormone release during the rat estrous cycle and after ovariectomy, as estimated with push-pull cannulae. *Endocrinology (Baltimore)* **111**, 1439–1448.

Lloyd, J. M., Hoffman, G. E., and Wise, P. M. (1994). Decline in immediate early gene expression in gonadotropin-releasing hormone neurons during proestrus in regularly cycling, middle-aged rats. *Endocrinology (Baltimore)* **134**, 1800–1805.

Lopez, F. J., Donoso, A. O., and Negro-Vilar, A. (1992). Endogenous excitatory amino acids and glutamate receptor subtypes involved in the control of hypothalamic LHRH secretion. *Endocrinology (Baltimore)* **130**, 1986–1992.

Luderer, U., Strobl, F. J., Levine, J. E., and Schwartz, N.B. (1993). Differential gonadotropin responses to *N*-methyl-D-aspartate (NMDA) in metestrous, proestrous, and ovariectomized rats. *Biol. Reprod.* **48**, 857–866.

Matt, D. W., Kauma, S. W., Pincus, S. M., Veldhuis, J. D., and Evans, W. S. (1998). Characteristics of luteinizing hormone secretion in younger versus older premenopausal women. *Am. J. Obstet. Gynecol.* **178**, 504–510.

Miller, G. M., and Gibson, M. J. (1994). Opioidergic modulation of N-methyl-D, L-aspartic-acid-stimulated LH release in young adult but not older male mice. *Neuroendocrinology* **59**, 277–284.

Miller, M. M., Tousignant, P., Yang, U., Pedvis, S., and Billiar, R. B. (1995). Effects of age and long-term ovariectomy on the estrogen-receptor containing sub-poulations of beta-endorphin-immunoreactive neurons in the arcuate nucleus of female C57BL/6J mice. *Neuroendocrinology* **61**, 542–551.

Mohankumar, P. S., Thyagarajan, S., and Quadri, S. K. (1994). Correlations of catecholamine release in the medial preoptic area with proestrous surges of luteinizing hormone and prolactin: Effects of aging. *Endocrinology (Baltimore)* **135**, 119–126.

Moore-Ede, M. C., Sulzman, F. M., and Fuller, C. A. (1982). Characteristics of circadian clocks. *In* ''The Clocks that Time Us'' (M. C. Moore-Ede, F. M. Sulzman, and C. A. Fuller, eds.), pp. 30–112. Harvard University Press, Cambridge, MA.

Nass, T. E., Lapolt, P. S., Judd, H. L., and Lu, J. K. H. (1984). Alterations in ovarian steroid and gonadotrophin secretion preceding the cessation of regular oestrous cycles in ageing female rats. *J. Endocrinol.* **100**, 43–50.

Nelson, J. F., Bender, M., and Schachter, B. S. (1988). Age-related changes in proopiomelanocortin messenger ribonucleic acid levels in hypothalamus and pituitary of female C57BL/6J mice. *Endocrinology (Baltimore)* **123**, 340–344.

Ondo, J. G., Wheeler, D. D., and Dom, R. M. (1988). Hypothalamic site of action for *N*-methyl-D-aspartate (NMDA) on LH secretion. *Life Sci.* **43**, 2283–2286.

Parker, C. R., Jr., and Porter, J. C. (1984). Luteinizing hormone-releasing hormone and thyrotropin-releasing hormone in the hypothalamus of women: Effects of age and reproductive status. *J. Clin. Endocrinol. Metab.* **58**, 488–491.

Peng, M.-T., and Huang, H.-H. (1972). Aging of hypothalamic-pituitary-ovarian function in the rat. *Fertil. Steril.* **23**, 535–542.

Petersen, S. L., Keller, M. L., Carder, S. A., and McCrone, S. (1993). Differential effects of estrogen and progesterone on levels of POMC mRNA levels in the arcuate nucleus: Relationship to the timing of LH surge release. *J. Neuroendocrinol.* **15**, 643–648.

Ping, L., Mahesh, V. B., and Brann, D. W. (1994a). Release of glutamate and aspartate from the preoptic area during the progesterone-induced LH surge: In vivo microdialysis studies. *Neuroendocrinology* **59**, 318–324.

Ping, L., Mahesh, V. B., and Brann, D. W. (1994b). A physiological role for *N*-methyl-D-aspartic acid and non-*N*-methyl-D-aspartic acid receptors in pulsatile gonadotropin secretion in the adult female rat. *Endocrinology (Baltimore)* **135**, 113–118.

Piva, F., Maggi, R., Limonta, P., Motta, M., and Martini, L. (1985). Effect of naloxone on luteinizing hormone, follicle-stimulating hormone, and

prolactin secretion in the different phases of the estrous cycle. *Endocrinology (Baltimore)* **117**, 766–772.

Quadri, S. K., Kledzik, G. S., and Meites, J. (1973). Reinitiation of estrous cycles in old constant-estrous rats by central-acting drugs. *Neuroendocrinology* **11**, 248–255.

Rance, N., Wise, P. M., Selmanoff, M. K., and Barraclough, C. A. (1981). Catecholamine turnover rates in discrete hypothalamic areas and associated changes in median eminence luteinizing hormone-releasing hormone and serum gonadotropins on proestrus and diestrous day 1. *Endocrinology (Baltimore)* **108**, 1795–1802.

Rance, N. E., and Uswandi, S. V. (1996). Gonadotropin-releasing hormone gene expression is increased in the medial basal hypothalamus of postmenopausal women. *J. Clin. Endocrinol. Metab.* **81**, 3540–3546.

Reame, N. E., Kelch, R. P., Beitins, I. Z., Yu, M.-Y., Zawacki, C. M., and Padmanabhan, V. (1996). Age effects on follicle-stimulating hormone and pulsatile luteinizing hormone secretion across the menstrual cycle of premenopausal women. *J. Clin. Endocrinol. Metab.* **81**, 1512–1518.

Rettori, V., Belova, N., Dees, W. L., Nyberg, C. L., Gimeno, M., and McCann, S. M. (1993). Role of nitric oxide in the control of luteinizing hormone-releasing hormone release in vivo and in vitro. *Proc. Natl. Acad. Sci. U.S.A* **90**, 10130–10134.

Reymond, M. J., Arita, J., Dudley, C. A., Moss, R. L., and Porter, J. C. (1984). Dopaminergic neurons in the mediobasal hypothalamus of old rats: Evidence for decreased affinity of tyrosine hydroxylase for substrate and cofactor. *Brain Res.* **304**, 215–223.

Rubin, B. S., and Bridges, R. S. (1989). Alterations in luteinizing hormone-releasing hormone release from the mediobasal hypothalamus of ovariectomized, steroid-primed middle-aged rats as measured by push-pull perfusion. *Neuroendocrinology* **49**, 225–232.

Rubin, B. S., Mitchell, S., Lee, C. E., and King, J. C. (1995). Reconstructions of populations of LHRH neurons in young and middle-aged rats reveal progressive increases in subgroups expressing fos protein on proestrus and age-related deficits. *Endocrinology (Baltimore)* **136**, 3823–3830.

Rubin, B. S., Lee, C. E., Ohtomo, M., and King, J. C. (1997). Luteinizing hormone-releasing hormone gene expression differs in young and middle-aged females on the day of a steroid-induced LH surge. *Brain Res.* **770**, 267–276.

Sabatino, F. D., Collins, P., and McDonald, J. K. (1989). Neuropeptide-Y stimulation of luteinizing hormone-releasing hormone secretion from the median eminence in vitro by estrogen-dependent and extracellular Ca2+-independent mechanisms. *Endocrinology (Baltimore)* **124**, 2089–2098.

Sahu, A., and Kalra, S. P. (1998). Absence of increased neuropeptide Y neuronal activity before and during the luteinizing hormone (LH) surge may underlie the attenuated preovulatory LH surge in middle-aged rats. *Endocrinology (Baltimore)* **139**, 696–702.

Sahu, A., Crowley, W. R., and Kalra, S. P. (1994). Hypothalamic neuropeptide-Y gene expression increases before the onset of the ovarian steroid-induced luteinizing hormone surge. *Endocrinology (Baltimore)* **134**, 1018–1022.

Sanella, M. I., and Petersen, S. L. (1997). Dual label in situ hybridization studies provide evidence that LHRH neurons do not synthesize mRNA for mu, kappa, or delta opiate receptors. *Endocrinology (Baltimore)* **138**, 1667–1672.

Sarkar, D. K., Gottschall, P. E., and Meites, J. (1982). Damage to hypothalamic dopaminergic neurons is associated with development of prolactin-secreting pituitary tumors. *Science* **218**, 684–686.

Sawchenko, P. E., Swanson, L. W., Grzanna, R., Howe, P. R. C., Bloom, S. R., and Polak, J. M. (1985). Colocalization of neuropeptide Y immunoreactivity in brainstem catecholaminergic neurons that project to the paraventricular nucleus of the hypothalamus. *J. Comp. Neurol.* **241**, 138–153.

Scarbrough, K., and Wise, P. M. (1990). Age-related changes in the pulsatile pattern of LH release precede the transition to estrous acyclicity and depend upon estrous cycle history. *Endocrinology (Baltimore)* **126**, 884–890.

Shivers, B. D., Harlan, R. E., Morrell, J. I., and Pfaff, D. W. (1983). Absence of oestradiol concentration in cell nuclei of LHRH-immunoreactive neurones. *Nature (London)* **304**, 345–347.

Silverman, A.-J. (1994). The gonadotropin-releasing hormone (GnRH) neuronal systems: Immunocytochemistry and in situ hybridization. *In* "The Physiology of Reproduction" (E. Knobil and J. Neill, eds.), pp. 1683–1710. Raven Press, New York.

Simpkins, J. W. (1984). Regional changes in monoamine metabolism in the aging constant estrous rat. *Neurobiol. Aging* **5**, 309–313.

Simpkins, J. W., Mueller, G. P., Huang, H. H., and Meites, J. (1977). Evidence for depressed catecholamine and enhanced serotonin metabolism in aging male rats: Possible relation to gonadotropin secretion. *Endocrinology (Baltimore)* **100**, 1672–1678.

Skynner, M. J., Sim, S. A., and Herbison, A. E. (1999). Detection of estrogen receptor α and β mRNA in adult gonadotropin-releasing hormone neurons. *Endocrinology (Baltimore)* **140**, 5195–5201.

Suh, J., Song, E. S., Kim, C., Yu, M. H., and Kim, K. (1994). Cross-talk between *N*-methyl-D-aspartate and adrenergic neurotransmission in the regulation of hypothalamic GnRH gene expression. *Brain Res.* **645**, 36–40.

Terasawa, E., Krook, C., Hei, D. L., Gearing, M., Schultz, N. J., and Davis, G. A. (1988). Norepinephrine is a possible neurotransmitter stimulating pulsatile release of luteinizing hormone-releasing hormone in the rhesus monkey. *Endocrinology (Baltimore)* **123**, 1808–1816.

Tsuruo, Y., Kawano, H., Kagotani, Y., Hisano, S., Daikoku, S., Chihara, K., Zhang, T., and Yanaihara N. (1990). Morphological evidence for neuronal regulation of luteinizing hormone-releasing hormone-containing neurons by neuropeptide Y in the rat septo-preoptic area. *Neurosci. Lett.* **110**, 261–266.

Turek, F. W. (1985). Circadian neural rhythms in mammals. *Annu. Rev. Physiol.* **47**, 49–64.

Turek, F. W., and Van Cauter, E. (1994). Rhythms in reproduction. *In* "The Physiology of Reproduction" (E. Knobil and J. Neill, eds.), pp. 487–540. Raven Press, New York.

van der Beek, E. M., Wiegant, V. M., van der Donk, H. A., van den Hurk, R., and Buijs, R. M. (1993). Lesions of the suprachiasmatic nucleus indicate the presence of a direct vasoactive intestinal polypeptide-containing projection to gonadotropin-releasing hormone neurons in the female rat. *J. Neuroendocrinol.* **5**, 137–144.

van der Beek, E. M., Horvath, T. L., Wiegant, V. M., van den Hurk, R., and Buijs, R. M. (1997). Evidence for a direct neuronal pathway from the suprachiasmatic nucleus to the gonadotropin-releasing hormone system: Combined tracing and light and electron microscopic immunocytochemical studies. *J. Comp. Neurol.* **384**, 569–579.

Van Vugt, D. A., Aylsworth, C. F., Sylvester, P. W., Leung, F. C., and Meites, J. (1981). Evidence for hypothalamic noradrenergic involvement in naloxone-induced stimulation of luteinizing hormone release. *Neuroendocrinology* **33**, 261–264.

vom Saal, F. S., Finch, C. E., and Nelson, J. F. (1994). Natural history and mechanisms of reproductive aging in humans, laboratory rodents, and other selected vertebrates. *In* "The Physiology of Reproduction" (E. Knobil and J. D. Neill, eds.), pp. 1213–1314. Raven Press, New York.

Walker, R. F., Cooper, R. L., and Timiras, P. S. (1980). Constant estrus: Role of rostral hypothalamic monoamines in development of reproductive dysfunction in aging rats. *Endocrinology (Baltimore)* **107**, 249–255.

Watanobe, H., and Takebe, K. (1992). Evidence that neuropeptide Y secretion in the median eminence increases prior to the luteinizing hormone surge in ovariectomized steroid-primed rats: Estimation by push-pull perfusion. *Neurosci. Lett.* **146**, 57–59.

Wehrenberg, W. B., Corder, R., and Gaillard, R. C. (1989). A physiological role for neuropeptide Y in regulating the estrogen/progesterone induced luteinizing hormone surge in ovariectomized rats. *Neuroendocrinology* **49**, 680–682.

Weick, R. F. (1978). Acute effects of adrenergic receptor blocking drugs and neuroleptic agents on pulsatile discharges of luteinizing hormone in the ovariectomized rat. *Neuroendocrinology* **26**, 108–117.

Weiland, N. G., and Wise, P. M. (1990). Aging progressively decreases the densities and alters the diurnal rhythm of alpha-1-adrenergic receptors in selected hypothalamic regions. *Endocrinology (Baltimore)* **126**, 2392–2397.

Weiland, N. G., Scarbrough, K., and Wise, P. M. (1992). Aging abolishes the estradiol-induced suppression and diurnal rhythm of proopiomelanocortin gene expression in the arcuate nucleus. *Endocrinology (Baltimore)* **131**, 2959–2964.

Wise, P. M. (1982a). Norepinephrine and dopamine activity in microdissected brain areas of the middle-aged and young rat on proestrus. *Biol. Reprod.* **27**, 562–574.

Wise, P. M. (1982b). Alterations in proestrous LH, FSH, and prolactin surges in middle-aged rats. *Proc. Soc. Exp. Biol. Med.* **169**, 348–354.

Wise, P. M. (1984). Estradiol-induced daily luteinizing hormone and prolactin surges in young and middle-aged rats: Correlations with age-related changes in pituitary responsiveness and catecholamine turnover rates in microdissected brain areas. *Endocrinology* **115**, 801–809.

Wise, P. M., Rance, N., and Barraclough, C. A. (1981). Effects of estradiol and progesterone on catecholamine turnover rates in discrete hypothalamic regions in ovariectomized rats. *Endocrinology (Baltimore)* **108**, 2186–2193.

Wise, P. M., Scarbrough, K., Weiland, N. G., and Larson, G. H. (1990). Diurnal pattern of proopiomelanocortin gene expression in the arcuate nucleus of proestrous, ovariectomized, and steroid-treated rats: A possible role in cyclic luteinizing hormone secretion. *Mol. Endocrinol.* **4**, 886–892.

Wise, P. M., Kashon, M. L., Krajnak, K. M., Rosewell, K. L., Cai, A., Scarbrough, K., Harney, J. P., McShane, T., Lloyd, J., and Weiland, N. G. (1997). Aging of the female reproductive system: A window into brain aging. *Recent Prog. Horm. Res.* **52**, 279–305.

Witkin, J. W. (1987). Aging changes in synaptology of luteinizing hormone-releasing hormone neurons in male rat preoptic area. *Neuroscience* **22**, 1003–1013.

Zhen, S., and Gallo, R. V. (1995). The role of norepinephrine in mediating luteinizing hormone release in response to blockade of k-opioid receptors in the medial preoptic area. *Brain Res.* **698**, 121–129.

Zuo, Z., Mahesh, V. B., Zamorano, P. L., and Brann, D. W. (1996). Decreased gonadotropin-releasing hormone neurosecretory response to glutamate aganists in middle-aged female rats on proestrus afternoon: A possible role in reproductive aging? *Endocrinology (Baltimore)* **137**, 2334–2338.

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57

Hypothalamic Changes Relevant to Reproduction in Aging Male Rodents

As a recipient of sex steroid feedback and neuromodulatory input from other central nervous system areas, the gonadotropin-releasing hormone (GnRH) neuronal system forms a final common pathway for brain regulation of reproduction. Therefore, aging-related changes in GnRH secretion could be due either to intrinsic alterations in GnRH neuronal function or alterations in hormonal feedback and other neuromodulatory influences that affect GnRH secretion. Currently available evidence indicates that GnRH gene expression and peptide content are decreased in the medial preoptic area of aging male rodents, consistent with a decrease in GnRH synthetic capacity with aging. Furthermore, this decrease is independent of testicular feedback factors such as sex steroids.

In addition to these age-related changes in GnRH neurons themselves, changes in other neurons that project to GnRH neurons may contribute to declining GnRH secretion with aging. For example, hypothalamic neuropeptide Y (NPY) gene expression declines with aging, suggesting that decreased NPY neuronal input contributes to a decrease in GnRH and luteinizing hormone (LH) secretion with aging. However, no one component of the complex, interactive network of neurons and hormones regulating GnRH secretion has been identified that fully accounts for declining GnRH secretion with aging. Indeed, it is likely that altered GnRH neuronal function with aging reflects the integration of all changes in neuromodulatory influences affecting GnRH-containing neurons, together with intrinsic changes in GnRH-containing neurons themselves. Further studies are needed to determine the relative importance of each of these factors, and these studies should optimally be performed in models that are validated for reproductive aging studies and exhibit reproductive aging changes that are similar to those occurring in humans, e.g., the male Brown Norway rat.

I. Introduction

The hypothalamus plays a vital role in the regulation of reproductive function. Gonadotropin-releasing hormone (GnRH), a decapeptide synthesized in the brain, stimulates pituitary secretion of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which in turn stimulate testicular testosterone production and spermatogenesis. GnRH secretion is regulated by testosterone feedback from the testes and by excitatory and inhibitory neurotransmitters and neuromodulatory substances within the central nervous system. As a recipient of sex steroid feedback and neuromodulatory input from other central nervous system sources, the GnRH neuronal system forms a final common pathway for brain regulation of reproduction.

In recent years, our understanding of the regulation of GnRH function in adult animal models has become increasingly sophisticated. Neuroanatomical studies have not only identified and mapped neuronal pathways impinging on GnRH-containing neurons and demonstrated their interrelationships, but have also shown that in some cases more than one neurotransmitter or neuropeptide may be colocalized within the same neurons. For example, neuropeptide Y (NPY) and γ-aminobutyric acid (GABA) are colocalized within a subset of neurons in the arcuate nucleus of the hypothalamus (Horvath *et al*., 1997), suggesting the potential for coordinated regulation of GnRH release by these neurons (Kalra *et al*., 1997). At the same time, an increasing number of substances modulating GnRH secretion have been identified, including the neuropeptide galanin (Cheung *et al*., 1996), the excitatory amino acid glutamate (Brann, 1995) and nitric oxide, a diffusable gas that may mediate neuroendocrine signals from other neuromodulators (Bonavera *et al*., 1993; Kalra *et al*, 1997).

Research into the effects of aging on neuroendocrine regulation of reproduction has benefited from these advances in our knowledge of reproductive regulation in adult animals, but our understanding of aging processes is considerably less complete. Beginning with studies of aging effects on indirect indices of GnRH pulse generator function such as pulsatile LH release and *in vitro* studies of basal and stimulated GnRH release, investigators later turned to tools such as mRNA hybridization techniques to describe changes in gene expression under baseline conditions as an index of GnRH synthetic capacity and release. Further studies of aging have focused on

GnRH gene expression in response to experimental manipulation of variables affecting GnRH neuronal function, such as steroid hormonal milieu and other neurotransmitters and neuropeptides. Based on these studies, it is now possible to begin to determine the relative contributions of intrinsic changes in GnRH secretory capacity, changes in hormonal milieu, and altered neuropeptide and neurotransmitter input affecting GnRH neurons to the overall alterations in hypothalamic reproductive regulation with aging.

An important consideration for aging studies involving animal models is to ensure that the model selected is appropriate for the physiological system under study. Historically, however, little attention has been paid to controlling for potentially confounding disease-related variables in most animal studies, which as discussed below turns out to be crucial in separating the effects of aging per se from the effects of age-associated pathologies. In addition, it is desirable to select a model with attributes that resemble the human condition as closely as possible, in order to provide insights relevant to human aging. For these reasons, this chapter will emphasize studies involving the male Brown Norway rat, which appears to be the best available rodent model for studies of male reproductive system aging as evidenced in the following discussion.

A. Rodent Models of Male Reproductive Aging

1. Primary and Secondary Testicular Failure

In man, aging of the reproductive system is associated with both primary and secondary testicular dysfunction. Primary testicular failure is evidenced by low bioavailable testosterone levels in many healthy elderly men (Swerdloff and Wang, 1993), together with a decreased testosterone response to exogenous LH administration (Harman and Tsitouras, 1980) and diminished Leydig cell mass (Vermeulen, 1991). As a result of reduced testosterone negative feedback, serum gonadotropin levels show a compensatory elevation, but the degree of this increase is inappropriately low compared to that seen in younger men with low serum testosterone levels (Vermeulen, 1991), and the frequency of pulsatile LH release relative to the level of circulating testosterone is abnormally slow (Deslypere *et al*., 1987). These findings indicate that hypothalamic/pituitary control of testicular function is also altered in aging men.

In contrast, most of the studies in male rat models of aging such as the Sprague–Dawley and Fischer 344 (F344) rat have demonstrated only secondary testicular failure (i.e., hypothalamic/pituitary dysfunction). For example, in Sprague–Dawley rats, testicular Leydig cell mass tends to rise with increasing age (Kaler and Neaves, 1981), and testosterone production in response to human chorionic gonadotropin stimulation *in vitro* is unchanged with aging, whereas serum LH levels at baseline and in response to exogenous GnRH are decreased in old compared to younger rats (Pirke *et al*., 1978a). Similarly, both gonadotropin and testosterone levels decrease substantially with aging in male F344 rats (Gruenewald *et al*., 1992), indicating marked hypogonadotropic (i.e., secondary) hypogonadism.

2. Other Problems with Aging Rat Models

An additional problem with aging rat models for the study of reproductive system aging is the common occurrence of pituitary adenomas and other tumors of the endocrine system (Hollander, 1976). For example, aging outbred Sprague–Dawley rats commonly develop both pituitary adenomas and pancreatic islet cell tumors (Hollander, 1976; Cohen *et al*., 1978; Sandusky *et al*., 1988). Indeed, all rats have a degree of susceptibility to pituitary adenomas (Hazzard *et al*., 1992), indicating that evaluation for these tumors at postmortem examination is essential to exclude affected individuals. Regardless of the presence of macroscopic pituitary adenomas, hyperprolactinemia is a potentially confounding variable in studies of reproductive aging, because of the inhibitory effect of elevated prolactin levels upon GnRH and gonadotropin secretion (McNeilly, 1987). Hyperprolactinemia has been reported in several studies involving aging male rats of strains including F344 (Bethea and Walker, 1979), Long–Evans (Demarest *et al*., 1980), and Wistar rats (Simpkins *et al*., 1977). Despite this, prolactin levels have usually not been reported in studies of reproductive system aging that used these models.

Of even greater concern is the marked tendency of male F344 rats to develop testicular Leydig cell tumors with advancing age (Turek and Desjardins, 1979). These tumors are often functional, and may secrete large quantities of progesterone and other steroids (Amador *et al*., 1985; Gruenewald *et al*., 1992). We found that in old F344 rats, orchidectomy caused circulating gonadotropins and prepro-GnRH mRNA content in the medial preoptic area of the brain to be similar to that in young orchidectomized rats, and that circulating levels of progesterone were inversely correlated with serum gonadotropin levels (Gruenewald *et al*., 1992). These findings suggested that excessive progesterone secretion from testicular tumors in aging male F344 rats exerts a significant negative feedback effect on the reproductive hypothalamic–pituitary axis. Based on these findings, the intact F344 rat is inappropriate for studies of normal male reproductive aging.

The aging male F344/Brown Norway F1 hybrid model is another potential model for male reproductive aging studies, and has the advantage of a low incidence of age-related pathology (Sprott, 1991). However, like the male F344, these rats develop Leydig cell tumors with aging, although these tumors are less frequent and occur at older ages than in the F344 (Bronson, 1989; Masoro, 1991; Thurman *et al*., 1995). Age-related changes in steroid hormone secretion have not yet been fully characterized in the male F344/Brown Norway F1 rat, therefore elevated circulating levels of progesterone and other steroids secreted by Leydig cell tumors may be a confounding factor in aging studies using this model. Further studies are needed to rule out excessive steroid secretion from Leydig cell tumors before the aging male F344/Brown Norway F1 rat model can be considered appropriate for studies of reproductive axis aging.

3. Male Brown Norway Rat—Best Available Rat Model of Male Reproductive System Aging

Based on the foregoing, studies of normal male reproductive system aging in rodents require the use of a model that is relatively free of these confounding variables, and approximates as closely as possible the hormonal milieu of aging men. To this end, we (Gruenewald *et al*., 1994a) and others (C. Wang *et al*.,

1993; Zirkin *et al*., 1993; Chen *et al*., 1994) have evaluated the effects of aging on reproductive function in the male Brown Norway rat. In this inbred strain, testis function is relatively preserved until old age, when old (approximately 24-month-old) and senescent (approximately 30-month-old) rats exhibit decreased serum testosterone levels and Leydig cell testosterone production and histological evidence of testicular atrophy in the face of stable LH and progressively rising FSH levels with aging, consistent with primary testicular failure. Moreover, hypothalamic/pituitary dysfunction is also evident in the failure of LH levels to increase in intact aging rats despite low testosterone levels, and the progressive decrease in LH and FSH in orchidectomized aging BN rats (Gruenewald *et al*., 1994a). This combination of primary and secondary testicular dysfunction is more analogous to the situation in aging men than the other available rodent models of aging.

In addition, the Brown Norway rat has other advantages for aging studies, including a long, relatively disease-free survival rate; the relative absence of specific endocrine disorders such as hyperprolactinemia and high circulating progesterone levels, pituitary adenomas and Leydig cell hyperplasia and tumors; the genetic homogeneity of this inbred strain; and the ready availability of rats at different ages that are barrier-reared under specific pathogen-free conditions. Localized bladder cancer is the most common major disease of the aging Brown Norway rat, occurring in 35% of male rats at an average age of 27 months (Burek and Hollander, 1977). Most other significant diseases such as cardiomyopathies and other neoplasms do not occur in this rat strain until 26 to 30 months of age (Bronson, 1989). Considering all of these advantages together with the relatively few disadvantages, the Brown Norway rat appears to be the best available rat model for studies of male reproductive aging.

4. Mouse Models

The (C57BL/6N $\times$ C3H/HeN)F_1 hybrid (B6C3F_1) mouse is commonly used for aging studies. Its advantages include long survival (approximately 80% at 26 months), comparable to F344 rats (Cameron *et al*., 1985). In males, the most common age-related pathologies are hepatic adenomas and hepatocellular carcinomas (which are metastatic in 12%), malignant lymphomas and leukemias, lung adenomas, and adenocarcinomas (Tamano *et al*., 1988).

In aging male CBF_1 mice, progressive reductions in mating success and sperm production have been described with aging, which correlate with decreased serum LH and testosterone, consistent with secondary (hypothalamic/pituitary) testicular failure (Bronson and Desjardins, 1977). Moreover, the frequency of pulsatile LH release is decreased with aging in these mice (Coquelin and Desjardins, 1982), consistent with impaired hypothalamic GnRH pulse generator activity with aging. Thus, these mice demonstrate a hormonal profile consistent with predominantly secondary testicular failure, similar to most rat strains other than the Brown Norway rat. To our knowledge, no studies have been published that describe alterations in GnRH or its regulation in aging male mice.

Based on the foregoing discussion of the importance of the model in studies of male reproductive aging, these considerations will be included in the following sections covering age-related changes in hypothalamic regulation of reproductive function in male rodents.

II. The GnRH Neuronal System

A. Indirect Indicators of Aging Effects on GnRH Neurons

1. Aging and the Frequency of Pulsatile Gonadotropin Secretion

Aging effects on hypothalamic GnRH pulse generator function may be inferred from indirect measures such as changes in the frequency of pulsatile gonadotropin release. In the 1980s, studies were performed in male Sprague–Dawley rats to investigate the role of hypothalamic/pituitary alterations in mediating the decline in testosterone levels in old rats. In one study, sampling of blood was performed every 10 min through indwelling venous catheters for 8 hr in unanesthetized, intact 3- and 22-month-old male rats for measurement of LH levels by radioimmunoassay (RIA) (Steiner *et al*., 1984). The frequency of pulsatile LH release was found to be decreased in old rats, and compared to young rats the frequency was inappropriately low for the level of circulating testosterone. This decrease in pulsatile LH release suggested that hypothalamic GnRH pulse generator activity was impaired with aging in these rats. In a second study, 3-, 18-, and 26-month-old male Sprague–Dawley rats were orchidectomized, and frequent blood sampling for LH levels was performed 3 weeks later (Karpas *et al*., 1983). In the absence of testosterone negative feedback, LH pulse frequency was significantly decreased in old compared to middle-aged and young rats, indicating that this effect of aging on the hypothalamic pulse generator was not merely a result of alterations in testosterone feedback sensitivity with aging. In addition, the amplitude of LH pulses was also decreased with aging in both of the above studies, but this could have been the result of either decreased GnRH secretion, decreased pituitary responsiveness to GnRH, or both. Prolactin levels were not reported in either study, although in the study of orchidectomized rats, animals were free of gross evidence of pituitary tumors.

Similar to these studies in rats, Coquelin and Desjardins (1982) found a decrease in the frequency and amplitude of pulsatile LH secretion in 27- to 28-month-old compared to 6- to 8-month-old male CBF_1 mice, again suggesting an impairment in hypothalamic GnRH pulse generator activity with aging.

In contrast to this apparent reduction in GnRH pulse generator activity in aging mice and Sprague–Dawley rats, in intact young (3- to 4-month), middle-aged (12- to 13-month), and old (21- to 22-month) male Brown Norway rats, FSH pulse frequency increased with aging and LH pulse frequency tended to increase with aging, although the latter was not significant (Bonavera *et al*., 1997). Alterations in pulsatile gonadotropin secretion was not investigated in orchidectomized animals, so age-related changes in GnRH pulse generator activity in the absence of testosterone negative feedback are not known in this strain. However, despite the tendency for LH pulse frequency to increase in old rats, the amplitude and the total area under the LH pulses were reduced, indicating a decrease in the amount of LH secreted per pulse (Fig. 57.1). These findings

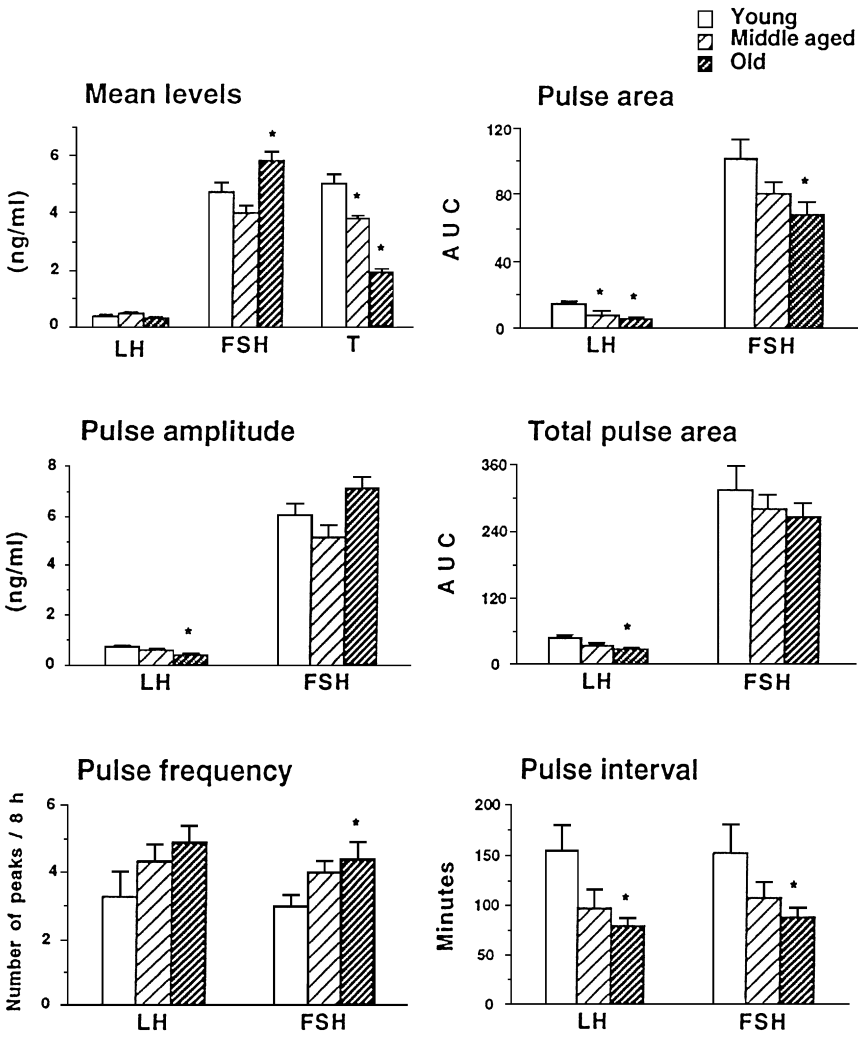

FIG. 57.1. Effects of age on mean hormone levels and on luteinizing hormone (LH) and follicle-stimulating hormone (FSH) pulsatile parameters over an 8-hr sampling period in young (3- to 4-month), middle-aged (12- to 13-month), and old (21- to 22-month) male Brown Norway rats. Data are expressed as the mean ± SEM (*$P < 0.05$ vs 3- to 4-month-old rats). Used with permission (Bonavera *et al.*, 1997).

indicate that although the effects of aging on pulsatile gonadotropin secretion differ between male Brown Norway rats and the other models, hypothalamic–pituitary regulation of the gonadal axis is altered with aging in all of these models.

2. Aging and Circadian Variations in Testosterone and LH Levels

Circadian variations in hormone secretion are thought to be regulated by the central nervous system, specifically, the suprachiasmatic nucleus of the hypothalamus. In men, aging is associated with a dampening of a number of circadian biorhythms, including those of pulsatile LH and testosterone secretion, which is thought to reflect, in part, an alteration in circadian rhythmicity of the GnRH pulse generator with aging (Tenover *et al.*, 1988).

In male Brown Norway rats, Bonavera *et al.* (1997) found that testosterone levels were higher in the morning and early afternoon than in the late afternoon in young but not in older animals (Fig. 57.2), suggesting a loss of circadian variation in testosterone levels analogous to that occurring in aging men. We also assessed age-related changes in the circadian rhythm of circulating testosterone levels in male Brown Norway rats, by measuring testosterone levels every 6 hr over a 24 hr period in rats of different ages. Three- to 4-, 12- to 13-, and 23- to 24-month-old rats were randomly assigned to one of five groups ($n = 8–9$ per group for each age), to be sacrificed every 6 hr over a 24-hr period. The differences between baseline and peak testosterone levels were used as an index of testosterone circadian variation and were compared between age groups. Testosterone levels over 24 hr declined progressively with aging, and showed a bimodal diurnal variation in young rats that was not evident in older animals. The magnitude of the diurnal variation as determined by the differences between peak and baseline testosterone levels over 24 hr decreased progressively from young (3.9 ± 0.7 ng/ml) to middle-aged

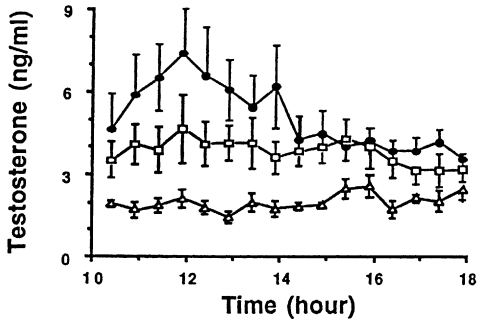

FIG. 57.2. Mean plasma testosterone levels between 10:00 and 18:00 hr in 3- to 4-month (filled circles), 12- to 13-month (open squares), and 21- to 22-month (open triangles) old male Brown Norway rats. Data are expressed as the mean ± SEM. Used with permission (Bonavera *et al*., 1997).

(1.7 ± 0.4 ng/ml) to old rats (0.8 ± 0.2 ng/ml, $P<0.01$ for young vs middle-aged and old rats) (Fig. 57.3). As in the report by Bonavera *et al*., (1997), testosterone levels were higher in the late morning than in the late afternoon in young but not in older male Brown Norway rats. However, unlike Bonavera *et al*., we sampled blood over a 24 hr period, revealing an apparent late evening surge in testosterone levels in young Brown Norway rats that was not present in older animals.

We also observed an apparent LH circadian rhythmicity in young rats that diminished with aging, although we were unable to show a statistically significant difference between age groups in the amplitude of LH circadian rhythmicity over a 24 hr period (Fig. 57.3). This loss of the circadian variation in testosterone and apparently LH levels with aging may reflect altered central nervous system control of the LH circadian rhythm (Tenover *et al*., 1988). It is possible that the variations in testosterone levels we observed in young rats were due to pulsatile secretion of testosterone, and loss of the apparent diurnal variation in testosterone levels with aging could reflect a decrease in the amplitude of pulsatile testosterone secretion. Nevertheless, the fact that we and others have found higher testosterone levels in the late morning than in the late afternoon in young but not in old rats suggests that the age-related reductions in testosterone were due, at least in part, to alterations in the circadian rhythm of testosterone levels.

Indirect studies of pulsatile gonadotropin release and circadian rhythmicity such as these suggest altered hypothalamic regulation of reproductive axis function with aging. However, these studies do not indicate whether such age-related changes are due to intrinsic alterations in GnRH-containing neurons with reduced GnRH synthetic capacity, or to changes in other neurotransmitters and neuropeptides affecting reproductive axis function (e.g., neuropeptide Y, catecholamines, and endogenous opioid peptides). Other approaches are needed to address these questions.

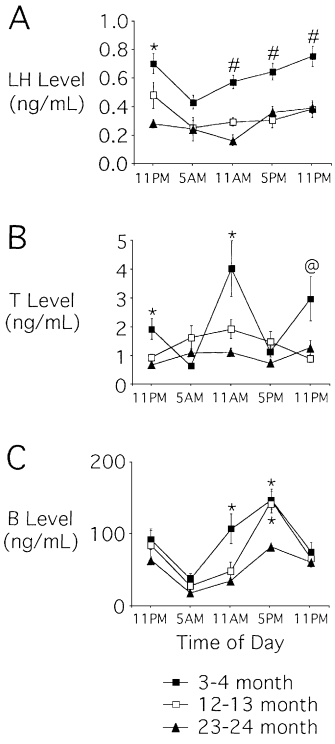

FIG. 57.3. Circadian variation in serum luteinizing hormone (LH; A), testosterone (T; B), and corticosterone (B; C) levels measured by radioimmunoassay in young (3- to 4-month-old, filled squares), middle-aged (12- to 13-month-old, open squares), and old (23 hr to 24-month-old, filled triangles) male Brown Norway rats sacrificed at 6 hr intervals over a 24 hr period ($n = 8$–9/time point for each age group). Values shown are means ± SEM ($^{*}P<0.05$ vs old; $^{\#}P<0.01$ vs middle-aged and old; $^{@}P=0.05$ vs old). Note that testosterone levels exhibited a bimodal diurnal variation in young but not older rats, and B levels exhibited a circadian rhythm with a decreasing amplitude in old compared to younger rats. Used with permission (Gruenewald *et al*., 1999).

3. Aging and Androgen Feedback Sensitivity and Action in the Brain

An age-related increase in the sensitivity of gonadotropin secretion to androgenic feedback has been reported in a number of studies in male rats (Shaar *et al*., 1975; Pirke *et al*., 1978b; Gray *et al*., 1980) and men (Winters *et al*., 1984; Deslypere *et al*., 1987). The primary observation in support of increased feedback sensitivity with aging in most of these studies was that circulating gonadotropin levels were suppressed to a greater degree by androgen replacement in old than in younger males. Furthermore, studies in men reported a greater inhibitory effect of androgens on gonadotropin pulse frequency in old than in younger men (Winters *et al*., 1984), indicating involvement of the hypothalamic GnRH pulse generator. Potential contributors to increased androgenic feedback sensitivity with aging have been suggested, including increased endogenous opiate activity, age-related hyperprolactinemia, impairment of pituitary gonadotropin secretory function, and impaired central nervous system stimulation of the pituitary–gonadal axis (Gray *et al*., 1980). However, the actual mechanism(s) responsible for this increased hypothalamic androgen feedback sensitivity with aging have remained unclear. Data from our laboratory suggest the possibility that this may be due to changes at the level of the hypothalamus, and specifically GnRH gene expression (see section II.C.2, below).

Alterations in androgen feedback sensitivity and action in the brain with aging could be due to changes in sex steroid hor-

mone receptor binding or active androgen metabolism (e.g., aromatization to estradiol (E_2) or 5α-reduction to dihydrotestosterone (DHT)) within the central nervous system. In male F344 rats, Chambers *et al*. (1991) reported that nuclear androgen receptor binding was decreased in the hypothalamus and preoptic area of old rats and activity of aromatase (the enzyme that converts androgens to estrogens) in the preoptic area declined with aging. To determine whether these deficits in androgen receptor binding and active metabolism of testosterone were due to low testosterone levels in the old rats, young and old rats were orchidectomized and given testosterone replacement, resulting in testosterone levels that were similar in the two age groups but higher than in intact young rats. Nuclear androgen receptor binding was similar in old orchidectomized/testosterone-replaced compared to young intact rats, although binding levels were not as high as in young orchidectomized/testosterone-replaced animals. Thus, age-associated alterations in androgen receptor binding appear to be due in part to testosterone deficiency. There were no age-related differences in the induction of aromatase activity by testosterone, implying that testosterone deficiency does not account for the decreased aromatase activity in intact old rats. Functionally, none of the old intact rats ejaculated during tests of sexual behavior, whereas all of the young rats ejaculated. When animals were orchidectomized and testosterone replaced, young rats continued to exhibit ejaculation in all cases, whereas 25% of the old rats ejaculated. Thus, there was some improvement in sexual functioning with restoration of testosterone levels, but not to levels observed in young rats.

In further studies by this group, old (24.5 months) male F344 rats exhibited lower nuclear estrogen receptor levels in the amygdala than young (5 months) animals, but this was not a consequence of decreased circulating estradiol levels which were similar in the two age groups (Roselli *et al*., 1993). Administration of testosterone to orchidectomized old males restored testosterone to normal young adult levels but failed to increase nuclear estrogen receptor levels in the amygdala to levels observed in young rats. Taken together, these findings suggested that the inability of testosterone treatment to increase aromatase activity in the hypothalamus and nuclear estrogen receptor levels in the amygdala may contribute to the impairment in sexual performance observed in old males. Furthermore, the decrease in central nervous system synthesis and binding of estrogen derived from circulating testosterone may lead to decreased androgen responsiveness with aging.

It has been suggested that some androgen actions in brain and feedback effects of testosterone on gonadotropin secretion are mediated, in part, by conversion of testosterone to DHT and 5α-androstane-3α,17β-diol (3α-diol), which are produced through the actions of 5α-reductase and 3α-hydroxysteroid dehydrogenase, respectively (Piva *et al*., 1993). Using tissues from young (3-month-old) and old (20+-month-old) male rats, Piva *et al*. found no age-related impairment in the ability of hypothalamic tissue *in vitro* to metabolize testosterone into either DHT or 3α-diol (Piva *et al*., 1993). Furthermore, testosterone replacement of old rats did not exert any effect on levels of these metabolites of testosterone. These data suggest that alterations in testosterone feedback sensitivity with aging are not due to alterations in the hypothalamic metabolism of testosterone to either DHT or 3α-diol.

B. Morphological Considerations

The perikarya of GnRH-containing neurons in the medial preoptic area (MPOA) of the male F344 rat do not appear to deteriorate with age, but morphologic evidence of nerve terminal deterioration in the median eminence has been reported (Sladek *et al*., 1980), suggesting the existence of a primary age-related defect in the secretory capacity of GnRH-containing neurons. However, a 10-fold increase in synaptic input onto GnRH perikarya was observed in the brains of aging male F344 rats, with an increase in the proportion of synaptic boutons associated with an inhibitory morphology (Witkin, 1987). To determine whether this apparent increase in inhibitory input was due to increased input from GABAergic neurons, a follow-up study was performed in aging virgin male Sprague–Dawley rats (Witkin, 1992a) employing dual label immunocytochemistry to identify neurons positive for GnRH and glutamic acid decarboxylase, the enzyme required for GABA synthesis. A threefold increase in synaptic density was observed in old rats, similar to the previous findings in aging F344 rats. However, the density of GABAergic synaptic input was similar in young and old male Sprague–Dawley rats, and GABAergic input decreased with aging as a percentage of the total synaptic input onto GnRH neurons. Further complicating the interpretation of these studies was the finding that synaptic input onto GnRH-containing neurons did not increase in retired breeder males of the Sprague–Dawley strain (Witkin, 1992b), indicating an important effect of reproductive experience on the density of synaptic input onto GnRH-containing neurons in these animals. Nevertheless, it is intriguing that synaptic input to GnRH-containing neurons increases with aging in some male rat models, suggesting that some of the changes in hypothalamic regulation of reproduction with aging may be due to altered neurotransmitter or neuropeptide input affecting GnRH-containing neurons.

To our knowledge, no similar studies have been performed in Brown Norway rats, nor are quantitative data available regarding aging effects on synaptic input by other excitatory or inhibitory neuron types onto GnRH neurons (e.g., opioid peptide-containing and catecholaminergic neurons).

C. Aging and GnRH Synthetic and Secretory Capacity

Many of the early studies intended to directly determine the effects of aging on GnRH-containing neurons involved the measurement of hypothalamic GnRH content in aging animals. GnRH content was reported to be stable (Dorsa *et al*., 1984) or decreased (Riegle *et al*., 1977; Bedrak *et al*., 1983) in the hypothalamus of aging male rats and decreased in senescent white-footed mice (Steger *et al*., 1980a). In addition, GnRH content in the median eminence, the location of the terminal neuroendocrine boutons of GnRH neurons, was reported to be decreased in old male rats (Simpkins *et al*., 1977). However, the interpretation of studies of GnRH peptide content is difficult, because a decrease in GnRH content, for example, could represent either a decrease in GnRH synthesis or an increase in GnRH release or metabolism. Nevertheless, these alterations in hypothalamic GnRH content suggested that age-related changes in GnRH synthetic or secretory capacity might contribute to altered pituitary–gonadal function with aging.

1. Basal and Stimulated GnRH Release *in Vitro*

Further investigations were performed *in vitro*, to determine the effects of aging on GnRH release from superfused hypothalami. These studies, performed under both baseline and stimulated conditions, have yielded contradictory results. In one study, the mediobasal hypothalamus was removed from 6- and 18-month-old male Sprague–Dawley rats, and were perfused *in vitro* (Zanisi *et al*., 1987). The levels of GnRH were measured in the effluent, under basal conditions and after exposure to a depolarizing concentration of K^+ (110 mM). The mean basal concentration of GnRH was similar in young and old rats. Exposure to K^+ enriched medium for 5 min every 30 min resulted in an increase in GnRH levels in the effluent in each of the six exposure periods (Fig. 57.4). The magnitude of the GnRH response was less in old than in young animals, but this was not statistically significant.

In a second study, GnRH release was measured *in vitro* from the median eminence of 4-, 11-, 18-, and 27-month-old male F344 rats under basal conditions and after 30 min of exposure to a stimulating concentration (60 μM) of norepinephrine (Steger *et al*., 1985). In this study, basal concentrations of GnRH in the effluent increased progressively with aging, although only the 4- and 27-month-old groups were significantly different. Norepinephrine tended to stimulate GnRH release in all age groups except the 11-month-old rats, but the increase in GnRH release over the baseline values in the same age group was significant only for the 27-month-old rats.

Finally, a third group compared GnRH release *in vitro* from the medial basal hypothalamus of 2- and 23-month old male F344 rats, under basal conditions and in response to stimulating concentrations (20 μM) of norepinephrine followed by 40 μM of naloxone (Jarjour *et al*., 1986). The pituitary fossa was inspected at the time animals were sacrificed, and no macroscopic evidence of tumor was found. Basal GnRH levels in the effluent were significantly lower in old than in young rats (Table 57.1). Both young and old rats responded to stimulation with norepinephrine and naloxone, with a similar relative and absolute magnitude of response to stimulation to these agents in young and old rats. However, like the basal GnRH levels, the stimulated GnRH output from the medial basal hypothalamus of old rats was less than from the young rats.

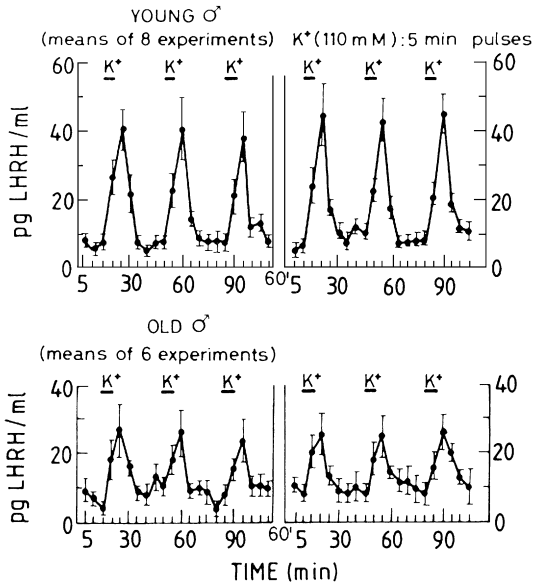

FIG. 57.4. GnRH release *in vitro* from mediobasal hypothalami from 6-month (top)- and 18-month (bottom)-old male Sprague–Dawley rats under basal conditions and after perfusion with potassium-enriched medium (110 mM). Values shown are means of eight and six experiments, respectively. Used with permission (Zanisi *et al*., 1987).

TABLE 57.1 Basal and Stimulated GnRH Output from Medial Basal Hypothalamus (MBH) of 2- and 23-Month-Old Male Fischer 344 Rats *in Vitro*

	2-Month	23-Month
Basal	33.5*	13.1
	(25.8–43.5)	(11.8–14.5)
Norepinephrine (20 μM)	98.1*,#	38.5#
	(57.9–166.3)	(21.9–67.8)
Naloxone (40 μM)	57.9*,#	36.6#
	(37.5–89.4)	(27.1–49.5)

Note. Data adapted from Jarjour *et al*. (1986); used with permission. Values shown are geometric means (95% confidence limits) of GnRH output from chambers (four young and four old) in pg/6 MBH/min.
*$P < 0.05$ vs 2-month-old.
#$P < 0.01$ vs basal.

In summary, basal and stimulated GnRH release from hypothalamic fragments *in vitro* have been reported to be unchanged, increased, and decreased with aging, depending on the rat strain and secretagogue used. Two of these studies used the aging male F344 rat model; therefore, as noted above, data from these studies may be confounded by the effects of excessive sex steroid feedback from testicular tumors on GnRH secretion. The remaining study performed in Sprague–Dawley rats suggests no major change in GnRH release with aging. However, a trend was observed toward a decrease in GnRH release and postperfusion GnRH content with aging, consistent with a modest decrease in GnRH secretory capacity. To our knowledge, no studies of this type have been performed in the aging male Brown Norway rat.

2. Prepro-GnRH mRNA and GnRH Peptide Content

To further investigate the possibility of altered GnRH synthetic capacity in aging animals, we tested the hypothesis that the age-related decrease in GnRH secretion is due to decreased GnRH gene expression. In intact 3- and 24-month-old male F344 rats, we used *in situ* hybridization histochemistry (ISHH) to compare prepro-GnRH (ppGnRH) mRNA in the MPOA and the diagonal band of Broca and measured GnRH peptide content in microdissected regions of the arcuate nucleus and median eminence by radioimmunoassay (Gruenewald and Matsumoto, 1991). ppGnRH mRNA levels were quantified in anatomically matched sections of the MPOA and the diagonal band of Broca by using the number of silver grains per cell as an index of cellular ppGnRH mRNA content and the number of labeled cells counted per brain section as an index of the number of neurons expressing the GnRH gene.

In rats, most of the cell bodies of GnRH-producing neurons are localized in the MPOA, and to a lesser extent in the diag-

onal band of Broca and medial septal nucleus, rather than in the hypothalamus. In addition, many but not all of the GnRH neurons located in septal, preoptic, and hypothalamic structures have been found to project to the median eminence (Silverman *et al*., 1987; Merchenthaler *et al*., 1989) and are therefore thought to be directly involved in the regulation of gonadotropin secretion. However, deafferentation and lesioning studies suggest that preoptic, but not septal nuclei are essential for normal gonadotropin secretion (Koves and Molnar, 1986; Silverman *et al*., 1987). Thus, by combining measurements of ppGnRH mRNA levels in the MPOA and the diagonal bond of Broca with GnRH peptide content measured in the arcuate nucleus and median eminence, which contain the infundibular tract and terminal neuroendocrine boutons of GnRH neurons, we were able to obtain a more complete picture of changes in synthetic capacity of GnRH neurons directly regulating gonadotropin secretion than with either technique alone.

We found an age related decrease in the number of neurons expressing the GnRH gene in the MPOA of intact (sham-operated) male F344 rats, although cellular ppGnRH mRNA content was unchanged with age. Furthermore, GnRH peptide content was decreased with aging in the arcuate nucleus and tended to decrease with aging in the median eminence. Finally, serum gonadotropin levels were lower in old than in young rats. Taken together, these findings suggested that GnRH synthetic capacity is decreased with aging.

In addition, since sex steroids modulate GnRH secretion, we determined hypothalamic–pituitary responsiveness to removal of testicular feedback by comparing ppGnRH mRNA and gonadotropin levels in intact (sham-operated) and orchidectomized 3- and 24-month-old F344 rats. In contrast to intact rats, neither the number of cells expressing GnRH nor cellular ppGnRH mRNA content were altered with aging in the MPOA of orchidectomized rats, suggesting that the age-related decrease in GnRH synthetic capacity was dependent upon testicular feedback. The age-related decrease in ppGnRH mRNA and gonadotropins observed in intact but not in orchidectomized rats could have been due to increased hypothalamic sensitivity to testosterone negative feedback with age. The concept of increased testosterone feedback sensitivity with aging has experimental support from other studies in aging male rats and humans (Pirke *et al*., 1978b; Steiner *et al*., 1984; Winters *et al*., 1984), as noted above (see section II.A.2). Alternatively, we postulated that increased production of factors other than testosterone by the aging testis may have inhibited hypothalamic GnRH synthesis in intact but not orchidectomized old rats. Our subsequent work examining the effects of excessive progesterone secretion from testicular tumors on gonadotropin levels in the aging male F344 rat (see section I.A.2, above) (Gruenewald *et al*., 1992) seemed to support the latter explanation and suggested that the apparent decline in GnRH synthetic capacity and reproductive function with aging may have been due to a suppressive effect of excessive progesterone secretion on the reproductive hypothalamic/pituitary axis rather than aging per se.

Based on this major concern regarding potential confounding factors in the F344 model, we performed further studies of aging and hypothalamic GnRH gene expression in the male Brown Norway rat. We compared ppGnRH mRNA in the MPOA and the diagonal band of Broca by ISHH, and GnRH peptide content in microdissected brain areas by radioimmunoassay in intact (sham-operated) 3- to 4-, 12- to 13-, and 22- to 24-month-old male Brown Norway rats (Gruenewald *et al*., 1999). We found that in intact male Brown Norway rats, the number of neurons expressing GnRH decreased progressively from young (11.8 ± 0.4 cells/section) to middle-aged (11.1 ± 0.6 cells/section) to old (9.9 ± 0.4 cells/section) rats ($P < 0.05$ for young vs middle-aged and old rats) (Fig. 57.5A and 57.5B). The cellular ppGnRH mRNA content was also decreased with aging and was significantly lower in 22- to 24-month-old (102.6 ± 2.8 grains/cell) than in 3- to 4-month-old (110.3 ± 1.9 grains/cell) or 12- to 13-month-old (109.1 ± 4.0 grains/cell) animals ($P < 0.05$).

We also determined hypothalamic–pituitary responsiveness to removal of testicular feedback by comparing ppGnRH mRNA and gonadotropin levels in intact (sham-operated) and orchidectomized young, middle-aged, and old Brown Norway rats. In contrast to aging male F344 rats, cellular ppGnRH mRNA content decreased progressively with aging in young (118.8 ± 2.8 grains/cell), middle-aged (115.9 ± 1.8 grains/cell), and old (109.6 ± 4.4 grains/cell, $P < 0.05$) orchidectomized as in intact rats (Figs. 5C and 5D), although the number of neurons expressing ppGnRH mRNA was unchanged with aging in these rats (young, 11.2 ± 0.7 cells/section; middle-aged, 11.1 ± 0.3 cells/section; old, 10.8 ± 0.5 cells/section).

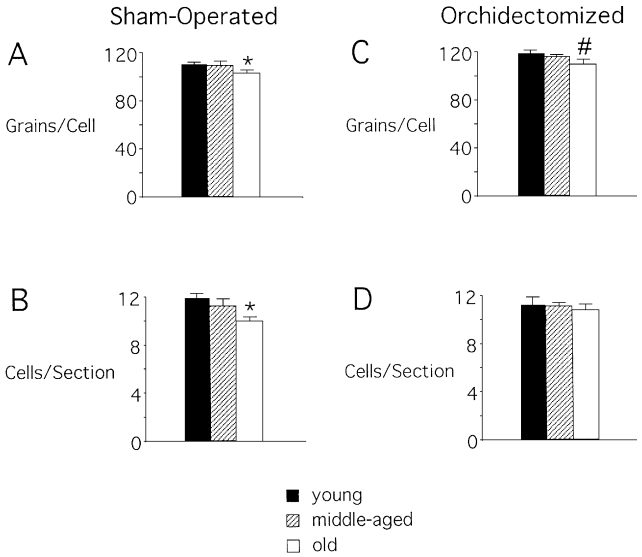

FIG. 57.5. ppGnRH mRNA content measured by *in situ* hybridization histochemistry in the medial preoptic area (MPOA) and diagonal band of Broca (DBB) of sham-operated (A, B) and orchidectomized (C, D) young (3-month-old, solid bars, $n = 8$ and 8, respectively), middle-aged (13-month-old, hatched bars, $n = 8$ and 7, respectively), and old (23-month-old, open bars, $n = 7$ and 7, respectively) male Brown Norway rats. ppGnRH mRNA content was quantitated by measurement of the number of silver grains per cell (A, C) and the number of labeled cells per section (B, D). Values shown are means ± SEM. The number of grains per cell was significantly reduced in old compared to young and middle-aged rats in both the sham-operated and orchidectomized groups. The number of labeled cells per section was significantly decreased in old compared to young and middle-aged sham-operated, but not orchidectomized animals (*$P < 0.05$ vs young and middle-aged rats). Used with permission (Gruenewald *et al*., 1999).

Thus, the reduction in GnRH gene expression with aging is not dependent on testicular feedback factors in the male Brown Norway rat. These finding suggest that excessive progesterone secretion in aging F344 rats may have confounded our earlier studies.

We also found that ppGnRH mRNA levels were not markedly affected by orchidectomy in young rats. However, with aging, both the cellular ppGnRH mRNA content and the number of neurons expressing the GnRH gene were increased with orchidectomy, suggesting that testicular factors may play a greater role in regulating GnRH gene expression in old than in younger animals. This increased effect of orchidectomy on GnRH gene expression with aging as well as the age-related decline in GnRH gene expression itself could be explained by an increase in testosterone negative feedback sensitivity with aging. Additional studies to determine the dose–response effects of testosterone replacement on hypothalamic ppGnRH mRNA levels in aging orchidectomized Brown Norway rats will be required to test this hypothesis.

III. Modulation of GnRH Neuronal Activity by Other Neurotransmitters and Neuropeptides

Our understanding of reproductive system aging must account not only for intrinsic alterations in GnRH neuronal function and changes in gonadal feedback, but also for alterations in other neuromodulatory influences that affect GnRH secretion. In this regard, our findings in Brown Norway rats suggest that the age-related decline in gonadotropin secretion is not fully accounted for by changes in GnRH gene expression. ppGnRH mRNA levels in intact male Brown Norway rats decreased by 20 to 25% between young and old animals, if changes in both the cellular ppGnRH mRNA content and the number of neurons expressing the GnRH gene are combined (Gruenewald *et al*., 1999). In contrast, GnRH peptide content in the MPOA and median eminence declined by 35–40% in old Brown Norway rats, suggesting that factors other than altered GnRH gene expression contribute to the age-related dysregulation of reproductive axis function. At the same time, pituitary responsiveness to GnRH stimulation is intact with aging in male Brown Norway rats (Bonavera *et al*., 1998; Gruenewald *et al*., 1999), indicating a defect in brain regulation rather than at the pituitary level.

Pulsatile GnRH secretion is thought to be governed by an interactive network of neurons secreting a variety of neurotransmitters and neuromodulators (Kalra *et al*., 1997). The list of candidate substances that may mediate the effects of aging on GnRH neurons includes (but is not limited to) neuropeptide Y, the excitatory amino acid glutamine, the endogenous opioid peptide β-endorphin, and the diffusable gas nitric oxide, among many others. However, demonstration of an age-related change in neurons that are thought to affect GnRH neurons does not prove that the impairment in GnRH synthesis and secretion is due to the change in these ''upstream'' neurons. Rather, it seems likely that as the final common pathway of hypothalamic regulation of reproduction, age-related changes in GnRH neuronal function reflect the integration of all changes in neuromodulatory systems impacting GnRH neurons, together with intrinsic changes in GnRH neurons themselves. Sorting out the relative contributions of each of these elements is a formidable challenge indeed.

A. Neuropeptide Y

Neuropeptide Y (NPY) has been implicated in the control of gonadotropin secretion. In adult male rats, intracerebroventricular administration of NPY stimulates LH release (Allen *et al*., 1985), although chronic intracerebroventricular NPY infusion results in a marked inhibition of pituitary–testicular axis function (Pierroz *et al*., 1996). Furthermore, NPY neurons synapse on GnRH neuron perikarya (Tsuruo *et al*., 1990), suggesting that these stimulatory and inhibitory effects of NPY on LH are mediated in part by corresponding actions of NPY on GnRH neurons. The actions of NPY on the hypothalamic/pituitary/gonadal axis appear to depend on the presence of sex steroids. For example, NPY readily stimulates GnRH release *in vitro* from the mediobasal hypothalamus or the median eminence of intact adult male rats (Kalra *et al*., 1990), and intracerebroventricular (or intravenous) NPY induces an increase in GnRH gene expression in preoptic area neurons of adult male rats (Li *et al*., 1994). However, GnRH responsiveness to NPY is reduced in tissues obtained from orchidectomized rats (Urban *et al*., 1996), and in contrast to intact rats, NPY administered intravenously or icv decreases circulating LH levels in orchidectomized rats (Kerkerian *et al*., 1985).

NPY neurons appear to be an important target of sex steroid feedback (Sar *et al*., 1990; Kalra *et al*., 1997). NPY release and gene expression in the arcuate nucleus is modulated by testosterone, with castration decreasing NPY release and arcuate nucleus prepro-NPY messenger RNA (ppNPY mRNA) levels, and testosterone replacement increasing ppNPY mRNA back to levels observed in intact rats (Sahu *et al*., 1992; Urban *et al*., 1993).

Based on the foregoing, it has been proposed that decreased LH and GnRH secretion in aging rats is due at least in part to declining NPY neuronal activity and that decreased circulating testosterone levels with aging may contribute to declining NPY neuron secretory activity (Sahu *et al*., 1988; Kalra *et al*., 1993). An initial study to assess age-related changes in hypothalamic NPY activity involved the measurement of NPY peptide content in discrete hypothalamic nuclei and the ability of potassium-induced depolarization to cause NPY release from the mediobasal hypothalamus of male Sprague–Dawley rats *in vitro* (Sahu *et al*., 1988). Compared to 2.5-month-old rats, 8- and 13-month-old rats released significantly less NPY from the mediobasal hypothalamus after potassium-induced depolarization *in vitro*, and NPY content was reduced in several hypothalamic nuclei containing the perikarya and nerve terminals of NPY neurons, including the arcuate nucleus, MPOA, median eminence, and other nuclei.

A subsequent study of the effects of aging, orchidectomy, and testosterone replacement on NPY peptide content in hypothalamic nuclei revealed that in young rats, orchidectomy was associated with a decrease in NPY content in the ventromedial nucleus, arcuate nucleus, and median eminence that was prevented with testosterone replacement (Sahu *et al*., 1990) (Fig. 57.6). However, no effect of orchidectomy was observed in four other nuclei (MPOA, suprachiasmatic nucleus,

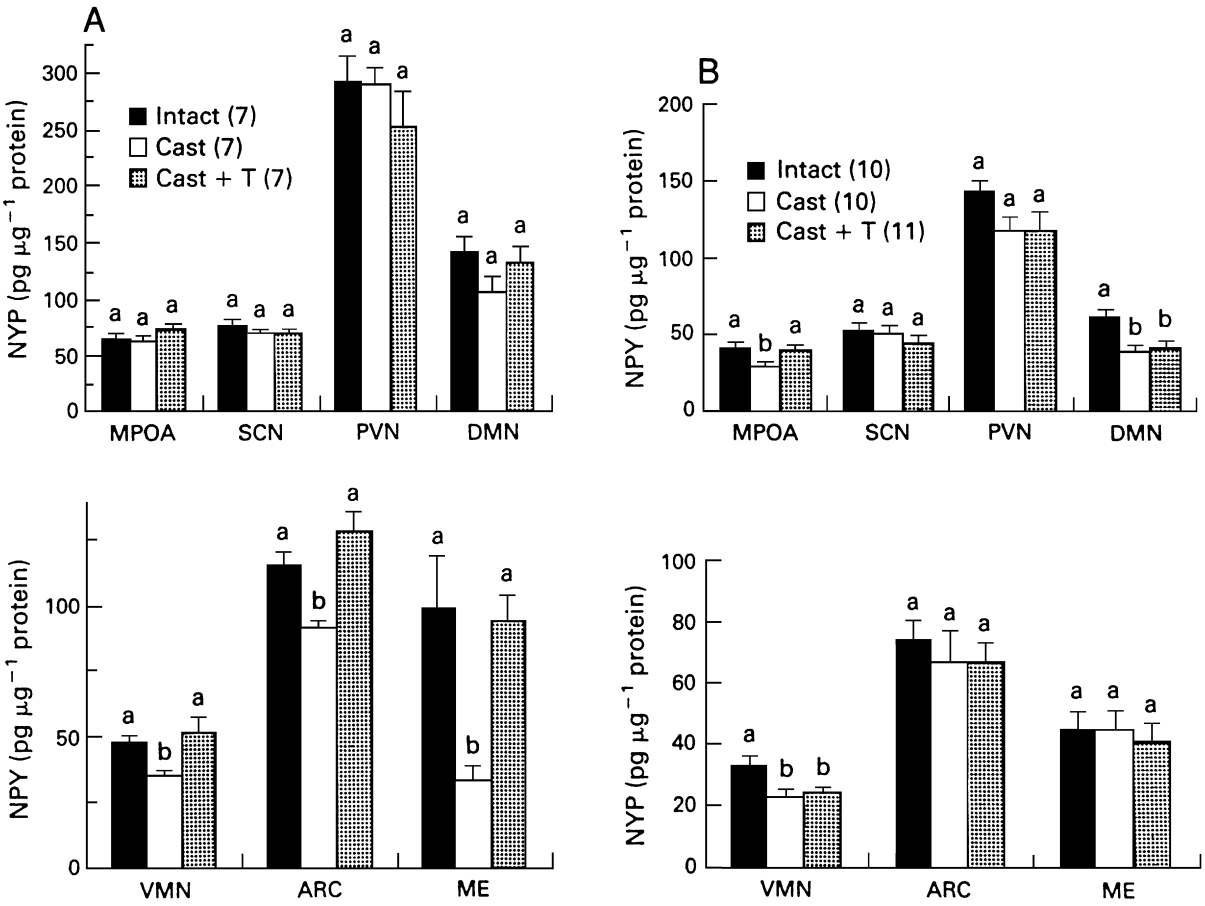

FIG. 57.6. Effects of orchidectomy (Cast) and testosterone (T) replacement on the neuropeptide Y (NPY) content by radioimmunoassay in various hypothalamic sites of (A) 2.5-month- and (B) 15-month-old male Crl:CD(SD)Br rats. ARC, arcuate nucleus; DMN, dorsomedial nucleus; ME, median eminence; MPOA, medial preoptic area; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; VMN, ventromedial nucleus. Figures in parentheses denote number of rats. Values shown are means ± SEM. Bars with dissimilar superscripts are significantly different from each other for that nucleus ($P < 0.05$). Used with permission (Sahu *et al*., 1990).

paraventricular nucleus, and dorsomedial nucleus). In contrast, old rats did not exhibit a decrease in NPY content with orchidectomy in the median eminence or the arcuate nucleus, whereas a decrease was observed in the ventromedial nucleus, MPOA, and dorsomedial nucleus. Furthermore, with the exception of the MPOA (the site of most GnRH neuron cell bodies), testosterone replacement did not prevent the decrease in NPY content with orchidectomy in old rats. These findings suggested regional specificity in the effects of orchidectomy on NPY neurons and loss of NPY responsiveness to testosterone action with aging.

As noted previously, it is not possible to determine whether a change in peptide content is due to an effect on peptide synthesis or an opposite effect on peptide release or degradation. Therefore, we performed additional studies in our laboratory in aging male Brown Norway rats to determine whether the decreased NPY content and *in vitro* release with aging were associated with a decrease in hypothalamic NPY synthetic capacity (Gruenewald *et al*., 1994b). Furthermore, we assessed the potential role of testicular feedback in contributing to age-related alterations in NPY synthetic capacity.

We hypothesized that reduced NPY secretion with aging is due to decreased NPY gene expression and that this decrease is independent of testicular feedback. To test this hypothesis, arcuate nucleus ppNPY mRNA levels measured by *in situ* hybridization and serum gonadotropin and testosterone levels determined by radioimmunoassay were compared in sham-operated and orchidectomized young (3 month), middle-aged (13 month), and old (23 month) male Brown Norway rats. Hybridization area and average optical density were used as indices of arcuate nucleus ppNPY mRNA content. In sham-operated rats, both ppNPY mRNA hybridization area and optical density decreased progressively with aging (Fig. 57.7), while serum testosterone levels were decreased in old compared to middle-aged and young rats. In orchidectomized rats, ppNPY mRNA hybridization area also decreased significantly with aging, although optical density did not change significantly (Fig. 57.8). This observation of an age-associated reduction in ppNPY mRNA levels in both sham-operated and orchidectomized rats suggested that NPY synthetic capacity is decreased with aging independently of the effects of testicular feedback. Furthermore, ppNPY mRNA levels were

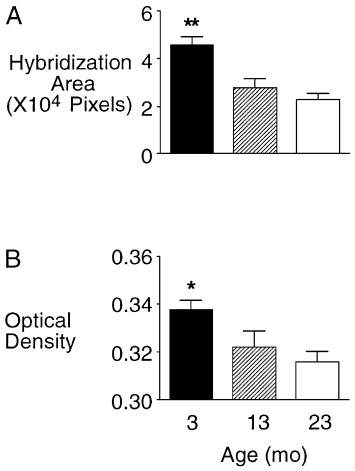

FIG. 57.7. ppNPY mRNA content measured by *in situ* hybridization histochemistry in the arcuate nucleus of sham-operated young (3-month-old, $n=8$, solid bars), middle-aged (13-month-old, $n=6$, hatched bars), and old (23-month-old, $n=7$, open bars) male Brown Norway rats. ppNPY mRNA levels were quantitated by film autoradiography over the entire arcuate nucleus of each rat brain. Total hybridization area (A) and average optical density (B) were used as indices of total arcuate nucleus ppNPY mRNA levels. Values shown are means ± SEM. Note that optical density is a logarithmic function, and that optical density scale does not include zero. Both hybridization area and optical densities were significantly decreased in old and middle-aged compared to young rats (**$P<.001$ vs middle-aged and old; *$P<0.05$ vs middle-aged and old). Used with permission (Gruenewald *et al*., 1994b).

lower in orchidectomized than in sham-operated young and middle-aged rats, while they were similar in orchidectomized and sham-operated old rats, indicating a lack of effect of orchidectomy upon ppNPY mRNA levels in older animals.

Our findings are consistent with the hypothesis that declining NPY synthetic capacity and secretion contributes to a decrease in GnRH and LH secretion with aging. Although ppNPY mRNA levels were markedly decreased in middle-aged animals, we found that LH and testosterone levels at a single time point were not decreased in middle-aged compared to young intact Brown Norway rats, (Gruenewald *et al*., 1994b), suggesting a lack of concordance between ppNPY mRNA levels on the one hand and circulating gonadotropin and testosterone levels on the other. However, we subsequently found that circulating levels of LH and testosterone determined over a 24-hr period decreased progressively with aging, apparently the result of an age-related decrease in diurnal variation of these hormones in male Brown Norway rats (Gruenewald *et al*., 1999). These recent findings indicate that the age-related reductions in NPY synthetic capacity are associated with reductions in GnRH, LH, and testosterone secretion in this model.

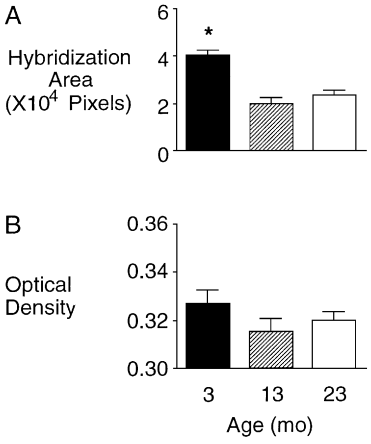

FIG. 57.8. ppNPY mRNA content measured by *in situ* hybridization histochemistry in the arcuate nucleus of orchidectomized young (3-month-old, $n=8$, solid bars), middle-aged (13-month-old, $n=7$, hatched bars), and old (23-month-old, $n=7$, open bars) male Brown Norway rats. Values shown are means ± SEM. In these orchidectomized rats, total hybridization area (A) was significantly decreased in old and middle-aged compared to young rats (*$P<.001$ vs middle-aged and old), while average optical density (B) was unchanged with aging. Used with permission (Gruenewald *et al*., 1994b).

Some of our findings suggest that declining testosterone levels may not be a major cause of decreased NPY synthetic capacity with aging. First, NPY hybridization area decreased with aging in both orchidectomized and in sham-operated animals (Figs. 57.7 and 57.8). Second, the reduction in NPY gene expression we observed with orchidectomy (10–32% depending upon age group) was not as marked as the decline occurring with aging (40–50%), even though the reduction in testosterone levels was much greater with orchidectomy than aging. Therefore, the mechanisms underlying the decreases in NPY gene expression with aging and orchidectomy may be different, and the effects of aging upon ppNPY mRNA levels are not due only to declining testosterone levels with aging.

A subsequent study of aging and ppNPY gene expression in male F344 × Brown Norway rats by Li *et al*. (1998) reported that ppNPY mRNA levels did not decrease from 3 to 24 to 31 months of age. However, Li *et al*. sacrificed their rats at a different time in the light–dark cycle, 2 hr after lights-off, in contrast to our practice of sacrificing animals during the light phase. ppNPY mRNA levels in young adult animals exhibit circadian variation, with a sustained increase during the second half of the light phase and a sharp decrease at the time of lights off (Akabayashi *et al*., 1994). When rats were killed during the light phase, Li *et al*. were able to confirm a decrease in ppNPY mRNA with aging, similar to our results (unpublished data, see Li *et al*., 1998). Based on these findings, future studies of NPY gene expression should take careful account of this circadian variation in ppNPY mRNA (and circulating hormone) levels, and further studies are indicated to determine whether the circadian variation observed in young adult animals is affected by aging.

B. Excitatory and Inhibitory Amino Acids

Acidic amino acids such as glutamate and aspartate activate central nervous system neurons and are thought to be important excitatory neurotransmitters in the mammalian central nervous system. Moreover, excitatory amino acids are thought to have an important role in the regulation of GnRH and LH secretion. For example, *N*-methyl-D-aspartate (NMDA), an analog of the putative neurotransmitter aspartate, increases LH secretion in male rats after systemic or central administration (Schainker and Cicero, 1980; Ondo *et al*., 1988). This

effect of NMDA on the hypothalamic–pituitary–gonadal axis is thought to be mediated by specific receptors within the central nervous system rather than at the pituitary level (Schainker and Cicero, 1980; Ondo *et al*., 1988; Brann and Mahesh, 1994), with NMDA administration stimulating hypothalamic GnRH release that in turn leads to LH release. Furthermore, ppGnRH mRNA levels increase rapidly following NMDA administration (Petersen *et al*., 1991), suggesting that NMDA stimulates GnRH neuron synthetic capacity.

With aging, decreases have been reported in binding to and number of hippocampal NMDA receptors in various rodent models (Ingram *et al*., 1992; Magnusson and Cotman, 1993). To determine whether alterations in excitatory amino acids acting through NMDA receptors could in part explain alterations in GnRH release with aging, GnRH release from hypothalamic explants from young (3- to 6-month-old) and old (20- to 22-month-old) male Wistar-Kyoto rats was measured *in vitro* after administration of the excitatory amino acids NMDA, glutamate, and kainate (Sortino *et al*., 1996). These different glutamate receptor agonists were administered because although the role of glutamate in the control of GnRH release is well-established, the involvement of various subtypes of glutamate receptors (e.g., NMDA, kainate and D,L-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors) in this process is more controversial. GnRH release in response to glutamate was similar in young and old rats, whereas NMDA elicited a greater GnRH response in young than in old rats. In contrast, GnRH release after kainate administration was greater in the old than in the young animals. These findings suggest that the involvement of the various excitatory amino acid receptor subtypes in the regulation of GnRH secretion may vary with aging.

The role of excitatory amino acids in mediating age-related changes in reproductive axis regulation was further evaluated by Bonavera *et al*. (1998) in young (3- to 4-month-old), middle-aged (12- to 13-month-old), and old (21- to 22-month-old) male Brown Norway rats. The increase in circulating LH levels after NMDA administration *in vivo* was less in old than in younger rats (Fig. 57.9), and GnRH release from mediobasal hypothalamus/preoptic area fragments *in vitro* after NMDA was also decreased in old compared to younger rats (Fig. 57.10). Finally, glutamine content in mediobasal hypothalamus/preoptic area fragments was decreased in old compared to younger rats. These findings support an important role for excitatory amino acids acting through NMDA receptors in mediating the age-related decline in GnRH secretion and extend these observations to the aging male Brown Norway rat model.

This group further investigated the potential mechanisms underlying hypothalamic–pituitary dysregulation of reproductive function, by evaluating age-related changes in nitric oxide synthesis in the preoptic area and hypothalamus of male Brown Norway rats (Vernet *et al*., 1998). Nitric oxide (NO) is a neurotransmitter that is thought to play a physiological role in stimulating the release of a number of hypothalamic neuropeptides including GnRH. Under normal conditions, NO is thought to mediate the effects of glutamate acting through NMDA receptors in the hypothalamus; glutamate stimulates neuronal NO synthase, and NO synthase inhibitors block the ability of glutamate to stimulate GnRH release

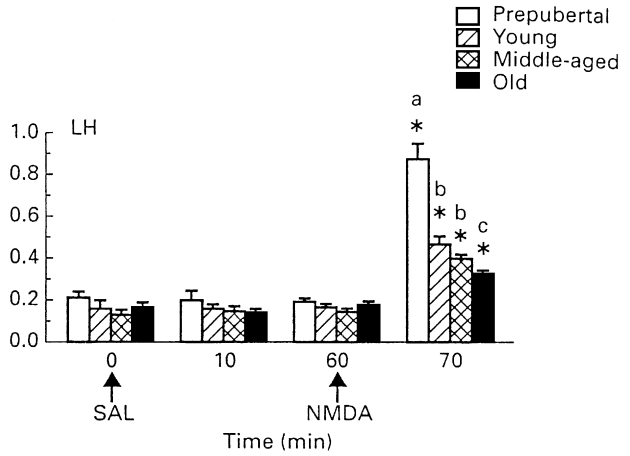

FIG. 57.9. Effect of NMDA on LH secretion in prepubertal (35-day), young (3- to 4-month), middle-aged (12- to 13-month) and old (21- to 23-month) male Brown Norway rats *in vivo*. Saline (SAL) and NMDA (5 mg/kg) were injected iv at 0 and at 60 min, respectively. Note that NMDA but not SAL produced significant increases in LH at 70 min (denoted by an asterisk, $P < 0.05$ vs SAL). Different superscripts above the bars denote significant differences ($P < 0.05$) among different age groups at the same time point. Used with permission (Bonavera *et al*., 1998).

(Rettori *et al*., 1994). Thus, an aging-related decrease in NO synthesis due to reduced excitatory amino acid binding to NMDA receptors could contribute to decreased GnRH secretion with aging. On the other hand, if glutamate stimulation of NO synthesis were excessively increased with aging, this could lead to accumulation of NO metabolites, possibly resulting in cytotoxic effects on hypothalamic neurons and impairment of GnRH neuronal function.

To determine whether aging is associated with alterations in NO synthesis in the hypothalamus that could contribute to reproductive axis dysfunction, hypothalamic NO synthase activity was measured in 3- and 24-month-old male Brown Norway rats using the arginine/citrulline assay (Vernet *et al*., 1998). A relatively modest (67%) increase in hypothalamic neuronal NO synthase activity was observed in old compared to young rats, but there was no change in neuronal NO synthase content by Western blot with aging, and hypothalamic NMDA receptor content and binding were decreased by 34 and 66%, respectively, in old rats. In contrast, hypothalamic activity of inducible NO synthase, the isoform induced in inflammatory or degenerative processes, was increased nearly fourfold in old compared to young adult rats. Thus, hypothalamic NO production appears to increase with aging, but there is no corresponding increase in the number of NMDA receptors. These findings argue against the hypothesis that reduced GnRH secretion with aging is due to a decrease in excitatory amino acid binding to NMDA receptors and subsequent reduction in NO synthesis. Furthermore, it is unlikely that excessive excitatory amino acid stimulation acting through the NMDA receptor is the cause of elevated NO synthase activity with aging, and NMDA receptors are probably not a major participant in the degenerative hypothalamic changes occurring in aging rats. However, the marked increase in inducible NO synthase activity with aging suggests that elevated NO levels could contribute to neurotoxicity by other mechanisms, possi-

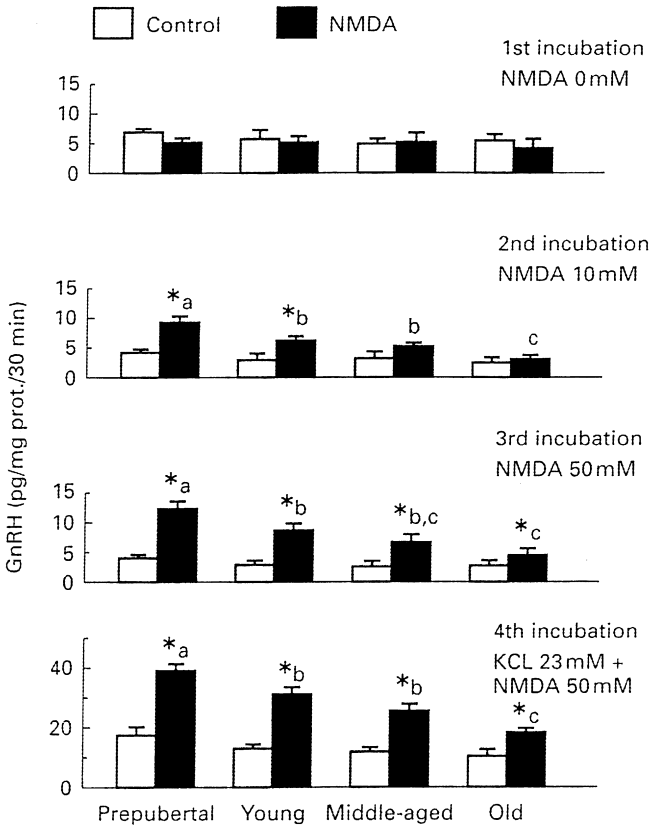

FIG. 57.10. Effect of NMDA on basal and KCl-induced GnRH release *in vitro* by fragments of preoptic area/mediobasal hypothalamus (POA-MBH) from prepubertal (35-day), young (3- to 4-month), middle-aged (12- to 13-month), and old (21- to 23-month) male Brown Norway rats. Each treated fragment (filled bars) was incubated consecutively with medium containing 0 mM (basal, first incubation), 10 mM (second incubation), 50 mM NMDA (third incubation), and 50 mM NMDA + 23 mM KCl (fourth incubation). Control POA-MBH fragments (clear bars) were incubated with Krebs-Ringer phosphate bicarbonate in the first three incubations and with Krebs-Ringer containing 23 mM KCl in the fourth incubation. Significant differences between control and NMDA treated fragments within each age group at the same incubation time are denoted by an asterisk ($P < 0.05$; unpaired student t test). Bars with different superscripts indicate significant differences ($P < 0.05$) among NMDA-treated fragments from different age groups at the same incubation time. Used with permission (Bonavera *et al.*, 1998).

bly leading to impairment in GnRH neuronal function in old animals.

Alterations in inhibitory acids may also play a role in mediating aging-related changes in GnRH and gonadotropin secretion. GABA is thought to be important in the regulation of gonadotropin secretion in young adult male rats. In studies *in vitro*, for example, the GABA-A and GABA-B receptor agonists muscimol and baclofen, respectively, decreased GnRH and glutamate release from hypothalamic perfusates, whereas the GABA-A and GABA-B receptor antagonists bicuculline and phaclofen, respectively, enhanced GnRH and glutamate release (Feleder *et al.*, 1996). With aging, concentrations of GABA in the hypothalamus are significantly decreased in male Brown Norway (Bonavera *et al.*, 1998) and F344 (Banay-Schwartz *et al.*, 1989) rats, and a reduction in [^{3}H]muscimol binding to GABA-A receptors was observed in all brain areas examined in male F344 rats (Araki *et al.*, 1996). However, the effects of these age-related changes in brain GABA on GnRH and LH secretion are unknown.

C. β-Endorphin

β-Endorphin is an endogenous opioid peptide that is thought to exert a tonic inhibitory influence upon GnRH secretion and to be an important regulator of reproductive function (Cicero *et al.*, 1979; Delitala *et al.*, 1983). β-Endorphin is derived from a larger precursor peptide, proopiomelanocortin (POMC), and is produced by neurons located in the arcuate nucleus, many of which synapse onto GnRH neurons in the MPOA.

Administration of the opiate antagonist naloxone in male Sprague–Dawley rats was found to induce a marked increase in serum LH levels in young animals, which was attenuated with aging (Steger *et al.*, 1980b). However, more recently, *in vitro* studies in males of the same rat strain found that less naloxone was needed to induce GnRH release from hypothalami of older compared to young adult rats (Nazian *et al.*, 1998). The reasons for this apparent discrepancy are unclear. Hypothalamic β-endorphin content decreases with age in various rat strains such as Wistar (Dax *et al.*, 1988), Sprague–Dawley (Gambert *et al.*, 1980; Barden *et al.*, 1981; Forman *et al.*, 1981), and Long–Evans (Dorsa *et al.*, 1984) rats, which could represent either a decrease in β-endorphin production, or an increase in β-endorphin release or metabolism. Alterations in posttranslational processing of hypothalamic POMC occur in aging male F344 rats, with increased amounts of acetylated β-endorphin peptides and shorter forms of β-endorphin (βE_{1-27} and βE_{1-26}) (Wilkinson and Dorsa, 1986; Dax *et al.*, 1988). β-endorphin is thought to be an endogenous ligand for μ-opioid receptors, which are thought to be involved in the regulation of LH secretion (Panerai *et al.*, 1985), and the number of hypothalamic μ-opioid receptors was found to decrease with aging in male Sprague–Dawley rats (Piva *et al.*, 1987). However, the effects of decreased hypothalamic β-endorphin content, altered posttranslational processing of β-endorphin and reduced μ-receptor binding with aging on opiate tone and GnRH and gonadotropin secretion are unclear.

We tested the hypothesis that the age-related decrease in GnRH secretion in male rats is due to increased β-endorphin synthesis, by comparing prepro-POMC (ppPOMC) mRNA levels in the arcuate nucleus of intact young (3-month-old), middle-aged (11-month-old), and old (23-month-old) male F344 rats. ppPOMC mRNA levels were quantified by *in situ* hybridization histochemistry. We found a significant decrease in both cellular ppPOMC mRNA content and in the number of labeled POMC neurons in old compared to younger rats.

These findings in male F344 rats suggest that β-endorphin synthetic capacity is decreased with aging. However, as for the effects of aging on GnRH, these observations in aging male F344 rats are potentially confounded by elevated progesterone levels. To our knowledge, no studies of aging effects on POMC gene expression in the arcuate nucleus or other aspects of β-endorphin regulation of reproductive axis aging have been performed in aging male Brown Norway rats, but our results in the F344 model suggest that the decline in GnRH secretion in old male rats is not a result of increased β-endorphin synthesis.

D. Catecholamines and Serotonin

Dopamine is a catecholamine neurotransmitter synthesized from tyrosine. The rate-limiting step in dopamine biosynthesis is tyrosine conversion to L-dopa, which is catalyzed by tyrosine hydroxylase. Tuberoinfundibular dopaminergic neurons in the periventricular and arcuate nuclei and dopaminergic neurons of the rostral periventricular nuclei that project to the MPOA and other hypothalamic nuclei are thought to regulate reproductive function (Kalra and Kalra, 1983). However, the role of dopamine in the regulation of gonadotropin secretion is controversial, because dopamine has been found to both stimulate and inhibit GnRH and gonadotropin secretion (Schneider and McCann, 1969; Kamberi *et al*., 1970; Fuxe *et al*., 1976; Negro-Vilar *et al*., 1979; Gallo, 1980).

Dopamine released by tuberoinfundibular dopaminergic neurons tonically inhibits pituitary prolactin secretion. In turn, prolactin stimulates dopamine secretion from tuberoinfundibular dopaminergic neurons, forming a feedback loop (Moore, 1987; Toney *et al*., 1991). Increased prolactin levels occur in aging male rats of several strains, including F344 (Bethea and Walker, 1979; Turek and Desjardins, 1979), Wistar (Simpkins *et al*., 1977), and Long–Evans (Riegle and Meites, 1976), but not Brown Norway (Gruenewald *et al*., 1994a) or Sprague–Dawley (Amoroso *et al*., 1987) rats. Some investigators have found that chronic hyperprolactinemia occurring in some aging animal models is associated with suppressed tuberoinfundibular dopaminergic activity (Sarkar *et al*., 1984; Simpkins and Gabriel, 1984), suggesting that the hyperprolactinemia is caused by age-related reduction in the activity of tuberoinfundibular dopaminergic neurons in these rat strains. However, elevated PRL levels inhibit gonadotropin secretion (McNeilly, 1987; Fox *et al*., 1987) and ppGnRH mRNA levels (Selmanoff *et al*., 1991), therefore, excessive prolactin is a potentially confounding variable in reproductive aging studies in these rat strains. Furthermore, clinically significant hyperprolactinemia does not occur with normal aging in humans; therefore, studies performed in rats that do not develop hyperprolactinemia with aging may provide information more analogous to the situation in aging humans.

Most studies have reported a decrease in dopamine content in the hypothalamus and median eminence of aging male rats, including Long–Evans (Demarest *et al*., 1980), Sprague–Dawley (Carfagna *et al*., 1985), F344 (Steger *et al*., 1985) and Wistar (Simpkins *et al*., 1977; Bhaskaran and Radha, 1983) rats and C57BL/6J mice (Finch, 1973), although levels of hypothalamic dopamine were found to be similar in young and old Brown Norway rats (Gilad *et al*., 1993). Measurements of hypothalamic dopamine turnover rates, which provide more information about dopaminergic neuron activity than content measurements, were found to decrease with aging in Long–Evans (Demarest *et al*., 1980) and Wistar (Simpkins *et al*., 1977) rats, but were unchanged with aging in Sprague–Dawley rats (Carfagna *et al*., 1985). Diminished dopamine release *in vitro* with aging has also been reported (Goldman *et al*., 1987; Gregerson and Selmanoff, 1990), although no age-related changes were found in Sprague–Dawley rats (Amoroso *et al*., 1987). *In vivo*, secretion of dopamine into hypophysial portal blood has been found to decrease with aging (Gudelsky *et al*., 1981) or to increase with aging (Hotta *et al*., 1991) in Wistar rats that were hyperprolactinemic. Hypothalamic tyrosine hydroxylase activity appears to be unchanged with aging (Ponzio *et al*., 1982; Fernandez-Ruiz *et al*., 1992). Moreover, tyrosine hydroxylase gene expression in the hypothalamus was unchanged with aging in male Long–Evans rats (Kedzierski and Porter, 1990). However, activity of hypothalamic monoamine oxidase, an enzyme involved in catecholamine removal, increases with aging (Bhaskaran and Radha, 1983).

Few studies have assessed the effects of these age-related changes in dopamine secretion on reproductive axis function. L-Dopa-induced hypothalamic LH release in young adult female rats is attenuated with aging, but in male rats L-dopa injections failed to increase serum LH in either young or old rats (Riegle and Meites, 1976). Administration of pergolide, a D2 dopamine receptor agonist, throughout adult life in male F344 rats prevented the age-related decline in circulating FSH levels typically seen in this rat strain (Felten *et al*., 1992). Intriguingly, long-term (12 months) testosterone replacement in 24-month-old male Wistar rats increased dopamine release in the MPOA, while at the same time restoring sexual behavior nearly to levels observed in 3-month-old rats (Sato *et al*., 1998). These findings suggest that dopamine may be involved in the age-related decline in male reproductive function, and that this impairment is reversible with chronic testosterone replacement therapy. To our knowledge, the effect of age-related changes in hypothalamic dopamine function on GnRH secretion have not been directly determined, nor are data available regarding the effects of aging on hypothalamic dopamine turnover and release, or tyrosine hydroxylase activity in male Brown Norway rats. Finally, it is important to note that many of the studies of age-related effects on dopamine activity mentioned above were performed in rats that either developed hyperprolactinemia with aging or in whom prolactin levels were not measured, raising questions regarding potential confounding effects of chronic hyperprolactinemia on hypothalamic dopamine activity and/or GnRH secretion.

Levels of hypothalamic norepinephrine have been found either to decrease (Miller *et al*., 1976; Simpkins *et al*., 1977; Bhaskaran and Radha, 1983; Steger *et al*., 1985) or to remain unchanged (Lorens *et al*., 1990; Rodriguez-Gomez *et al*., 1995) with aging in male rats. Most studies have found a decrease in hypothalamic norepinephrine turnover with aging in male rats (Simpkins *et al*., 1977; Ponzio *et al*., 1978) and mice (Finch, 1973), although some have reported no change in norepinephrine turnover with aging (Carfagna *et al*., 1985). Noradrenergic neurons are thought to stimulate GnRH release (Kalra and Kalra, 1983), suggesting that a decline in noradrenergic stimulation with aging could contribute to the age-related decline in GnRH secretion.

The effects of serotonin or 5-hydroxytryptamine on gonadotropin secretion are controversial, with reports of stimulatory, inhibitory, or no effects (Vitale and Chiocchio, 1993). Many studies have reported that serotonin levels in the hypothalamus are unchanged in aging male rats (Simpkins *et al*., 1977; Ponzio *et al*., 1982; Bhaskaran and Radha, 1983), although others have found increased (Steger *et al*., 1985; Rodriguez-Gomez *et al*., 1995) or decreased (Gozlan *et al*., 1990) levels with aging. These inconsistencies may be due in part to a marked circadian variation in hypothalamic serotonin neuronal activity, especially in the suprachiasmatic nucleus (Simpkins and Mill-

ard, 1987). However, hypothalamic serotonin turnover has been more consistently reported to increase with aging (Simpkins *et al*., 1977; Gozlan *et al*., 1990; Rodriguez-Gomez *et al*., 1995).

The reader is referred elsewhere for more in-depth reviews of aging and hypothalamic neurotransmitters (Simpkins and Millard, 1987; Meites, 1991).

E. Other Neuromodulators

Corticotropin-releasing hormone (CRH) is thought to inhibit the secretion of gonadotropins through both an endogenous opioid-dependent inhibition of GnRH secretion and a direct, opioid-independent effect on GnRH neurons (Almeida *et al*., 1988; MacLusky *et al*., 1988). With aging, hypothalamic CRH content has been found to decrease in male Wistar (Kowalski *et al*., 1992) and F344 × Brown Norway F1 hybrid (Cizza *et al*., 1994) rats, and was decreased in the median eminence of F344 × Brown Norway F1 hybrid rats (Hauger *et al*., 1994). CRH binding was decreased in the hypothalamus of old male F344 rats (Heroux *et al*., 1991). CRH mRNA levels in the paraventricular nucleus of the hypothalamus was found to decrease (Cizza *et al*., 1994) or to be unchanged (Hauger *et al*., 1994) in aging male F344 × Brown Norway F1 hybrid rats, and was decreased with aging in male F344 rats (Kasckow *et al*., 1999). Based on these data that generally indicate a decrease in hypothalamic CRH activity with aging, together with the findings of an inhibitory effect of CRH on GnRH and gonadotropin secretion, it seems unlikely that CRH plays a major role in the age-related impairment in reproductive axis regulation in the male rat.

Galanin is a neuropeptide that stimulates LH release, apparently by potentiating the effect of GnRH (Lopez *et al*., 1991). Galanin and GnRH are colocalized in a subset of preoptic neurons in the rat brain, and in adult female rats galanin gene expression in GnRH neurons is induced by ovarian steroids in association with the LH surge (Rossmanith *et al*., 1996), suggesting an important role for galanin in the regulation of reproductive functions in females. However, less attention has been paid to its role in the regulation of male reproductive function. The number of galanin-immunoreactive cells in the medial septal nucleus and the diagonal band of Broca is decreased in 16- to 30-month-old compared to 3- to 6-month old male rats (de Bilbao *et al*., 1991). However, no studies have determined whether the number of neurons colocalizing galanin and GnRH declines, nor has the effect of declining numbers of galanin-immunoreactive neurons on reproductive function been assessed in aging male rats.

The effects of Substance P on gonadotropin secretion are controversial. Dees *et al*. (1985) reported that injections of either a substance P antagonist or an antiserum into the lateral ventricle suppressed circulating LH levels in orchidectomized male rats. In contrast, Picanco-Diniz *et al*. (1990) found that injection of substance P into the MPOA of either intact or orchidectomized male rats significantly decreased circulating gonadotropin levels. Hypothalamic substance P content has been reported to decline with aging in male Sprague–Dawley rats (Z. P. Wang *et al*., 1993), but the role of substance P in mediating age-related changes in male reproductive axis function has not been determined.

IV. Experimental Approaches to "Reversal" of Age-Related Hypothalamic Reproductive Dysfunction

A. Hormone Supplementation

There has recently been considerable interest in the potential role of declining levels of hormones such as testosterone, growth hormone, dehydroepiandrosterone (DHEA), and melatonin in causing or contributing to the adverse effects of aging. In contrast to aging-related estrogen deficiency in females, the age-related decreases in the levels of these other hormones occur gradually, and the rate of decline is often highly variable between individuals. Indeed, it is unclear whether declining hormone levels with aging constitute a hormone "deficiency" state or whether the decrease in hormones may protect against other adverse effects of aging.

It has been hypothesized that the age-related decline in hormones such as the ones described above may contribute to the age-related decrease in GnRH neuronal activity with aging, and that replacement of these hormones may at least in part reverse the age-related decline in reproductive function (Li *et al*., 1997a,b). For example, although melatonin has been observed to have antigonadotrophic effects in some species, particularly those with marked seasonal variations in gonadal function, in young adult male rats, melatonin was found to stimulate GnRH gene expression as determined by ISHH (Li and Pelletier, 1995). Based on observations that administration of melatonin to female rodents increased the longevity of these animals, Li and Pelletier determined the effect of melatonin administration on age-related changes in ppGnRH mRNA levels in male Sprague–Dawley rats (Li *et al*., 1997b). Either vehicle or 24 mg/kg melatonin was administered intraperitoneally twice a day for 2.5 days to 50- to 54-day-old and 18-month-old rats, followed by sacrifice and measurement of cellular ppGnRH mRNA content (number of silver grains per labeled cell) by ISHH in the basal forebrain. Cellular ppGnRH mRNA content declined by 13% in old compared to young vehicle-treated rats, similar to results we reported in both F344 and Brown Norway rats (Gruenewald and Matsumoto, 1991; Gruenewald *et al*., 1999). In young rats, melatonin administration increased cellular ppGnRH mRNA content by 11% compared to vehicle-treated rats (Fig. 57.11). In old rats, melatonin administration increased cellular ppGnRH mRNA content by 17% compared to old vehicle-treated rats, restoring mRNA levels to the level of young vehicle-treated rats but not reaching levels observed in young melatonin-treated rats.

A similar study methodology was used to determine the effect of DHEA administration (12 mg/kg body weight sc twice a day) for 2.5 days in 50- to 54-day-old and 18-month-old male Sprague–Dawley rats. As in the melatonin study, a 10% decrease in cellular ppGnRH mRNA content was observed in old compared to young control rats. Whereas DHEA administration resulted in an 18% decrease in cellular ppGnRH mRNA content in young rats, in older animals DHEA restored cellular ppGnRH mRNA content to levels slightly higher than those in young vehicle-treated rats.

Based on the foregoing, it was suggested that declining melatonin levels may be involved in the reduction in GnRH neuronal activity with aging, and that melatonin or DHEA

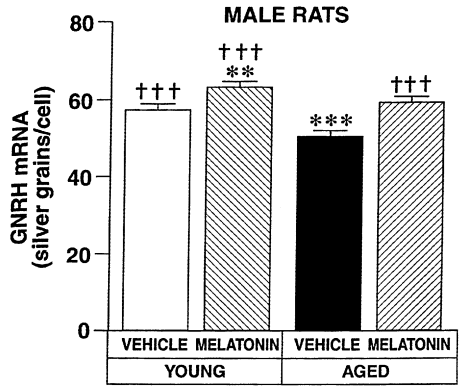

FIG. 57.11. Effect of five injections of melatonin (24 mg/kg body weight) administered intraperitoneally every 12 hr on ppGnRH mRNA levels as measured by the number of grains overlying labeled neurons by *in situ* hybridization histochemistry in young (50- to 54-day) and aged (18-month) male Sprague–Dawley rats (***$P<0.001$, **$P<0.01$ vs vehicle-treated young rats; $^{+++}P<0.001$ vs vehicle-treated old rats). Control animals received vehicle (0.9% NaCl and 5% ethanol) instead of melatonin. Rats were sacrificed 6 hr after the last injection. Used with permission (Li *et al*., 1997b).

administration might be useful in preventing or reversing age-related deficits in reproductive function. However, the dose of melatonin administered to these animals was pharmacological rather than physiological; by comparison, in humans, administration of 0.1 to 0.3 mg in the daytime results in peak serum concentrations that are within the normal nighttime range (Brzezinski, 1997). In these rat studies, no data were provided regarding circulating endogenous melatonin levels, nor were circulating LH and testosterone levels measured. It is possible that melatonin treatment decreased testosterone levels, which could in turn increase GnRH synthesis and secretion as a result of reduced testosterone negative feedback. Therefore, the physiological role of melatonin in the aging process cannot be determined from these studies alone. This will require further investigation.

As for DHEA, in contrast to humans, rodents have negligible production of adrenal androgens such as DHEA. Indeed, DHEA concentrations are so low in rodents that it is unknown whether they decline with aging as in humans (Baulieu, 1996). Therefore, the role of this compound is purely pharmacological in rodents, and these results have limited applicability to the human condition. In addition, as in the melatonin study, the dose of DHEA that was used is large; human studies involving 100 mg per day or more have been associated with increased circulating concentrations of androgenic and estrogenic steroids (Yen *et al*., 1995). Furthermore, because DHEA is metabolized to active androgenic and estrogenic steroids such as testosterone and estradiol, including within the brain, it is unclear whether the effects of DHEA are due to a direct effect of DHEA or to the actions of one or more of its metabolites. Finally, in both the melatonin and the DHEA studies, the experimental protocol involved administering these substances for only 2.5 days; therefore the relevance of these findings to longer term changes of hypothalamic aging is unclear.

Finally, as noted above, long-term testosterone replacement from 12 to 24 months of age in orchidectomized male rats was found to restore the mount rate of these 24-month-old rats nearly to levels observed in 3-month-old rats (Sato *et al*., 1998). Furthermore, dopamine release in the MPOA after infusion of *N*-methyl-D-aspartate was increased in testosterone-replaced old rats. These findings suggest that the aging-related impairment in sexual functioning is reversible with chronic testosterone replacement and that dopamine may play a role in mediating the effects of aging on reproductive function in the male.

B. Grafting of Fetal Neurons

Another potential experimental strategy to reverse the decline in hypothalamic regulation of reproductive axis function with aging is to surgically implant juvenile neuronal tissues in the hypothalamic area of experimental animals. Huang *et al*. (1987) transplanted the anterior hypothalamus from male Long–Evans rat fetuses at 17–19 days of gestation into the third ventricles of 18- to 20-month-old impotent male rats. Compared to control rats of the same age that were either untreated or that received grafts of cerebral cortex, 7 of 10 hypothalamic grafted rats experienced restoration of sexual function and were able to father pups when placed together with a proestrous female rat, whereas 0 of 7 untreated and 1 of 4 cortex-grafted rats experienced restoration of sexual function. Furthermore, circulating LH and testosterone levels were significantly higher 2 to 3 months after hypothalamic grafting in animals that exhibited increased sexual functioning (Table 57.2), whereas hormone levels declined in rats whose sexual activity was not increased with hypothalamus grafting and in untreated and cortex-grafted rats. Histological evaluation demonstrated that well-vascularized hypothalamic (and cortex) grafts were growing in the third ventricle of the host brains, apparently in all grafted rats. These findings indicate

TABLE 57.2 Circulating Luteinizing Hormone (LH) and Testosterone (T) Levels by Radioimmunoassay in Impotent 18- to 20-Month-Old Male Long–Evans Rats before and 2 to 3 Months after Receiving Third Ventricular Transplantation of Anterior Hypothalamus from Male Long–Evans Rat Fetuses at 17–19 Days of Gestation

	Transplanted rats		Untreated controls	
	Baseline	After grafting	Baseline	Baseline +2–3 months
LH (ng/ml)	0.68 ± 0.13	0.98 ± 0.09*	0.76 ± 0.16	0.45 ± 0.07*
T (ng/ml)	0.29 ± 0.04	1.04 ± 0.16*	0.67 ± 0.26	0.24 ± 0.07*

Note. Control rats of the same age that did not receive surgery are included for comparison. The experimental data shown are for the subset of rats that exhibited increased sexual functioning after transplantation. In contrast, rats that received third ventricular transplantation of fetal hypothalamus grafts but that did not exhibit increased sexual functioning after the grafts exhibited reduced LH and testosterone levels after 2 to 3 months (data not shown). Furthermore, another group of control rats that received third ventricular transplantation of cortex grafts from fetal rats exhibited declining LH and testosterone levels after 2 to 3 months, similar to that seen in untreated controls (data not shown). Values are expressed as the mean ± SEM. Data from Huang *et al*., 1987, used with permission.

*$P<0.05$ vs baseline for the same experimental group.

that transplantation of fetal hypothalamic grafts can survive and develop in the brains of aging rats and that these grafts can restore reproductive hormone levels and sexual function at least partially toward normal young adult levels. However, no young control rats were included for comparison, to determine whether full restoration of hormonal status or sexual functioning was achieved.

A similar study was subsequently performed by Hung *et al*. (1997), who grafted fetal preoptic area neurons into the preoptic area of 19- to 24-month-old male Long–Evans rats. Similar to the previous study, most of the grafted rats exhibited improved sexual motivation and copulatory activity, whereas sexual performance did not improve in rats grafted with cortex neurons or who received preoptic grafts into the ventromedial hypothalamus. Grafted rats that exhibited improved sexual performance also showed normalization of serum testosterone and LH levels that were not significantly different than levels in intact 6-month-old rats used as controls. These findings suggest that age-related alterations in reproductive neuroendocrine functions and sexual activity are due at least in part to loss of preoptic area function with aging.

C. Calorie Restriction

Chronic dietary calorie restriction has been shown to delay or prevent a wide variety of aging-associated pathologies in a number of rodent strains. In fact, it has been suggested that calorie-restricted rodents be used as a standard model for aging research (Masoro, 1993). Calorie restriction throughout adult life has been found to prevent aging-related reductions in circulating LH, FSH, and testosterone levels in male Sprague–Dawley and F344 rats (Merry and Holehan, 1981; Stokkan *et al*., 1991). Furthermore, 22-month-old male F344 rats that were calorie-restricted throughout adult life exhibited decreased hypothalamic dopamine, norepinephrine, and serotonin content compared to *ad libitum*-fed age-matched control rats (Kolta *et al*., 1989). However, the effects of chronic calorie restriction on aging-related changes in GnRH synthesis and secretion are unknown, and studies of the effects of calorie restriction on reproductive axis function have not been performed in aging male Brown Norway or F344 $\times$ Brown Norway F1 hybrid rats.

V. Conclusion

Based on the studies described above, it is apparent that age-related alterations in reproductive axis function in male rats are due in part to alterations in hypothalamic function. These changes are manifested at the level of GnRH gene expression in preoptic area GnRH-containing neurons. An important unresolved issue in reproductive neuroendocrinology is whether the decrease in GnRH gene expression with aging is due to an intrinsic change in GnRH neuronal function, to altered neuromodulatory input onto GnRH-containing neurons, or both. Currently available studies indicate that the activities of some of the neurons that synapse onto GnRH-containing neurons undergo important changes with aging that could account for decreased GnRH secretion, such as the NPY-containing neurons. Thus, there is probably an important role for altered neuromodulatory input affecting GnRH-containing neurons in contributing to aging-related reproductive decline. However, the data are not yet complete enough to construct a meaningful ''circuit diagram'' describing the effects of aging on a majority of the neuromodulatory influences affecting GnRH secretion that have been described in young adult male rodents. Clearly, further studies are needed to determine the relative importance of changes in the milieu of circulating hormones such as testosterone and alterations in neurotransmitter and neuropeptide input in mediating the effects of aging on GnRH secretion. These studies should use models of aging such as the male Brown Norway rat, in which the physiology of aging related changes in reproductive function is similar to the human, and in which the potential for confounding age-associated pathologies is minimized. Finally, remarkably few studies of the hypothalamic regulation of reproductive aging in the male have been performed in rodent models other than the aging rat. It would be beneficial to validate the findings from rat studies in other rodent models, especially the mouse, to determine whether these results are more broadly generalizable. It is likely that this research will ultimately provide important clues that will add to our understanding of the effects of aging on neuroendocrine regulation in humans.

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References

Akabayashi, A., Levin, N., Paez, X., Alexander, J. T., and Leibowitz, S. F. (1994). Hypothalamic neuropeptide Y and its gene expression: Relation to light/dark cycle and circulating corticosterone. *Mol. Cell. Neurosci*. **5**, 210–218.

Allen, L. G., Kalra, P. S., Crowley, W. R., and Kalra, S. P. (1985). Comparison of the effects of neuropeptide Y and adrenergic transmitters on LH release and food intake in male rats. *Life Sci*. **37**, 617–623.

Almeida, O. F. X., Nikolarakis, K. E., and Herz, A. (1988). Evidence for the involvement of endogenous opioids in the inhibition of luteinizing hormone by corticotropin-releasing factor. *Endocrinology (Baltimore)* **122**, 1034–1041.

Amador, A., Steger, R. W., Bartke, A., Johns, A., Siler-Khodr, T. M., Parker, C. R., Jr., and Shepherd, A. M. (1985). Testicular LH receptors during aging in Fisher 344 rats. *J. Androl*. **6**, 61–64.

Amoroso, S., Di Renzo, G. F., Maurano, F., Maida, P., Taglialatela, M., and Annunziato, L. (1987). Lack of evidence for an impairment of tuberoinfundibular dopaminergic neurons in aged male rats of the Sprague-Dawley strain. *Exp. Aging Res*. **13**, 85–87.

Araki, T., Kato, H., Fujiwara, T., and Itoyama, Y. (1996). Regional age-related alterations in cholinergic and GABAergic receptors in the rat brain. *Mech. Ageing Dev.* **88**, 49–60.

Banay-Schwartz, M., Lajtha, A., and Palkovits, M. (1989). Changes with aging in the levels of amino acids in rat CNS structural elements. I. glutamate and related amino acids. *Neurochem. Res*. **14**, 555–562.

Barden, N., Dupont, A., Labrie, F., Merand, Y., Rouleau, D., Vaudry, H., and Boissier, J. R. (1981). Age-dependent changes in the beta-endorphin content of discrete rat brain nuclei. *Brain Res*. **208**, 209–212.

Baulieu, E.-E. (1996). Editorial: Dehydroepiandrosterone (DHEA): A fountain of youth? *J. Clin. Endocrinol. Metab.* **81**, 3147–3151.

Bedrak, E., Chap, Z., and Brown, R. (1983). Age-related changes in the hypothalamic-pituitary-testicular function of the rat. *Exp. Gerontol*. **18**, 95–104.

Bethea, C. L., and Walker, R. F. (1979). Age-related changes in reproductive hormones and in Leydig cell responsivity in the male Fischer 344 rat. *J. Gerontol*. **34**, 21–27.

Bhaskaran, D., and Radha, E. (1983). Monoamine levels and monoamine oxidase activity in different regions of rat brain as a function of age. *Mech. Ageing Dev.* **23**, 151–160.

Bonavera, J. J., Sahu, A., Kalra, P. S., and Kalra, S. P. (1993). Evidence that nitric oxide (NO) may mediate the ovarian steroid-induced luteinizing hormone surge: Involvement of excitatory amino acids. *Endocrinology (Baltimore)* **133**, 2481–2487.

Bonavera, J. J., Swerdloff, R. S., Leung, A., Lue, Y. H., Baravarian, S., Superlano, L., Sinha-Hikim, A. P., and Wang, C. (1997). In the male Brown-Norway (BN) rat, reproductive aging is associated with decreased LH pulse amplitude and area. *J. Androl*. **18**, 359–365.

Bonavera, J. J., Swerdloff, R. S., Sinha Hikim, A. P., Lue, Y. H., and Wang, C. (1998). Aging results in attenuated gonadotropin releasing hormone-luteinizing hormone axis responsiveness to glutamate receptor agonist *N*-methyl-D-aspartate. *J. Neuroendocrinol*. **10**, 93–99.

Brann, D. W. (1995). Glutamate: A major excitatory transmitter in neuroendocrine regulation. *Neuroendocrinology* **61**, 213–225.

Brann, D. W., and Mahesh, V. B. (1994). Excitatory amino acids: Function and significance in reproduction and neuroendocrine regulation. *Front. Neuroendocrinol*. **15**, 3–49.

Bronson, F. H., and Desjardins, C. (1977). Reproductive failure in aged CBF1 male mice: Interrelationships between pituitary gonadotropic hormones, testicular function, and mating success. *Endocrinology (Baltimore)* **101**, 939–945.

Bronson, R. T. (1989). Rate of occurrence of lesions in 20 inbred and hybrid genotypes of rats and mice sacrificed at 6 month intervals during the first years of life. *In* "Genetic Effects on Aging II" (D. E. Harrison, ed.), pp. 279–358. Telford Press, Caldwell, NJ.

Brzezinski, A. (1997). Melatonin in humans. *N. Engl. J. Med*. **336**, 186–195.

Burek, J. D., and Hollander, C. F. (1977). Incidence patterns of spontaneous tumors in BN/Bi rats. *J. Natl. Cancer Inst*. **58**, 99–105.

Cameron, T. P., Hickman, R. L., Kornreich, M. R., and Tarone, R. E. (1985). History, survival, and growth patterns of B6C3F1 mice and F344 rats in the National Cancer Institute Carcinogenesis Testing Program. *Fundam. Appl. Toxicol*. **5**, 526–538.

Carfagna, N., Trunzo, F., and Moretti, A. (1985). Brain catecholamine content and turnover in aging rats. *Exp. Gerontol*. **20**, 265–269.

Chambers, K. C., Thornton, J. E., and Roselli, C. E. (1991). Age-related deficits in brain androgen binding and metabolism, testosterone, and sexual behavior of male rats. *Neurobiol. Aging* **12**, 123–130.

Chen, H., Hardy, M. P., Huhtaniemi, I., and Zirkin, B. R. (1994). Age-related decreased Leydig cell testosterone production in the Brown-Norway rat. *J. Androl*. **15**, 551–557.

Cheung, C. C., Clifton, D. K., and Steiner, R. B. (1996). Galanin: An unassuming neuropeptide moves to center stage in reproduction. *Trends Endocrinol. Metab.* **7**, 301–306.

Cicero, T. J., Schainker, B. A., and Meyer, E. R. (1979). Endogenous opioids participate in the regulation of the hypothalamic-pituitary-luteinizing hormone axis and testosterone's negative feedback control of luteinizing hormone. *Endocrinology (Baltimore)* **104**, 1286–1291.

Cizza, G., Calogero, A. E., Brady, L. S., Bagdy, G., Bergamini, E., Blackman, M. R., Chrousos, G. P., and Gold, P. W. (1994). Male Fischer 344/N rats show a progressive central impairment of the hypothalamic-pituitary-adrenal axis with advancing age. *Endocrinology (Baltimore)* **134**, 1611–1620.

Cohen, B. J., Anver, M. R., Ringler, D. H., and Adelman, R. C. (1978). Age-associated pathological changes in rats. *Fed. Proc*. **37**, 2848–2850.

Coquelin, A., and Desjardins, C. (1982). Luteinizing hormone and testosterone secretion in young and old male mice. *Am. J. Physiol*. **243**, E257–E263.

Dax, E. M., Reichman, C., Fullerton, M., Wallace, C., Smith, A. I., and Funder, J. (1988). Beta endorphin and dynorphin levels in rat pituitary and hypothalamus: Age studies. *Neuroendocrinology* **47**, 241–248.

de Bilbao, F., Jazat, F., Lamour, Y., and Senut, M. C. (1991). Age-related changes in galanin-immunoreactive cells of the rat medial septal area. *J. Comp. Neurol*. **22**, 613–624.

Dees, W. L., Skelley, C. W., and Kozlowski, G. P. (1985). Central effects of an antagonist and an antiserum to substance P on serum gonadotropin and prolactin secretion. *Life Sci*. **37**, 1627–1631.

Delitala, G., Giusti, M., Mazzocchi, G., Granziera, L., Tarditi, W., and Giordano, G. (1983). Participation of endogenous opiates in regulation of the hypothalamic pituitary-testicular axis in normal men. *J. Clin. Endocrinol. Metab.* **57**, 1277–1281.

Demarest, K. T., Riegle, G. D., and Moore, K. E. (1980). Characteristics of dopaminergic neurons in the aged male rat. *Neuroendocrinology* **31**, 222–227.

Deslypere, J. P., Kaufman, J. M., Vermeulen, T., Vogelaers, D., Vandalem, J. L., and Vermeulen, A. (1987). Influence of age on pulsatile luteinizing hormone release and responsiveness of the gonadotrophs to sex hormone feedback in men. *J. Clin. Endocrinol. Metab.* **64**, 68–73.

Dorsa, D. M., Smith, E. R., and Davidson, J. M. (1984). Immunoreactive-beta-endorphin and LHRH levels in the brains of aged male rats with impaired sex behavior. *Neurobiol. Aging* **5**, 115–120.

Feleder, C., Jarry, H., Leonhardt, S., Wuttke, W., and Moguilevsky, J. A. (1996). The GABAergic control of gonadotropin-releasing hormone secretion in male rats during sexual maturation involves effects on hypothalamic excitatory and inhibitory amino acid systems. *Neuroendocrinology* **64**, 305–312.

Felten, D. L., Felten, S. Y., Fuller, R. W., Romano, T. D., Smalstig, E. B., Wong, D. T., and Clemens, J. A. (1992). Chronic dietary pergolide preserves nigrostriatal neuronal integrity in aged-Fischer-344 rats. *Neurobiol. Aging* **13**, 339–351.

Fernandez-Ruiz, J., De Miguel, R., Hernandez, M. L., Cebeira, M., and Ramos, J. A. (1992). Comparisons between brain dopaminergic neurons of juvenile and aged rats: Sex-related differences. *Mech. Ageing Dev.* **63**, 45–55.

Finch, C. E. (1973). Catecholamine metabolism in the brains of aging male mice. *Brain Res*. **52**, 271–276.

Forman, L. J., Sonntag, W. E., Van Vugt, D. A., and Meites, J. (1981). Immunoreactive beta-endorphin in the plasma, pituitary and hypothalamus of young and old male rats. *Neurobiol. Aging* **2**, 281–284.

Fox, S. R., Hoefer, M. T., Bartke, A., and Smith, M. S. (1987). Suppression of pulsatile LH secretion, pituitary GnRH receptor content and pituitary responsiveness to GnRH by hyperprolactinemia in the male rat. *Neuroendocrinology* **46**, 350–359.

Fuxe, K., Hökfelt, T. and Lofström, A., *et al*. (1976). On the role of neurotransmitters and hypothalamic hormones and their interactions in hypothalamic and extrahypothalamic control of pituitary function and sexual behavior. *In* "Subcellular Mechanisms in Reproductive Neuroendocrinology" (F. Naftolin, K. J. Ryan, and T. Davies, eds.), pp. 193–246. Elsevier, Amsterdam.

Gallo, R. V. (1980). Neuroendocrine regulation of pulsatile luteinizing hormone release in the rat. *Neuroendocrinology* **30**, 122–131.

Gambert, S. R., Garthwaite, T. L., Pontzer, C. H., and Hagen, T. C. (1980). Age-related changes in central nervous system beta-endorphin and ACTH. *Neuroendocrinology* **31**, 252–255.

Gilad, G. M., Li, R., Wyatt, R. J., and Tizabi, Y. (1993). Effects of genotype on age-related alterations in the concentrations of stress hormones in plasma and hypothalamic monoamines in rats. *J. Reprod. Fertil*. **46**, 119–130.

Goldman, J. M., Cooper, R. L., Rehnberg, G. L., Gabel, S., McElroy, W. K., Hein, J., and Conn, P. M. (1987). Age-related alterations in the stimulated release in vitro of catecholamines and luteinizing hormone-releasing hormone from the male rat hypothalamus. *Neurochem. Res*. **12**, 651–657.

Gozlan, H., Daval, G., Verge, D., Spampinato, U., Fattaccini, C. M., Gallissot, M. C., el Mestikawy, S., and Hamon, M. (1990). Aging associated changes in serotoninergic and dopaminergic pre-and postsynaptic neurochemical markers in the rat brain. *Neurobiol. Aging* **11**, 437–449.

Gray, G. D., Smith, E. R., and Davidson, J. M. (1980). Gonadotropin regulation in middle-aged male rats. *Endocrinology (Baltimore)* **107**, 2021–2026.

Gregerson, K. A., and Selmanoff, M. (1990). Changes in the kinetics of [3H]dopamine release from median eminence and striatal synaptosomes during aging. *Endocrinology (Baltimore)* **126**, 228–234.

Gruenewald, D. A., and Matsumoto, A. M. (1991). Age-related decreases in serum gonadotropin levels and gonadotropin-releasing hormone gene expression in the medial preoptic area of the male rat are dependent upon testicular feedback. *Endocrinology (Baltimore)* **129**, 2442–2450.

Gruenewald, D. A., Hess, D. L., Wilkinson, C. W., and Matsumoto, A. M. (1992). Excessive testicular progesterone secretion in aged male Fischer 344 rats: A potential cause of age-related gonadotropin suppression and a confounding variable in aging studies. *J. Gerontol.* **47**, B164–B170.

Gruenewald, D. A., Naai, M. A., Hess, D. L., and Matsumoto, A. M. (1994a). The Brown Norway rat as a model for male reproductive aging: Evidence for both primary and secondary testicular failure. *J. Gerontol.* **49**, B42–B50.

Gruenewald, D. A., Naai, M. A., Marck, B. T., and Matsumoto, A. M. (1994b). Age-related decrease in neuropeptide Y gene expression in the arcuate nucleus of the male rat brain is independent of testicular feedback. *Endocrinology (Baltimore)* **134**, 2383–2389.

Gruenewald, D. A., Naai, M. A., Marck, B. T., and Matsumoto, A. M. (2000). Age-related decrease in hypothalamic gonadotropin-releasing hormone (GnRH) gene expression, but not pituitary responsiveness to GnRH, in the male Brown Norway rat. *J. Androl*. **21**, 72–84.

Gudelsky, G. A., Nansel, D. D., and Porter, J. C. (1981). Dopaminergic control of prolactin secretion in the aging male rat. *Brain Res*. **204**, 446–450.

Harman, S. M., and Tsitouras, P. D. (1980). Reproductive hormones in aging men. I. Measurement of sex steroids, basal luteinizing hormone, and Leydig cell response to human chorionic gonadotropin. *J. Clin. Endocrinol. Metab.* **51**, 35–40.

Hauger, R. L., Thrivikraman, K. V., and Plotsky, P. M. (1994). Age-related alterations of hypothalamic-pituitary-adrenal axis function in male Fischer 344 rats. *Endocrinology (Baltimore)* **134**, 1528–1536.

Hazzard, D. G., Bronson, R. T., McClearn, G. E., and Strong, R. (1992). Guest editorial: Selection of an appropriate animal model to study aging processes with special emphasis on the use of rat strains. *J. Gerontol.* **47**, B63–B64.

Heroux, J. A., Grigoriadis, D. E., and De Souza, E. B. (1991). Age-related decreases in corticotropin-releasing factor (CRF) receptors in rat brain and anterior pituitary gland. *Brain Res*. **542**, 155–158.

Hollander, C. F. (1976). Current experience using the laboratory rat in aging studies. *Lab. Anim. Sci*. **26**, 320–328.

Horvath, T. L., Bechmann, I., Naftolin, F., Kalra, S. P., and Leranth, C. (1997). Heterogeneity in the neuropeptide Y-containing neurons of the rat arcuate nucleus: GABAergic and non-GABAergic subpopulations. *Brain Res.* **746**, 283–286.

Hotta, H., Ito, H., Matsuda, K., Sato, A., and Tohgi, H. (1991). Age-related changes in rates of basal secretion of immunoreactive vasoactive intestinal polypeptide and dopamine into pituitary stalk blood from the hypothalamus in anesthetized male rats. *Jpn. J. Physiol*. **41**, 317–325.

Huang, H. H., Kissane, J. Q., and Hawrylewicz, E. J. (1987). Restoration of sexual function and fertility by fetal hypothalamic transplant in impotent aged male rats. *Neurobiol. Aging* **8**, 465.

Hung, S.-H., Pi, W.-P., Tsai, Y.-F., and Peng, M.-T. (1997). Restoration of sexual behavior in aged male rats by intracerebral grafts of fetal preoptic area neurons. *J. Formosan Med. Assoc*. **96**, 812–818.

Ingram, D. K., Garofalo, P., Spangler, E. L., Mantione, C. R., Odano, I., and London, E. D. (1992). Reduced density of NMDA receptors and increased sensitivity to dizocilpine-induced learning impairment in aged rats. *Brain Res*. **580**, 273–280.

Jarjour, L. T., Handelsman, D. J., and Swerdloff, R. S. (1986). Effects of aging on the in vitro release of gonadotropin-releasing hormone. *Endocrinology (Baltimore)* **119**, 1113–1117.

Kaler, L. W., and Neaves, W. B. (1981). The androgen status of aging male rats. *Endocrinology (Baltimore)* **108**, 712–719.

Kalra, S. P., and Kalra, P. S. (1983). Neural regulation of luteinizing hormone secretion in the rat. *Endocr. Rev.* **4**, 311–351.

Kalra, S. P., Sahu, S., Kalra, P. S., and Crowley, W. R. (1990). Hypothalamic neuropeptide Y: A circuit in the regulation of gonadotropin secretion and feeding behavior. *Ann. N. Y. Acad. Sci*. **611**, 273–283.

Kalra, S. P., Sahu, A., and Kalra, P. S. (1993). Ageing of the neuropeptidergic signals in rats. *J. Reprod. Fertil*. **46**, 11–19.

Kalra, S. P., Horvath, T., Naftolin, F., Xu, B., Pu, S., and Kalra, P. S. (1997). The interactive language of the hypothalamus for the gonadotropin releasing hormone (GnRH) system. *J. Neuroendocrinol*. **9**, 569–576.

Kamberi, I. A., Nicol, R. S., and Porter, J. C. (1970). Effect of anterior pituitary perfusion and intraventricular injection of catecholamines and indoleamines on LH release. *Endocrinology (Baltimore)* **87**, 1–5.

Karpas, A. E., Bremner, W. J., Clifton, D. K., Steiner, R. A., and Dorsa, D. M. (1983). Diminished luteinizing hormone pulse frequency and amplitude with aging in the male rat. *Endocrinology (Baltimore)* **112**, 788.

Kasckow, J. W., Regmi, A., Mulchahey, J. J., Plotsky, P. M., and Hauger, R. L. (1999). Changes in brain corticotropin-releasing factor messenger RNA expression in aged Fischer 344 rats. *Brain Res*. **822**, 228–230.

Kedzierski, W., and Porter, J. C. (1990). Quantitative study of tyrosine hydroxylase mRNA in catecholaminergic neurons and adrenals during development and aging. *Mol. Brain Res*. **7**, 45–51.

Kerkerian, L., Guy, J., Lefevre, G., and Pelletier, G. (1985). Effects of neuropeptide Y (NPY) on the release of anterior pituitary hormones in the rat. *Peptides* **6**, 1201–1204.

Kolta, M. G., Holson, R., Duffy, P., and Hart, R. W. (1989). Effect of long-term caloric restriction on brain monoamines in aging male and female Fischer 344 rats. *Mech. Ageing Dev.* **48**, 191–198.

Koves, K., and Molnar, J. (1986). Effects of various hypothalamic deafferentations injuring different parts of GnRH pathway on ovulation, GnRH content of the median eminence and plasma LH and FSH levels. *Neuroendocrinology* **44**, 172–183.

Kowalski, C., Micheau, J., Corder, R., Gaillard, R., and Conte-Devolx, B. (1992). Age-related changes in corticotropin-releasing factor, somatostatin, neuropeptide Y, methionine enkephalin and beta-endorphin in specific rat brain areas. *Brain Res.* **582**, 38–46.

Li, H., Matheny, M., Tumer, N., and Scarpace, P. J. (1998). Aging and fasting regulation of leptin and hypothalamic neuropeptide Y gene expression. *Am. J. Physiol.* **275**, E405–E411.

Li, S., and Pelletier, G. (1995). Effects of pinealectomy and melatonin on gonadotropin-releasing hormone (GnRH) gene expression in the male rat brain. *Endocrine* **3**, 533–536.

Li, S., Hong, M., Fournier, A., St.-Pierre, S., and Pelletier, G. (1994). Role of neuropeptide Y in the regulation of gonadotropin-releasing hormone gene expression in the rat preoptic area. *Mol. Brain Res*. **26**, 69–73.

Li, S., Givalois, L., and Pelletier, G. (1997a). Dehydroepiandrosterone administration reverses the inhibitory influence of aging on gonadotrophin-releasing hormone gene expression in the male and female rat brain. *Endocrine* **6**, 265–270.

Li, S., Givalois, L., and Pelletier, G. (1997b). Effects of aging and melatonin administration on gonadotropin-releasing hormone (GnRH) gene expression in the male and female rat. *Peptides* **18**, 1023–1028.

Lopez, F. J., Merchenthaler, I., Ching, M., Wisniewski, M. G., and Negro-Vilar, A. (1991). Galanin: A hypothalamic-hypophysiotrophic hormone

modulating reproductive functions. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 4508–4512.

Lorens, S. A., Hata, N., Handa, R. J., Van de Kar, L. D., Guschwan, M., Goral, J., Lee, J. M., Hamilton, M. E., Bethea, C. L., Clancy, J. J., *et al.* (1990). Neurochemical, endocrine and immunological responses to stress in young and old Fischer 344 male rats. *Neurobiol. Aging* **11**, 139–150.

MacLusky, N. J., Naftolin, F., and Leranth, C. (1988). Immunocytochemical evidence for direct synaptic connections between corticotrophin-releasing factor (CRF) and gonadotrophin-releasing hormone (GnRH)-containing neurons in the preoptic area of the rat. *Brain Res.* **439**, 391–395.

Magnusson, K. R., and Cotman, C. W. (1993). Age-related changes in excitatory amino acid receptors in two mouse strains. *Neurobiol. Aging* **14**, 197–206.

Masoro, E. J. (1991). Use of rodents as models for the study of ''normal aging'': Conceptual and practical issues. *Neurobiol. Aging* **12**, 639–643.

Masoro, E. L. (1993). Nutrition, including diet restriction, in mammals. *Aging Clin. Exp. Res.* **5**, 269–275.

McNeilly, A. S. (1987). Commentary: Prolactin and the control of gonadotrophin secretion. *J. Endocrinol.* **115**, 1–5.

Meites, J. (1991). Role of hypothalamic catecholamines in aging processes. *Acta Endocrinol. (Copenhagen)* **125**, 98–103.

Merchenthaler, I., Setalo, G., Csontos, C., Petrusz, P., Flerko, B., and Negro-Vilar, A. (1989). Combined retrograde tracing and immunocytochemical identification of luteinizing hormone-releasing hormone- and somatostatin-containing neurons projecting to the median eminence of the rat. *Endocrinology (Baltimore)* **125**, 2812–2821.

Merry, B. J., and Holehan, A. M. (1981). Serum profiles of LH, FSH, testosterone and 5 α-DHT from 21 to 1000 days of age in ad libitum fed and dietary restricted rats. *Exp. Gerontol.* **16**, 431–444.

Miller, A. E., Shaar, C. J., and Riegle, G. D. (1976). Aging effects on hypothalamic dopamine and norepinephrine content in the male rat. *Exp. Aging Res.* **2**, 475–480.

Moore, K. E. (1987). Interactions between prolactin and dopaminergic neurons. *Biol. Reprod.* **36**, 47–58.

Nazian, S. J., Landon, C. S., and Dietz, J. R. (1998). Alterations in the opioid control of LHRH release from hypothalami isolated from aged male rats. *J. Gerontol.* **53**, B191–B195.

Negro-Vilar, A., Ojeda, S. R., and McCann, S. M. (1979). Catecholaminergic modulation of luteinizing hormone-releasing hormone release by median eminence terminals in vitro. *Endocrinology (Baltimore)* **104**, 1749–1757.

Ondo, J. G., Wheeler, D. D., and Dom, R. M. (1988). Hypothalamic site of action for *N*-methyl-D-aspartate (NMDA) on LH secretion. *Life Sci.* **43**, 2283–2286.

Panerai, A. E., Petraglia, F., Sacerdote, P., and Genazzani, A. R. (1985). Mainly μ-opiate receptors are involved in luteinizing hormone and prolactin secretion. *Endocrinology (Baltimore)* **117**, 1096–1099.

Petersen, S. L., McCrone, S., Keller, M., and Gardner, E. (1991). Rapid increase in LHRH mRNA levels following NMDA. *Endocrinology (Baltimore)* **129**, 1679–1681.

Picanco-Diniz, D. L. W., Valenca, M. M., Franci, C. R., and Antunes-Rodrigues, J. A. (1990). Role of substance P in the medial preoptic area in the regulation of gonadotropin and prolactin secretion in normal or orchidectomized rats. *Neuroendocrinology* **51**, 675–682.

Pierroz, D. D., Catzeflis, C., Aebi, A. C., Rivier, J. E., and Aubert, M. L. (1996). Chronic administration of neuropeptide Y into the lateral ventricle inhibits both the pituitary-testicular axis and growth hormone and insulin-like growth factor I secretion in intact adult male rats. *Endocrinology (Baltimore)* **137**, 3–12.

Pirke, K. M., Vogt, H. J., and Geiss, M. (1978a). In vitro and in vivo studies on Leydig cell function in old rats. *Acta Endocrinol. (Copenhagen)* **89**, 393–403.

Pirke, K. M., Geiss, M., and Sintermann, R. (1978b). A quantitative study on feedback control of LH by testosterone in young adult and old male rats. *Acta Endocrinol. (Copenhagen)* **89**, 789–795.

Piva, F., Maggi, R., Limonta, P., Dondi, D., and Martini, L. (1987). Decrease of mu opioid receptors in the brain and in the hypothalamus of the aged male rat. *Life Sci.* **40**, 391–398.

Piva, F., Celotti, F., Dondi, D., Limonta, P., Maggi, R., Messi, E., Negri-Cesi, P., Zanisi, M., Motta, M., and Martini, L. (1993). Ageing of the neuroendocrine system in the brain of male rats: Receptor mechanisms and steroid metabolism. *J. Reprod. Fertil.* **46**, 47–59.

Ponzio, F., Brunello, N., and Algeri, S. (1978). Catecholamine synthesis in brain of ageing rat. *J. Neurochem.* **30**, 1617–1620.

Ponzio, F., Calderini, G., Lumuscio, G., Vantini, G., Toffano, G., and Algeri, S. (1982). Changes in monoamines and their metabolite levels in some brain regions of aged rats. *Neurobiol. Aging* **3**, 23–29.

Rettori, V., Kamat, A., and McCann, S. M. (1994). Nitric oxide mediates the stimulation of luteinizing hormone-releasing hormone induced by glutamic acid in vitro. *Brain Res.* **33**, 501–503.

Riegle, G. D., and Meites, J. (1976). Effects of aging on LH and prolactin after LHRH, L-DOPA, methyl-dopa, and stress in male rats. *Proc. Soc. Exp. Biol. Med.* **151**, 507–511.

Riegle, G. D., Meites, J., Miller, A. E., and Wood, S. M. (1977). Effect of aging on hypothalamic LH-releasing and prolactin inhibiting activities and pituitary responsiveness to LHRH in the male laboratory rat. *J. Gerontol.* **32**, 13–18.

Rodriguez-Gomez, J. A., de la Roza, C., Machado, A., and Cano, J. (1995). The effect of age on the monoamines of the hypothalamus. *Mech. Ageing Dev.* **77**, 185–195.

Roselli, C. E., Thornton, J. E., and Chambers, K. C. (1993). Age-related deficits in brain estrogen receptors and sexual behavior of male rats. *Behav. Neurosci.* **107**, 202–209.

Rossmanith, W. G., Marks, D. L., Clifton, D. K., and Steiner, R. A. (1996). Induction of galanin mRNA in GnRH neurons by estradiol and its facilitation by progesterone. *J. Neuroendocrinol.* **8**, 185–191.

Sahu, A., Kalra, P. S., Crowley, W. R., and Kalra, S. P. (1988). Evidence that hypothalamic neuropeptide Y secretion decreases in aged male rats: Implications for reproductive aging. *Endocrinology (Baltimore)* **122**, 2199–2203.

Sahu, A., Kalra, S. P., Crowley, W. R., and Kalra, P. S. (1990). Aging in male rats modifies castration and testosterone-induced neuropeptide Y response in various microdissected brain nuclei. *Brain Res.* **515**, 287–291.

Sahu, A., Phelps, C. P., White, J. D., Crowley, W. R., Kalra, S. P., and Kalra, P. S. (1992). Steroidal regulation of hypothalamic neuropeptide Y release and gene expression. *Endocrinology (Baltimore)* **130**, 3331–3336.

Sandusky, G. E., Van Pelt, C. S., Todd, G. C., and Wightman, K. (1988). An immunocytochemical study of pituitary adenomas and focal hyperplasia in old Sprague-Dawley and Fischer 344 rats. *Toxicol. Pathol.* **16**, 376–380.

Sar, M., Sahu, A., Crowley, W. R., and Kalra, S. P. (1990). Localization of neuropeptide Y (NPY) immunoreactivity in estradiol concentrating cells in the hypothalamus. *Endocrinology (Baltimore)* **127**, 2752–2756.

Sarkar, D. K., Gottschall, P. E., and Meites, J. (1984). Decline of tuberoinfundibular dopaminergic function resulting from chronic hyperprolactinemia in rats. *Endocrinology (Baltimore)* **115**, 1269–1274.

Sato, Y., Shibuya, A., Adachi, H., Kato, R., Horita, H., and Tsukamoto, T. (1998). Restoration of sexual behavior and dopaminergic neurotransmission by long term exogenous testosterone replacement in aged male rats. *J. Urol.* **160**, 1572–1575.

Schainker, B. A., and Cicero, T. J. (1980). Acute central stimulation of luteinizing hormone by parenterally administered *N*-methyl-D,L-aspartic acid in the male rat. *Brain Res.* **184**, 425–437.

Schneider, H. P. B., and McCann, S. M. (1969). Possible role of dopamine as transmitter to promote discharge of LH-releasing factor. *Endocrinology (Baltimore)* **85**, 121–126.

Selmanoff, M., Shu, C., Petersen, S. L., Barraclough, C. A., and Zoeller, R. T. (1991). Single cell levels of hypothalamic messenger ribonucleic acid encoding luteinizing hormone-releasing hormone in intact,

castrated, and hyperprolactinemic male rats. *Endocrinology (Baltimore)* **128**, 459–466.

Shaar, C. J., Euker, J. S., Riegle, G. D., and Meites, J. (1975). Effects of castration and gonadal steroids on serum luteinizing hormone and prolactin in old and young rats. *J. Endocrinol.* **66**, 45–51.

Silverman, A. J., Jhamandas, J., and Renaud, L. P. (1987). Localization of luteinizing hormone-releasing hormone (LHRH) neurons that project to the median eminence. *J. Neurosci.* **7**, 2312–2319.

Simpkins, J. W., and Gabriel, S. M. (1984). Chronic hyperprolactinemia causes progressive changes in hypothalamic dopaminergic and noradrenergic neurons. *Brain Res.* **309**, 277–292.

Simpkins, J. W., and Millard, W. J. (1987). Influence of age on neurotransmitter function. *Clin. Endocrinol. Metab.* **16**, 893–917.

Simpkins, J. W., Mueller, G. P., Huang, H. H., and Meites, J. (1977). Evidence for depressed catecholamine and enhanced serotonin metabolism in aging male rats: Possible relation to gonadotropin secretion. *Endocrinology (Baltimore)* **100**, 1672–1678.

Sladek, J. R., Jr., Khachaturian, H., Hoffman, G. E., and Scholer, J. (1980). Aging of central endocrine neurons and their aminergic afferents. *Peptides* **1**, 141–157.

Sortino, M. A., Aleppo, G., Scapagnini, U., and Canonico, P. L. (1996). Different responses of gonadotropin-releasing hormone (GnRH) release to glutamate receptor agonists during aging. *Brain Res. Bull.* **41**, 359–362.

Sprott, R. L. (1991). Development of animal models of aging at the National Institute on Aging. *Neurobiol. Aging* **12**, 635–638.

Steger, R. W., Huang, H. H., Hodson, C. A., Leung, F. C., Meites, J., and Sacher, G. A. (1980a). Effects of advancing age on hypothalamic-hypophysial-testicular functions in the male white-footed mouse *(Peromyscus leucopus). Biol. Reprod.* **22**, 805–809.

Steger, R. W., Sonntag, W. E., Van Vugt, D. A., Forman, L. J., and Meites, J. (1980b). Reduced ability of naloxone to stimulate LH and testosterone release in aging male rats; possible relation to increase in hypothalamic met5-enkephalin. *Life Sci.* **27**, 747–753.

Steger, R. W., DePaolo, L. V., and Shepherd, A. M. (1985). Effects of advancing age on hypothalamic neurotransmitter content and on basal and norepinephrine-stimulated LHRH release. *Neurobiol. Aging* **6**, 113–116.

Steiner, R. A., Bremner, W. J., Clifton, D. K., and Dorsa, D. M. (1984). Reduced pulsatile luteinizing hormone and testosterone secretion with aging in the male rat. *Biol. Reprod.* **31**, 251–258.

Stokkan, K. A., Reiter, R. J., Vaughan, M. K., Nonaka, K. O., and Lerchl, A. (1991). Endocrine and metabolic effects of life-long food restriction in rats. *Acta Endocrinol. (Copenhagen)* **125**, 93–100.

Swerdloff, R. S., and Wang, C. (1993). Androgen deficiency and aging in men. *West. J. Med.* **159**, 579–585.

Tamano, S., Hagiwara, A., Shibata, M. A., Kurata, Y., Fukushima, S., and Ito, N. (1988). Spontaneous tumors in aging (C57BL/6N × C3H/HeN)F1 (B6C3F1) mice. *Toxicol. Pathol.* **16**, 321–326.

Tenover, J. S., Matsumoto, A. M., Clifton, D. K., and Bremner, W. J. (1988). Age-related alterations in the circadian rhythms of pulsatile luteinizing hormone and testosterone secretion in healthy men. *J. Gerontol.* **43**, M163–M169.

Thurman, J. D., Moeller, R. B., and Turturro, A. (1995). Proliferative lesions of the testis in ad libitum-fed and food-restricted Fischer-344 and FBNF1 rats. *Lab. Anim. Sci.* **45**, 635–640.

Toney, T. W., Lookingland, K. J., and Moore, K. E. (1991). Role of testosterone in the regulation of tuberoinfundibular dopaminergic neurons in the male rat. *Neuroendocrinology* **54**, 23–29.

Tsuruo, Y., Kawano, H., Kagotani, Y., Hisano, S., Daikoku, S., Chihara, K., Zhang, T., and Yanaihara, N. (1990). Morphological evidence for neuronal regulation of luteinizing hormone-releasing hormone-containing neurons by neuropeptide Y in the rat septo-preoptic area. *Neurosci. Lett.* **110**, 261–266.

Turek, F. W., and Desjardins, C. (1979). Development of Leydig cell tumors and onset of changes in the reproductive and endocrine systems of aging F344 rats. *J. Natl. Cancer Inst.* **63**, 969–975.

Urban, J. H., Bauer-Dantoin, A. C., and Levine, J. E. (1993). Neuropeptide Y gene expression in the arcuate nucleus: Sexual dimorphism and modulation by testosterone. *Endocrinology (Baltimore)* **132**, 139–145.

Urban, J. H., Das, I., and Levine, J. E. (1996). Steroid modulation of neuropeptide Y-induced luteinizing hormone releasing hormone release from median eminence fragments from male rats. *Neuroendocrinology* **63**, 112–119.

Vermeulen, A. (1991). Androgens in the aging male. *J. Clin. Endocrinol. Metab.* **73**, 221–224.

Vernet, D., Bonavera, J. J., Swerdloff, R. S., Gonzalez-Cadavid, N. F., and Wang, C. (1998). Spontaneous expression of inducible nitric oxide synthase in the hypothalamus and other brain regions of aging rats. *Endocrinology (Baltimore)* **139**, 3254–3261.

Vitale, M. L., and Chiocchio, S. R. (1993). Serotonin, a neurotransmitter involved in the regulation of luteinizing hormone release. *Endocr. Rev.* **14**, 480–493.

Wang, C., Leung, A., and Sinha-Hikim, A. P. (1993). Reproductive aging in the male Brown-Norway rat: A model for the human. *Endocrinology (Baltimore)* **133**, 2773–2781.

Wang, Z. P., Man, S. Y., and Tang, F. (1993). Age-related changes in the contents of neuropeptides in the rat brain and pituitary. *Neurobiol. Aging* **14**, 529–534.

Wilkinson, C. W., and Dorsa, D. M. (1986). The effects of aging on molecular forms of beta- and gamma-endorphins in rat hypothalamus. *Neuroendocrinology* **43**, 124–131.

Winters, S. J., Sherins, R. J., and Troen, P. (1984). The gonadotropin-suppressive activity of androgen is increased in elderly men. *Metab. Clin. Exp.* **33**, 1052–1059.

Witkin, J. W. (1987). Aging changes in synaptology of luteinizing hormone-releasing hormone neurons in male rat preoptic area. *Neuroscience* **22**, 1003–1013.

Witkin, J. W. (1992a). Increased synaptic input to gonadotropin-releasing hormone neurons in aged, virgin, male Sprague-Dawley rats. *Neurobiol. Aging* **13**, 681–686.

Witkin, J. W. (1992b). Reproductive history affects synaptology of GnRH neurons in the aging male rat. *J. Neuroendocrinol.* **4**, 427–432.

Yen, S. S. C., Morales, A. J., and Khorram, O. (1995). Replacement of DHEA in aging men and women: Potential remedial effects. *Ann. N. Y. Acad. Sci.* **774**, 128–142.

Zanisi, M., Messi, E., and Martini, L. (1987). In vitro release of luteinizing hormone-releasing hormone from the hypothalamus of old male rats. *Endocrinology (Baltimore)* **120**, 49–54.

Zirkin, B. R., Santulli, R., Strandberg, J. D., Wright, W. W., and Ewing, L. L. (1993). Testicular steroidogenesis in the aging Brown Norway rat. *J. Androl.* **14**, 118–123.

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Susan B. Roberts and Nicholas P. Hays

58

Regulation of Energy Intake in Old Age

Unexplained weight loss is common in late old age due to a decrease in energy intake relative to energy expenditure and is given the term ''anorexia of aging.'' There is evidence from multiple studies that a loss of the ability to accurately regulate food intake may be an important contributing factor, especially when combined with the adverse social and medical problems that are common in old age. The underlying reasons for the age-associated loss of the ability to regulate food intake are not well understood. However, likely candidates exist at all points in the cascade of mechanisms thought to be involved in energy regulation, including impaired taste and smell, delayed gastric emptying, and altered digestion-related hormone secretion and central responsiveness to those hormones. Further research is needed to identify which of the suspected mechanisms are quantitatively important and whether a loss of the ability to accurately regulate food intake can be offset by dietary or other lifestyle interventions. © 2001 Academic Press.

I. Introduction

Aging is known to be associated with alterations in body fat that have an important impact on health. Through middle age there is a doubling in body fat in men and women living in developed countries (Steen, 1988; Shimokata *et al*., 1989) which is associated with increased morbidity and mortality [U.S. Department of Health and Human Services (USDHHS), 1990]. In contrast, in late old age, body fat typically decreases even in healthy individuals (Steen *et al*., 1979; Chumlea *et al*., 1988; Steen, 1988; Shimokata *et al*., 1989) and unexplained weight loss leading to energy protein malnutrition becomes increasingly common in diverse groups of human subjects and also animal models (Fig. 58.1 and 58.2) (Mason, 1970; Fischer and Johnson, 1990; Miller *et al*., 1990; Coroni-Huntley *et al*., 1991; Fleming-Moran *et al*., 1991; Williamson, 1993; Launer *et al*., 1994; Scarlett *et al*., 1994; Armstrong and Lund, 1996; Ryan *et al*., 1996). Among the institutionalized elderly, as many as 30–50% are reported to suffer from protein-energy malnutrition (Rudman *et al*., 1990; Lipschitz, 1991). This loss of body fat is associated with premature death, micronutrient deficiencies, frailty, increased hospital admission, an increased risk of disability from falls, and it is also known to delay recovery from injury (Delmi *et al*., 1990; Tayback *et al*., 1990; Pamuk *et al*., 1992; Mowe *et al*., 1994).

Although negative energy balance, resulting from low energy intake relative to energy expenditure, is thought to be the usual cause of the loss of body fat in old age, the underlying causes of weight loss and negative energy balance in old age are not well understood. Nationwide studies have suggested that low dietary energy intake is widespread even among healthy elderly adults (USDHHS, 1983), while studies of total energy expenditure in the elderly (Prentice, 1992; Roberts, 1995; Roberts and Dallal, 1998) suggest low rather than high values. The combination of these findings suggest that inadequate energy intake, rather than increased energy expenditure, is the primary cause of weight loss in old age. However, whether this low energy intake is primarily due to social changes that decrease dietary intake or whether there are underlying metabolic changes that impair energy regulation in old age is still under investigation. This review and others (Doty *et al*., 1984; Morley *et al*., 1985; Leibowitz, 1988; Morley and Silver, 1988; Schiffman and Warwick, 1989; Morley, 1997; Schiffman, 1997; Blanton *et al*., 1999; Finkelstein and Schiffman, 1999; Roberts, 2000a–d) examines current evidence on the factors leading to late-life weight and fat loss.

II. Biobehavioral and Social Determinants of Energy Regulation in Older Adults

Many social changes associated with aging have been suggested to cause weight loss. As summarized in Table 58.1, adverse factors ranging from poverty and bereavement to poor dentition, chronic disease, and the use of multiple prescription medications may all potentially be important (Doty *et al*., 1984; Morley and Silver, 1988; Schiffman and Warwick, 1989; Morley, 1990, 1996, 1997; Gorbien, 1994). In addition, depression has been suggested as an important cause of weight loss among the elderly, a finding that has been confirmed in an analysis of cohort data (DiPietro *et al*., 1992). It is interesting to note that the study of DiPietro *et al*. (1992) showed that depression was associated with weight loss only in individuals ages 55 years or older and was actually associated with weight

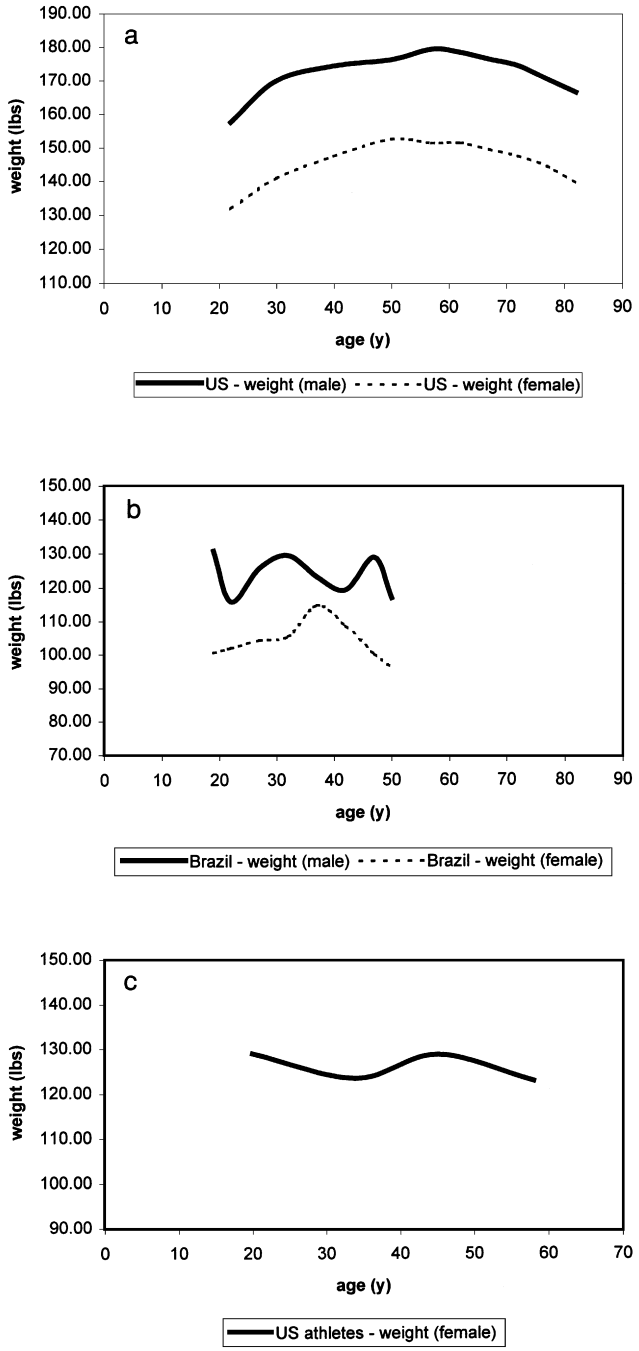

FIG. 58.1. Body weight change in men (—) and women (- - -) over the life span. (a) U.S. national survey data (Coroni-Huntley *et al*., 1991; Williamson, 1993). (b) Brazil Amerindian society study data (Fleming-Moran *et al*., 1991). (c) U.S. female athletes study data (Ryan *et al*., 1996).

gain in younger adults. One potential explanation for this finding (Roberts *et al*., 1994) is that social factors are potential catalysts for weight loss only when there is an underlying impairment in the regulation of food intake (see below) that allows impediments to eating to be expressed.

Two related social factors that may potentially reduce energy intake in old age and which have received relatively greater study are reduced dietary variety and social isolation.

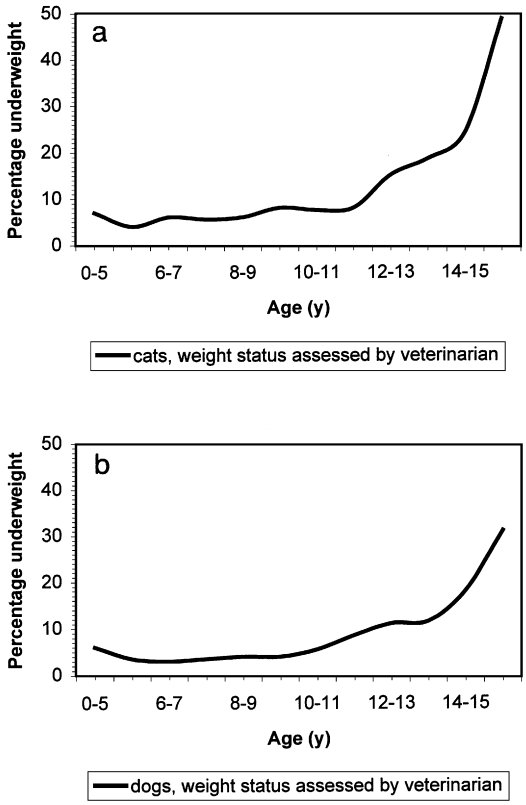

FIG. 58.2. Percentage of underweight cats (a) and dogs (b) by age group (Adapted from Armstrong and Lund, 1996, © Veterinary Practice Publishing Company).

Concerning variety, studies in laboratory rats, cats, and hamsters have shown that energy intake is greater when a variety of foods is provided compared to when only a single food is provided (McCrory *et al*., 1999b, 2000). Numerous single-meal studies in humans also show this phenomenon (Rolls, 1985; McCrory *et al*., 1999b). While two longer term studies have shown that laboratory rats have greater body fat and body fat gain when fed a variety of foods compared to when only a single food is fed (Rolls *et al*., 1983; Louis-Sylvestre *et al*., 1984), long-term studies in humans have been lacking until recently. Our laboratory recently reported on the long-term association between dietary variety and body fatness in healthy adult men and women (McCrory *et al*., 1999b), using 6 month dietary intake reports from a food frequency questionnaire (Block *et al*., 1986) and accurate measurements of body fatness by underwater weighing. In multiple regression analyses controlled for age and sex, dietary variety from a combined group of sweets, snacks, condiments, entrees, and carbohydrates was positively associated with body fatness and in the same model dietary variety from vegetables was negatively associated with body fatness. In other words, individuals who consumed a wide variety of higher energy foods coupled with a low variety of vegetables were relatively fat. The opposite was also true: individuals who consumed a low variety of sweets, snacks, condiments, entrees, and carbohydrates and a high variety of vegetables were relatively lean.

Although it was not possible to specifically examine the effects of age in that study, the results are relevant to aging because dietary variety comes primarily from high-energy

TABLE 58.1 Possible Causes of Age-Related Weight Loss[a]

Psychological
Depression
Bereavement
Alcoholism
Dementia/cognitive decline
Fatigue/apathy
Late life paranoia
Late life mania
Medical
Chronic disease
Infection
Wound healing
Pain
Hyperthyroidism
Malabsorption disorders
Medications that alter taste or appetite
Food/medication interactions
Poor dentition/swallowing problems
Anorexia
Overrestrictive therapeutic diets
Physical limitations when feeding self or preparing food
Impaired chemosensory function
Social
Poverty
Inability to shop for food
Social isolation at mealtime
Failure to cater to ethnic food preferences in institutionalized individuals
Elder abuse

[a]Adapted from Morley (1997), © American Society for Clinical Nutrition.

items, and there are reports suggesting that dietary variety typically decreases in old age (Brown, 1976; Fanelli and Stevenhagen, 1985). The reason for why dietary variety may decrease in old age is not known, but social factors such as poverty and living alone may be important. In addition, a study by Rolls and McDermott (1991) suggested that older adults have reduced "sensory specific satiety," a term used to describe the phenomenon of declining pleasantness of food as it is consumed. As summarized in Fig. 58.3, the adolescent and young adult subjects of Rolls and McDermott (1991) responded in the expected manner to a yogurt preload (i.e. decreased desire to eat yogurt but not other offered foods). However, older subjects did not respond and in fact reported an equivalent desire to eat yogurt and other test foods after the preload. Although necessarily short-term and requiring confirmation in a larger study, these data suggest that older adults lack normal patterns of sensory specific satiety that encourage wide dietary variety and thus provide a potential explanation for the observed reduction in dietary variety in old age (Brown, 1976; Fanelli and Stevenhagen, 1985). The underlying reason for decreased sensory specific satiety in old age requires investigation, but potentially may be traced back to the widely reported declines in taste and smell sensitivity associated with aging (Schiffman *et al*., 1976; Schiffman, 1977, 1994, 1997; Schiffman and Pasternak, 1979; Weiffenbach *et al*., 1982; Doty *et al*., 1984; Schiffman and Gatlin, 1993; Finkelstein and Schiffman, 1999). Most studies (Schiffman *et al*., 1976; Schiffman, 1977,1994,1997; Schiffman and Pasternak, 1979; Weiffenbach *et al*., 1982; Doty *et al*., 1984; Schiffman and Gatlin, 1993; Finkelstein and Schiffman, 1999) suggest that detection and recognition thresholds for salt and other specific tastes increase with age, in part because of a loss of functional taste buds and alterations in taste bud structure (Arey *et al*., 1936) and in part because of impaired olfaction (which contributes to the sensation of taste). In consequence, and as shown by Schiffman (1977), elderly individuals have a reduced ability to identify individual foods in blinded tests. If foods taste more similar, the pleasure of a diverse diet will presumably be reduced, thus tending to decrease dietary variety in the absence of social factors working in the opposite direction.

Concerning social isolation, de Castro and de Castro (1989) reported that less energy is eaten at meals taken alone compared to meals eaten in company, with the difference in energy intake between the two situations being a substantial 30%. Although such data are generally used to suggest that social eating is disadvantageous because it promotes overeating and obesity, the opposite (namely that eating alone leads to undereating and weight loss) may be an equally correct interpretation. This is especially true when it is considered that humans are a gregarious animal species, and naturally eat in social groups. This is directly relevant to the issue of low energy intake in older population, because bereavement and functional disabilities can limit social contact (Markson, 1997). Thus, an increased frequency of eating alone may be

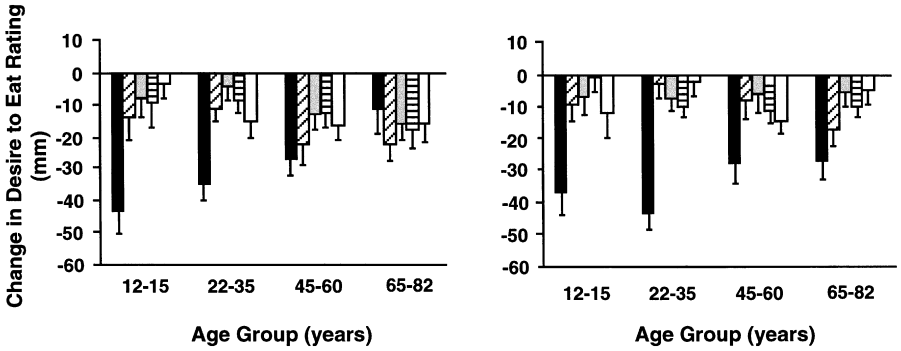

FIG. 58.3. Mean (± SEM) changes in ratings for each of the foods sampled of desire to eat that food from just before to just after eating either 300 g (left) or *ad libitum* (right) amounts of yogurt by the different age groups (years) (From Rolls and McDermott, 1991, © American Society for Clinical Nutrition). ■ Yogurt; ▨ tuna; ▩ cracker; ▤ carrot; □ pretzel.

one of the factors contributing to low energy intake in older adults. Furthermore, there is a positive association between the frequency of eating restaurant food and body fatness (McCrory *et al*., 1999a) and for reasons of social isolation and functional disabilities, older adults may eat out less frequently. The combination of these different observations and findings suggests a potentially important role for reductions in social meals and eating out in the low energy intake and body weight loss of older adults.

III. Impaired Regulation of Food Intake in Older Adults

The question of whether there might be a loss of the ability to regulate energy intake specifically associated with old age, rather than the adverse social factors and diseases common in the elderly, was investigated by our group (Roberts *et al*., 1994,1995; Saltzman and Roberts, 1996; Moriguti *et al*., 2000) and others (Rolls *et al*., 1995; Clarkston *et al*., 1997; Cook *et al*., 1997; Morley, 1997).

In our own studies, we tested the hypothesis that aging is associated with a reduced energy expenditure response to overfeeding or underfeeding and also an impaired subsequent regulation of food intake. As predicted, there were clear differences between age groups in both energy expenditure during the intervention and subsequent energy intake. Young men had a significant increase in basal metabolic rate during overfeeding and a significant decrease in resting energy expenditure during underfeeding, whereas elderly men did not (Saltzman and Roberts, 1996). Moreover, although body weight change during overfeeding or underfeeding was similar in the two age groups, young men subsequently tended to lose all the excess weight following overfeeding while the weight of the elderly men did not decrease significantly (Fig. 58.4). This difference was apparently due to the fact that the young men significantly decreased their voluntary energy intake following overfeeding, while the energy intake of the elderly men did not decrease significantly and indeed remained elevated relative to their previous weight-maintenance requirement (Fig. 58.5). Comparable results were obtained in the underfeeding component of the study. As with overfeeding, there was a similar weight loss during underfeeding in the young and elderly subjects (Fig. 58.6) but subsequently, young men gained back the weight lost during underfeeding while elderly men did not (Fig. 58.6). Again, this difference was apparently due to differences in voluntary food intake following the cessation of underfeeding, because the young men significantly increased their energy intake following underfeeding, although

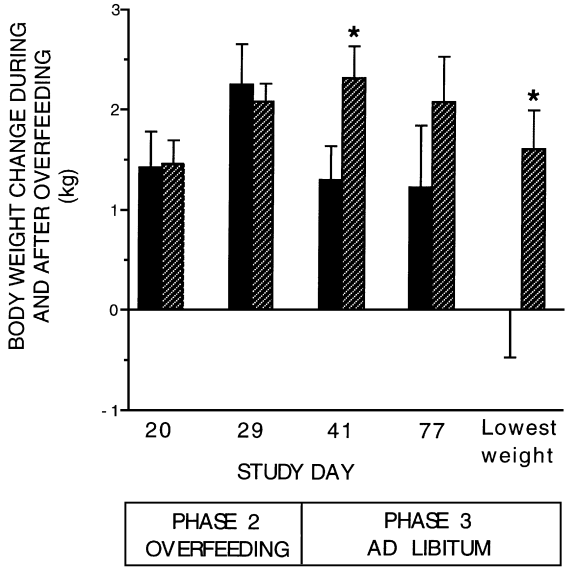

FIG. 58.4. Body weight change during 21 days of overfeeding and a subsequent 46-day period of *ad libitum* diet (From Roberts *et al*., 1994, © 1994 American Medical Association). Values are mean ± SEM for young (■) and older (▨) men. *$P<0.05$ relative to the young men.

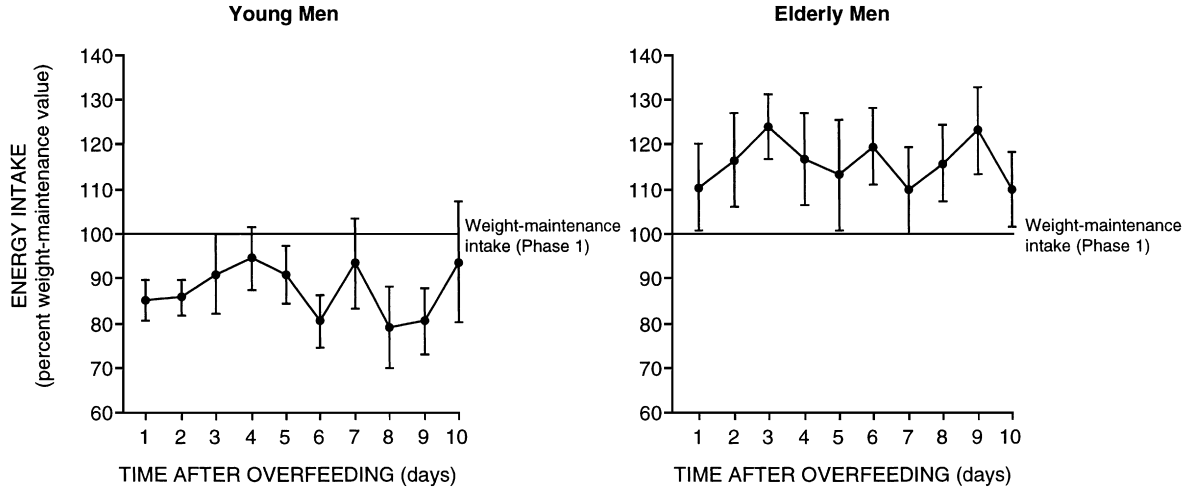

FIG. 58.5. Voluntary energy intake during a 10-day period following overfeeding in young and older men (From Roberts *et al*., 1994, © 1994 American Medical Association). Values are means ± SEM in comparison with initial weight maintenance energy requirements. The change in energy intake relative to initial weight maintenance requirements were significantly different between the age groups ($P = 0.006$).

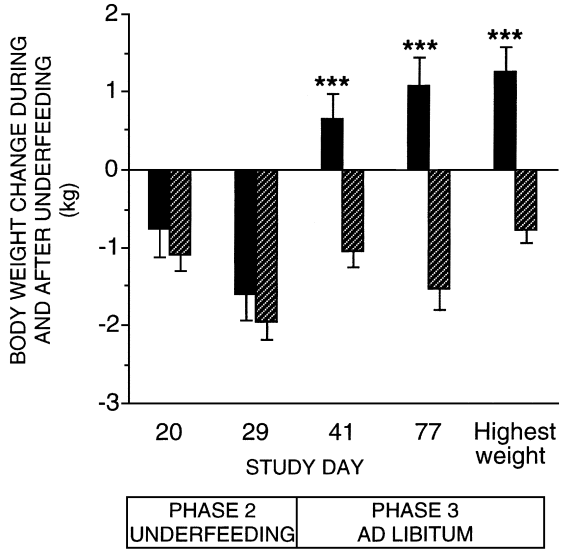

FIG. 58.6. Body weight change during 21 days of underfeeding and a subsequent 46-day period of *ad libitum* diet (From Roberts *et al*., 1994, © 1994 American Medical Association). Values are mean ± SEM for young (■) and older (▨) men. * $P<0.05$ relative to the young men. *** $P<0.001$ relative to the young men.

the energy intake of the elderly men did not increase significantly and actually remained somewhat depressed relative to their previous weight-maintenance requirement (Fig. 58.7).

Thus, in two separate protocols involving opposite, experimentally imposed changes in energy balance, older men had a substantial reduction in their ability to maintain a constant energy balance compared to young men. The fact that the same result was obtained under opposite experimental conditions (overfeeding and underfeeding) suggests a basic difference between age groups rather than an experimental artifact, and implies that aging is indeed associated with an impaired ability to accurately regulate food intake.

Consistent results have recently been reported in other studies. Rolls *et al*. (1995) and Morley (1997) both observed that elderly subjects are less able to accurately compensate for preloads at a subsequent meal than young subjects and report less desire to eat postprandially (Clarkston *et al*., 1997) and less hunger but not greater fullness (Clarkston *et al*., 1997). Intraduodenal nutrient infusion has also been reported to reduce hunger in young but not elderly adults (Cook *et al*., 1997). Finally, we have also obtained recent data (Moriguti 2000) suggesting that elderly men and women experience less frequent hunger during dieting than young individuals.

When interpreting these combined results, it is important to recognize that most of the studies were conducted in older men and women who reported having no problems with their appetite, and thus the extent to which an elderly individual with impaired energy regulation loses or gains weight may depend on social factors. For example, factors that have previously been thought to cause anorexia and weight loss (such as loss of teeth and depression) may precipitate a long-term reduction in body weight only when the metabolic signals that drive adaptive variations in energy intake are absent or reduced as they appear to be in the older subjects in the above investigations. In support of this suggestion, depression is associated with weight gain in young adults but weight loss in the elderly (DiPietro *et al*., 1992). Elderly individuals may also have an impaired ability to regain weight after periods of weight loss precipitated by disease.

IV. Mechanisms Underlying the Decreased Ability to Regulate Food Intake in Old Age

The mechanisms underlying successful energy regulation in young adults are poorly understood. It is generally believed that multiple overlapping mechanisms exist to regulate energy balance in young adults, with hunger and satiety being moni-

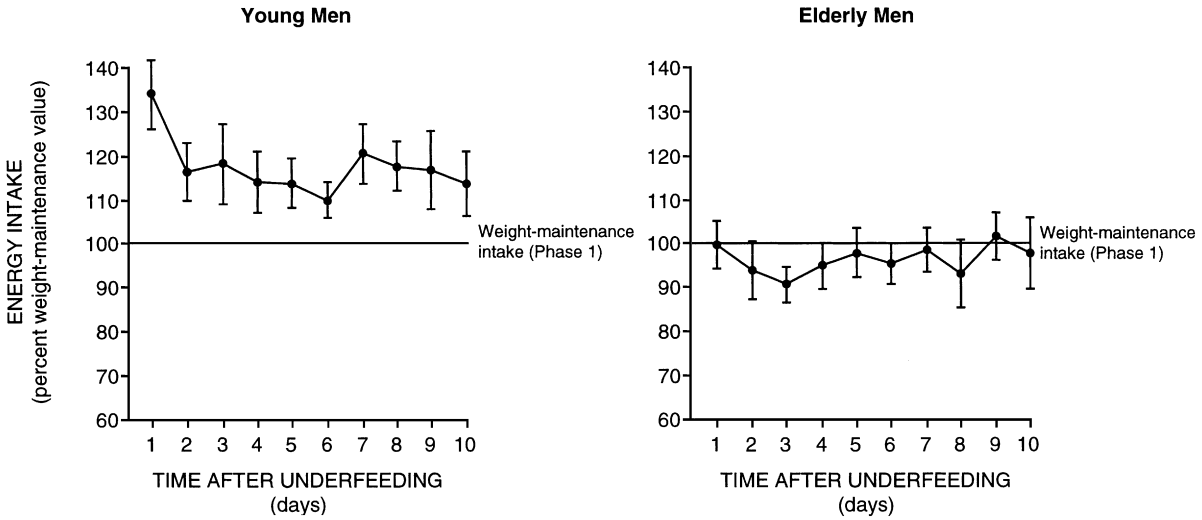

FIG. 58.7. Voluntary energy intake during a 10-day period following underfeeding in young and older men (From Roberts *et al*., 1994, © 1994 American Medical Association). Values are means ± SEM in comparison with initial weight maintenance energy requirements. The change in energy intake relative to initial weight maintenance requirements were significantly different between the age groups ($P = 0.016$).

tored both peripherally and centrally by several systems. As such, there are multiple candidates for age-related changes that could contribute to the anorexia of aging, and several of these have been outlined in recent reviews (see Morley *et al*., 1985; Blundell, 1988; Morley and Silver, 1988; Morley, 1997; Blanton *et al*., 1999). It is important to recognize that the likely redundancy in energy regulation mechanisms implies that multiple pathways need to fail before a measurable impairment in energy regulation is seen. Thus, quite a large number of candidate mechanisms may potentially be shown to be impaired in old age and work is needed to identify which mechanisms are quantitatively important. The strongest candidates based on current knowledge are summarized below, and are combined in a suggested model in Fig. 58.8.

Gastric emptying is one potentially important factor in the anorexia of aging. Most studies examining gastric emptying in relation to age have reported a decreased rate of gastric emptying in the elderly. In some cases delayed emptying of both liquids and solids has been observed (Richey and Bender, 1977; Horowitz *et al*., 1984; Wegener *et al*., 1988; Clarkston *et al*., 1997), while in other studies only delayed emptying of liquids (Evans *et al*., 1981; Moore *et al*., 1983) or normal emptying of solids (van Liere and Northup, 1941) has been reported. This is relevant because delayed gastric emptying has been linked to reduced hunger and increased satiety in several studies (Shafer *et al*., 1987; Sepple and Read, 1989; Bergmann *et al*., 1992; Horowitz *et al*., 1993; Clarkston *et al*., 1997) and thus may potentially contribute to increased satiety and satiation and/or decreased hunger.

The mechanisms by which reduced gastric emptying could directly cause alterations in energy regulation are not known, but there are several possibilities that require further study. For example, delayed gastric emptying presumably prolongs the period during which nutrients are absorbed, and this may translate into a prolonged period during which energy substrates (glucose, free fatty acids) remain in the circulation. Several models of energy regulation (Mayer, 1953; Flatt, 1987; Friedman, 1995) postulate a central role for substrate availability in energy regulation, and recent work on blood glucose and hunger has supported the concept of a role for low blood glucose in initiation of hunger signals in young adults (Campfield *et al*., 1996; Ludwig *et al*., 1999; Melanson *et al*., 1999). Although some aspects of the central detection and response to alterations in blood glucose (such as during hypoglycemia) seem to be impaired in elderly individuals (Brierley *et al*., 1995), the hunger response to hypoglycemia is reportedly intact (Brierley *et al*., 1995), and a prolonged age-associated elevation of blood glucose following consumption of meals has been reported in one recent study (Melanson *et al*., 1998). Gastric emptying presumably also results in a prolonged period of stomach distention, which may additionally prolong satiation directly through afferent vagal signals.

Concerning the underling causes of delayed gastric emptying in the elderly, more work in this area is needed, but several mechanisms have been suggested, including increased phasic pyloric pressure waves in response to nutrients in the duodenum (Cook *et al*., 1997) and impaired autonomic nervous system function which is common in the elderly (see Clarkston *et al*., 1997 and Chapter 64). Morley (1997) has also suggested that a reduction in nitric oxide production by the stomach in elderly adults may increase satiation by reducing relaxation of the fundus. In addition, the age-associated alterations in taste sensitivity (Schiffman *et al*., 1976; Schiffman, 1977, 1994, 1997; Schiffman and Pasternak, 1979; Weiffenbach

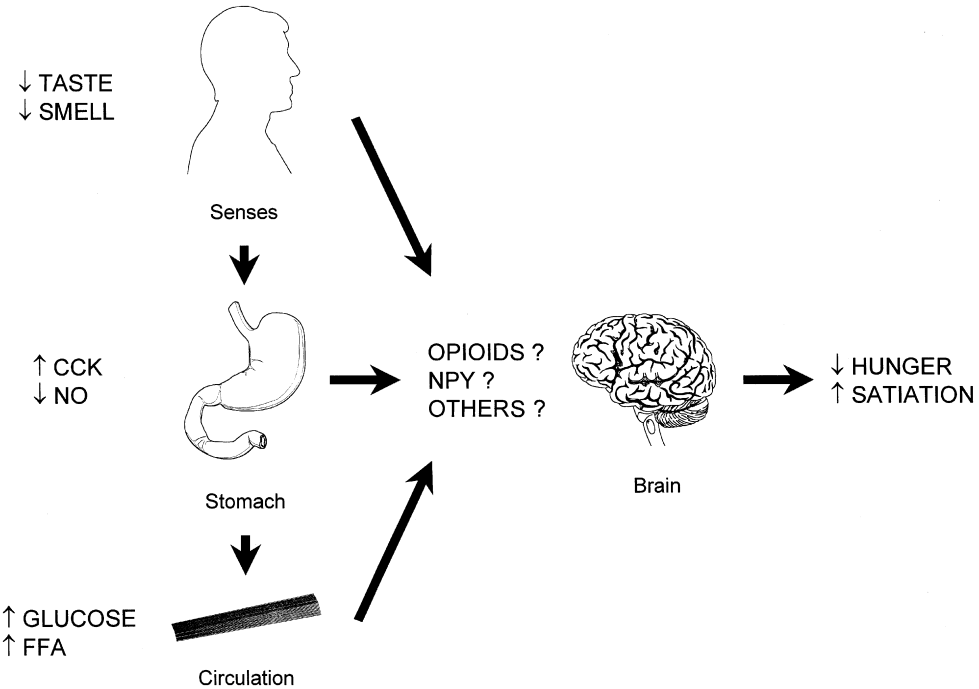

FIG. 58.8. Suggested model of metabolic impairments in the normal cascade of energy regulation mechanisms that could help explain impaired regulation of energy leading to an increased risk of weight loss in old age. FFA, free fatty acids; NO, nitric oxide; CCK, cholecystokinin; NPY, neuropeptide Y. Life ART Images © 1989-2000 by Lippincott Williams & Wilkins.

et al., 1982; Doty *et al*., 1984; Finkelstein and Schiffman, 1999), detailed above, may play a more central role than currently recognized. As reviewed elsewhere (Schiffman, 1997), intact senses of taste and smell are necessary for the celphalic phase of digestion, which includes the initial increases in salivary, gastric, pancreatic, and intestinal secretions designed to initiate digestion. We recently obtained preliminary evidence (Sawaya *et al*., 2000) suggesting that consumption of bland foods decreases the glycemic response to test meals compared to palatable meals of the same macronutrient composition, presumably as a result of a reduced rate of gastric emptying and hence digestion. If the losses in taste and smell associated with aging have an equivalent effect (i.e., making food seem more bland, as commonly reported), the decreased gastric emptying and delayed absorption of nutrients could help explain the reported increase in satiation and decrease in hunger in old age. Consistent with this suggestion, elderly individuals eat more of individual foods if they are flavor-enhanced (Schiffman and Warwick, 1993), and in another study smell was significantly correlated with hunger and appetite (de Jong *et al*., 1999). It should be noted that, in this latter study, there was no association between smell and energy intake; however, recognized inaccuracies in the measurement of energy intake (Schoeller and Fjeld, 1991) make the validity of such correlations uncertain.

In addition to its effects on taste, gastric emptying, and related variables, old age may also influence the production and detection of several digestion-related hormones thought to be involved in satiety and satiation. For example, glucagon has long been suggested to be one of the signals of satiation (Stunkard *et al*., 1955; Schulman *et al*., 1957; Geary, 1990), with its action mediated at least in part through vagal afferent signals from the liver and perhaps also by increasing blood glucose (which is another postulated signal, see above). Melanson *et al*. (1998) recently reported that elderly women have significantly elevated levels of glucagon in response to consumption of meals of 500 kcal or greater, suggesting a potential role for glucagon in the enhanced satiation associated with old age.

There is also evidence of both altered synthesis and impaired central responsiveness to gut hormones. For example, fasting levels of the postulated satiety hormone cholecystokinin are typically higher in elderly individuals than in young adults (Smith and Gibbs, 1988; Martinez *et al*., 1993; MacIntosh *et al*., 1999) and rise more in response to an isocaloric rate of intraduodenal fat infusion than in young adults (MacIntosh *et al*., 1999). Concerning evidence for impaired detection of gut hormone signals, the rise in cholecystokinin, glucagon-like peptide 1 and polypeptide YY associated with intraduodenal lipid infusion were significantly correlated with a decrease in hunger in young but not elderly individuals (MacIntosh *et al*., 1999). Consistent data were also obtained in animal models, with administration of exogenous cholecystokinin, bombesin, and calcitonin having a greater suppressing effect on food intake in young than in elderly mice (Silver *et al*., 1988).

Leptin (Zhang *et al*., 1994; Halaas *et al*., 1995) has also been proposed as a candidate hormone that may contribute to the anorexia of aging, but current data are conflicting. For example, there is a significant correlation between leptin and body fat in young adults (Considine *et al*., 1996; Roberts *et al*., 1997; Moller *et al*., 1998) and some (Moller *et al*., 1998) but not all (Roberts *et al*., 1997; Wolden-Hanson *et al*., 1999) investigations have suggested no similar correlation in elderly adults.

The fact that a variety of peripheral satiety signals, including cholecystokinin, leptin, and perhaps blood glucose are suggested to be imperfectly detected in old age may be related to several central mechanisms responsible for coordination of energy regulation signals. One potential candidate is neuropeptide Y (NPY), an integrator of metabolic endocrine and behavioral systems (Leibowitz, 1988, 1991) that acts through various hypothalamic nucleii, affects release of hormones that modulate energy metabolism such as insulin, and stimulates feeding behavior (Leibowitz, 1991). Currently, however, the data are conflicting. The finding of high levels of NPY in both plasma and cerebrospinal fluid of elderly anorectics implies that NPY expression does not relate to the anorexia of aging (since high NPY is predicted to increase feeding in young, see Martinez *et al*., 1993). On the other hand, the fasting-induced increases in both food intake and NPY gene expression normally seen in young rats are attenuated in aging rats (Gruenewald *et al*., 1996), suggesting that responsiveness to NPY may be impaired in old age. Consistent with this suggestion, the feeding and drinking responses to NPY injection in the paraventricular hypothalamic nucleus are attenuated in aged rats (Pich *et al*., 1992) but more work in this area is needed.

Finally, opioid peptides (Morley, 1980; Sanger, 1981; Morley and Levine, 1982) are also indirectly implicated in the anorexia of aging by the observation that the opioid agonist butorphanol increases feeding in young rats, and the effect is attenuated in elderly rats (Gosnell *et al*., 1983). Similarly, the opioid antangonist naloxone suppresses feeding in young rats but not in elderly rats (Gosnell *et al*., 1983).

V. Summary

Even healthy older adults appear to exhibit a significant loss of the ability to regulate food intake. When combined with a reduction in sensory specific satiety and disadvantageous social factors such as chronic illness, functional limitations and depression, this loss of the regulation of food intake may contribute to unexplained weight loss in elderly adults. Further research is needed to determine the quantitative importance of the different mechanisms (both biological and psychological) that are suggested to underlie age-related changes in the regulation of food intake, and to determine what practical steps can be taken to prevent weight loss and associated declines in physical function.

References

Arey, L. B., Tremaine, M. J., and Monzingo, F. L. (1936). The numerical and topographical relations of taste buds to human circumvallate papillae throughout the life span. *Anat. Rec*. **64**, 9–25.

Armstrong, P. J., and Lund, E. M. (1996). Changes in body composition and energy balance with aging. *Vet. Clin. Nutr.* **3**, 83–87.

Bergmann, J. F., Chassany, O., Petit, A., Triki, R., Caulin, C., and Segrestaa, J. M. (1992). Correlation between echographic gastric emptying and appetite: Influence of psyllium. *Gut* **33**, 1042–1043.

Blanton, C. A., Horwitz, B. A., and McDonald, R. B. (1999). Neurochemical alterations during age-related anorexia. *Proc. Soc. Exp. Biol. Med*. **221**, 153–165.

Block, G., Hartman, A. M., Dresser, C. M., Carroll, M. D., Gannon, J., and Gardner, L. (1986). A data-based approach to diet questionnaire design and testing. *Am. J. Epidemiol*. **124**, 453–469.

Blundell, J. E. (1988). Understanding anorexia in the elderly: Formulating biopsychological research strategies. *Neurobiol. Aging* **9**, 18–20.

Brierley, E. J., Broughton, D. L., James, O. F., and Alberti, K. G. (1995). Reduced awareness of hypoglycaemia in the elderly despite an intact counter-regulatory response. *Q. J. Med*. **88**, 439–445.

Brown, E. L. (1976). Factors influencing food choices and intake. *Geriatrics* **31**, 89–92.

Campfield, L. A., Smith, F. J., Rosenbaum, M., and Hirsch, J. (1996). Human eating: Evidence for a physiological basis using a modified paradigm. *Neurosci. Biobehav. Rev*. **20**, 133–137.

Chumlea, W. C., Garry, P. J., Hunt, W. C., and Rhyne, R. L. (1988). Distributions of serial changes in stature and weight in a healthy elderly population. *Hum. Biol*. **60**, 917–925.

Clarkston, W. K., Pantano, M. M., Morley, J. E., Horowitz, M., Littlefield, J. M., and Burton, F. R. (1997). Evidence for the anorexia of aging: Gastrointestinal transit and hunger in healthy elderly vs. young adults. *Am. J. Physiol*. **272**, R243–R248.

Considine, R. V., Sinha, M. K., Heiman, M. L., Kriauciunas, A., Stephens, T. W., Nyce, M. R., Ohannesian, J. P., Marco, C. C., McKee, L. J., Bauer, T. L., and Caro, J. F. (1996). Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N. Engl. J. Med*. **334**, 292–295.

Cook, C. G., Andrews, J. M., Jones, K. L., Wittert, G. A., Chapman, I. M., Morley, J. E., and Horowitz, M. (1997). Effects of small intestinal nutrient infusion on appetite and pyloric motility are modified by age. *Am. J. Physiol*. **273**, R755–R761.

Coroni-Huntley, J. C., Harris, T. B., Everett, D. F., Albanes, D., Micozzi, M. S., Miles, T. P., and Feldman, J. J. (1991). An overview of body weight of older persons, including the impact on mortality. *J. Clin. Epidemiol*. **44**, 743–753.

de Castro, J. M., and de Castro, E. S. (1989). Spontaneous meal patterns of humans: Influence of the presence of other people. *Am. J. Clin. Nutr*. **50**, 237–247.

de Jong, N., Mulder, I., de Graaf, C., and van Staveren, W. A. (1999). Impaired sensory functioning in elders: The relation with its potential determinants and nutritional intake. *J. Gerontol. A* **54A**, B324–B331.

Delmi, M., Rapin, C. H., Bengoa, J. M., Delmas, P. D., Vasey, H., and Bonjour, W. (1990). Dietary supplementation in elderly patients with fractured neck of the femur. *Lancet* **335**, 1013–1016.

DiPietro, L., Anda, R. F., Williamson, D. F., and Stunkard, A. J. (1992). Depressive symptoms and weight change in a national cohort of adults. *Int. J. Obes. Relat. Metab. Disord*. **16**, 745–753.

Doty, R. L., Shaman, P., Applebaum, S. L., Giberson, R., Siksorski, L., and Rosenberg, L. (1984). Smell identification ability: Changes with age. *Science* **226**, 1441–1443.

Evans, M. A., Triggs, E. J., Cheung, M., Broe, G. A., and Creasey, H. (1981). Gastric emptying rate in the elderly: Implications for drug therapy. *J. Am. Geriatr. Soc*. **29**, 201–205.

Fanelli, M. T., and Stevenhagen, K. J. (1985). Characterizing consumption patterns by food frequency methods: Core foods and variety of foods in diets of older Americans. *J. Am. Diet. Assoc*. **85**, 1570–1576.

Finkelstein, J. A., and Schiffman, S. S. (1999). Workshop on taste and smell in the elderly: An overview. *Physiol. Behav*. **66**, 173–176.

Fischer, J., and Johnson, M. A. (1990). Low body weight and weight loss in the aged. *J. Am. Diet. Assoc*. **90**, 1697–1706.

Flatt, J. P. (1987). The difference in the storage capacities for carbohydrate and for fat, and its implications in the regulation of body weight. *Ann. N. Y. Acad. Sci*. **499**, 104–123.

Fleming-Moran, M., Santos, R. V., and Coimbra, C. E. A. (1991). Blood pressure levels of the Surui and Zoro Indians of the Brazilian Amazon: Group- and sex-specific effects resulting from body composition, health status, and age. *Hum. Biol*. **63**, 835–861.

Friedman, M. I. (1995). Control of energy intake by energy metabolism. *Am. J. Clin. Nutr*. **62**, 1096S–1100S.

Geary, N. (1990). Pancreatic glucagon signals postprandial satiety. *Neurosci. Biobehav. Rev*. **14**, 323–338.

Gorbien, M. J. (1994). Anorexia of aging: A simple approach to management. *Cleveland Clin. J. Med*. **61**, 253.

Gosnell, B., Levine, A. S., and Morley, J. E. (1983). The effects of aging on opioid modulation of feeding in rats. *Life Sci*. **32**, 2793–2799.

Gruenewald, D. A., Marck, B. T., and Matsumoto, A. M. (1996). Fasting-induced increases in food intake and neuropeptide Y gene expression are attenuated in aging male Brown Norway rats. *Endocrinology (Baltimore)* **137**, 4460–4467.

Halaas, J. L., Gajiwala, K. S., Maffei, M., Cohen, S. L., Chait, B. T., Rabinowitz, D., Lallone, R. L., Burley, S. K. and Friedman, J. M. (1995). Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* **269**, 543–546.

Horowitz, M., Maddern, G. J., Chatterton, B. E., Collins, P. J., Harding, P. E., and Shearman, D. J. C. (1984). Changes in gastric emptying rates with age. *Clin. Sci*. **67**, 213–218.

Horowitz, M., Jones, K., Edelbroek, M. A. L., Smout, A. J. P. M., and Read, N. W. (1993). The effect of posture on gastric emptying and intragastric distribution of oil and aqueous meal components and appetite. *Gastroenterology* **105**, 382–390.

Launer, L. J., Harris, T., Rumpel, C., and Madans J. (1994). Body mass index, weight change, and risk of mobility disability in middle-aged and older women: The epidemiologic follow-up study of NHANES I. *JAMA, J. Am. Med. Assoc*. **271**, 1093–1098.

Leibowitz, S. F. (1988). Brain neurotransmitters and eating behavior in the elderly. *Neurobiol. Aging* **9**, 20–22.

Leibowitz, S. F. (1991). Brain neuropeptide Y: An integrator of endocrine, metabolic and behavioral processes. *Brain Res. Bull*. **27**, 333–337.

Lipschitz, D. A. (1991). Malnutrition in the elderly. *Semin. Dermatol*. **10**, 273–281.

Louis-Sylvestre, J., Giachetti, I., and Le Magnen, J. (1984). Sensory versus dietary factors in cafeteria-induced overweight. *Physiol. Behav*. **32**, 901–905.

Ludwig, D. S., Majzoub, J. A., Al-Zahrani, A., Dallal, G. E., Blanco, I., and Roberts, S. B. (1999). High glycemic index foods, overeating, and obesity. *Pediatrics* **103**, E26.

MacIntosh, C. G., Andrews, J. M., Jones, K. L., Wishart, J. M., Morris, H. A., Jansen, J. B. M. J., Morley, J. E., Horowitz, M., and Chapman, I. M. (1999). Effects of age on concentrations of plasma cholesystokinin, glucagon-like peptide 1, and peptide YY and their relation to appetite and pyloric motility. *Am. J. Clin. Nutr*. **69**, 999–1006.

Markson, A. W. (1997). Functional, social, and psychological disability as causes of loss of weight and independence in older community-living people. *Clin. Geriatr. Med*. **13**, 639–652.

Martinez, M., Hernanz, A., Gomez-Cerezo, J., Pena, J. M., Vazquez, J. J., and Arnalich, F. (1993). Alterations in plasma and cerebrospinal fluid levels of neuropeptides in idiopathic senile anorexia. *Regul. Pept*. **49**, 109–117.

Mason, E. (1970). Obesity in pet dogs. *Vet. Rec*. **86**, 612–616.

Mayer, J. (1953). Glucostatic mechanism of regulation of food intake. *N. Engl. J. Med*. **249**, 13–16.

McCrory, M. A., Fuss, P. J., Hays, N. P., Vinken, A. G., Greenberg, A. S., and Roberts, S. B. (1999a). Overeating in America: Association between restaurant food consumption and body fatness in healthy adult men and women aged 19 to 80. *Obes. Res*. **7**, 564–571.

McCrory, M. A., Fuss, P. J., McCallum, J. E., Yao, M., Vinken, A. G., Hays, N. P., and Roberts, S. B. (1999b). Dietary variety within food groups: Association with energy intake and body fatness in adult men and women. *Am. J. Clin. Nutr*. **69**, 440–447.

McCrory, M. A., Fuss, P. J., Saltzman, E., and Roberts, S. B. (2000). Dietary determinants of energy intake and weight regulation in healthy adults. *J. Nutr.* **130**, 276S–279S.

Melanson, K. J., Greenberg, A. S., Ludwig, D. S., Saltzman, E., Dallal, G. E., and Roberts S. B. (1998). Blood glucose and hormonal responses to small and large meals in healthy young and older women. *J. Gerontol. A* **53A**, B299–B305.

Melanson, K. J., Westerterp-Plantenga, M. S., Saris, W. H., Smith, F. J., and Campfield, L. A. (1999). Blood glucose patterns and appetite in time-blinded humans: Carbohydrate versus fat. *Am. J. Physiol* **277**, R337–R345.

Miller, D. K., Morley, J. E., Rubenstein, L. Z., Pietruszka, F. M., and Strome, L. S. (1990). Formal geriatric assessment instruments and the care of older general medical outpatients. *J. Am. Geriatr. Soc*. **38**, 645–651.

Moller, N., O'Brien, P., and Nair, K. S. (1998). Disruption of the relationship between fat content and leptin levels with aging in humans. *J. Clin. Endocrinol. Metab.* **83**, 931–934.

Moore, J. G., Tweedy, C., Christian, P. E., and Datz, F. L. (1983). Effect of age on gastric emptying of liquid-solid meals in man. *Dig. Dis. Sci*. **28**, 340–344.

Moriguti, J. C., Das, S. K., Saltzman, E., Corrales, A., McCrory, M. A., Greenberg, A. S., and Roberts, S. B. (2000). Effects of a 6-week hypocaloric diet on changes in body composition, hunger and subsequent weight regain in healthy young and older adults. *J. Gerontol. A* (in press).

Morley, J. E. (1980). The neuroendocrine control of appetite: The role of the endogenous opiates, cholecystokinin, TRH, gamma-amino-butyric-acid and the diazepam receptor. *Life Sci*. **27**, 355–368.

Morley, J. E. (1990). Anorexia in older patients: Its meaning and management. *Geriatrics* **45**, 59–66.

Morley, J. E. (1996). Anorexia in older persons: Epidemiology and optimal treatment. *Drugs Aging* **8**, 134–155.

Morley, J. E. (1997). Anorexia of aging: Physiologic and pathologic. *Am. J. Clin. Nutr.* **66**, 760–773.

Morley, J. E., and Levine, A. S. (1982). The role of the endogenous opiates as regulators of appetite. *Am. J. Clin. Nutr.* **35**, 757–761.

Morley, J. E. and Silver, A. J. (1988). Anorexia in the elderly. *Neurobiol. Aging* **9**, 9–16.

Morley, J. E., Levine, A. S., Gosnell, B. A., Mitchell, J. E., Krahn, D. D., and Nizielski, S. E. (1985). Peptides and feeding. *Peptides (N. Y.)* **6**, 181–192.

Mowe, M., Bohmer, T., and Kindt, E. (1994). Reduced nutritional status in an elderly population (>70 y) is probable before disease and possibly contributes to the development of disease. *Am. J. Clin. Nutr.* **59**, 317–324.

Pamuk, E. R., Williamson, D. F., Madans, J., Serdula, M. K., Kleinman, J. C., and Byers, T. (1992). Weight loss and mortality in a national cohort of adults, 1971–1987. *Am. J. Epidemiol*. **136**, 686–697.

Pich, E. M., Messori, B., Zoli, M., Ferraguti, F., Marrama, P., Biagini, G., Fuxe, K., and Agnati, L. F. (1992). Feeding and drinking responses to neuropeptide Y injections in the paraventricular hypothalamic nucleus of aged rats. *Brain Res*. **575**, 265–271.

Prentice, A. M. (1992). Energy expenditure in the elderly. *Eur. J. Clin. Nutr.* **46**, S21–S28.

Richey, D. P., and Bender, A. D. (1977). Pharmacokinetic consequences of aging. *Annu. Rev. Pharmacol. Toxicol*. **17**, 49–65.

Roberts, S. B. (1995). Effects of aging on energy requirements and the control of food intake in men. *J. Gerontol.* **50**, 101–106.

Roberts, S. B. (2000a). Energy regulation and aging: Recent findings and their implications. *Nutr. Rev.* **58**, 91–97.

Roberts, S. B. (2000b). Regulation of energy intake in older adults: recent findings and implications. *J. Nutr. Health Aging* **4**, 1–2.

Roberts, S. B. (2000c). Regulation of energy intake in relation to metabolic state and nutritional status. *Eur. J. Clin. Nutr.* **54**, S64–S69.

Roberts, S. B. (2000d). A review of age-related changes in energy regulation and suggested mechanisms. *Mech Ageing Dev.* (in press).

Roberts, S. B., and Dallal, G. E. (1998). Effects of age on energy balance. *Am. J. Clin. Nutr.* **68**, 975S–979S.

Roberts, S. B., Fuss, P., Heyman, M. B., Evans, W. J., Tsay, R., Rasmussen, H., Fiatarone, M., Cortiella, J., Dallal, G. E., and Young, V. R. (1994). Control of food intake in older men. *JAMA J. Am. Med. Assoc*. **272**, 1601–1606.

Roberts, S. B., Fuss, P., Heyman, M. B., and Young V. R. (1995). Influence of age on energy requirements. *Am. J. Clin. Nutr.* **62**, 1053S–1058S.

Roberts, S. B., Nicholson, M., Staten, M., Dallal, G. E., Sawaya, A. L., Heyman, M. B., Fuss, P., and Greenberg, A. S. (1997). Relationship between circulating leptin and energy expenditure in adult men and women aged 18 to 81 years. *Obes. Res*. **5**, 459–463.

Rolls, B. J. (1985). Experimental analyses of the effects of variety in a meal on human feeding. *Am. J. Clin. Nutr.* **42**, 932–939.

Rolls, B. J., and McDermott, T. M. (1991). Effects of age on sensory-specific satiety. *Am. J. Clin. Nutr.* **54**, 988–996.

Rolls, B. J., van Duijvenvoorde, P. M., and Rowe, E. A. (1983). Variety in the diet enhances intake in a meal and contributes to the development of obesity in the rat. *Physiol. Behav.* **31**, 21–27.

Rolls, B. J., Dimeo, K. A., and Shide, D. J. (1995). Age-related impairments in the regulation of food intake. *Am. J. Clin. Nutr.* **62**, 923–931.

Rudman, D., Arora, V. D., Feller, A. G., Nagraj, H. S., Lalitha, P. Y., and Cainidec, N. P. (1990). Epidemiology of malnutrition in nursing homes. *In* "Geriatric Nutrition: A Comprehensive Review" (J. E. Morley, Z. Glick, and Rubenstein, L. Z., eds.), pp. 325–332. Raven Press, New York.

Ryan, A. S., Nicklas, B. J., and Elahi, D. (1996). A cross-sectional study on body composition and energy expenditure in women athletes during aging. *Am. J. Physiol*. **271**, E916–E921.

Saltzman, E., and Roberts, S. B. (1996). Effects of energy imbalance on energy expenditure and respiratory quotient in young and older men: A summary of data from two metabolic studies. *Aging (Milano)* **8**, 370–378.

Sanger, D. J. (1981). Endorphinergic mechanisms in the control of food and water intake. *Appetite* **2**, 193–208.

Sawaya, A. L., Fuss, P. J., Dallal, G. E., McCrory, M. A., Young, V., and Roberts, S. B. (2000). Meal palatability, substrate oxidation and blood glucose in young and older men. Submitted for publication.

Scarlett, J. M., Donoghue, S., Saidla, J., and Wills, J. (1994). Overweight cats: Prevalence and risk factors. *Int. J. Obes. Relat. Metab. Disord.* **18**, S22–S28.

Schiffman, S. S. (1977). Food recognition by the elderly. *J. Gerontol.*

Schiffman, S. S. (1994). Changes in taste and smell: Drug interactions and food preferences. *Nutr. Rev.* **52**, S11–S14.

Schiffman, S. S. (1997). Taste and smell losses in normal aging and disease. *JAMA, J. Am. Med. Assoc*. **278**, 1357–1362.

Schiffman, S. S., and Gatlin, C. A. (1993). Clinical physiology of taste and smell. *Annu. Rev. Nutr.* **13**, 405–436.

Schiffman, S. S., and Pasternak, M. (1979). Decreased discrimination of food odors in the elderly. *J. Gerontol*. **34**, 73–79.

Schiffman, S. S., and Warwick, Z. S. (1989). Use of flavor-amplified foods to improve nutritional status in elderly persons. *Ann. N. Y. Acad. Sci*. **561**, 267–276.

Schiffman, S. S., and Warwick, Z. S. (1993). Effect of flavor enhancement of foods for the elderly on nutritional status: Food intake, biochemical indices, and anthropometric measures. *Physiol. Behav.* **53**, 395–402.

Schiffman, S. S., Moss, J., and Erickson, R. P. (1976). Thresholds of food odors in the elderly. *Exp. Aging Res*. **2**, 389–398.

Schoeller, D. A., and Fjeld, C. R. (1991). Human energy metabolism: What have we learned from the doubly labeled water method? *Annu. Rev. Nutr.* **11**, 355–373.

Schulman, J. L., Carleton, J. L., Whitney, G., and Whitehorn, J. C. (1957). Effect of glucagon on food intake and body weight in man. *J. Appl. Physiol*. **11**, 419–421.

Sepple, C. P., and Read, N. W. (1989). Gastrointestinal correlates of the development of hunger in man. *Appetite* **13**, 183–191.

Shafer, R. B., Levine, A. S., Marlette, J. M., and Morley, J. E. (1987). Effects of xylitol on gastric emptying and food intake. *Am. J. Clin. Nutr.* **45**, 744–747.

Shimokata, H., Tobin, J. D., Muller, D. C., Elahi, D., Coon, P. J., and Andres, R. (1989). Studies in the distribution of body fat: I. Effects of age, sex, and obesity. *J. Gerontol*. **44**, M66–M73.

Silver, A. J., Flood, J. F., and Morley, J. E. (1988). Effect of gastrointestinal peptides on ingestion in old and young mice. *Peptides* **9**, 221–225.

Smith, G. P., and Gibbs, J. (1988). The satiating effects of cholecystokinin. *Curr. Concepts Nutr.* **16**, 35–40.

Steen, B. (1988). Body composition and aging. *Nutr. Rev.* **46**, 45–51.

Steen, B., Isaksson, B., and Svanborg, A. (1979). Body composition at 70 and 75 years of age: A longitudinal population study. *J. Clin. Exp. Gerontol*. **1**, 185–192.

Stunkard, A. J., van Itallie, T. B., and Reis, B. B. (1955). The mechanism of satiety: Effect of glucagon on gastric hunger contractions in man. *Proc. Soc. Exp. Biol Med*. **89**, 258–261.

Tayback, M., Kumanyika, S., and Chee, E. (1990). Body weight as a risk factor in the elderly. *Arch. Intern. Med*. **150**, 1065–1072.

U.S. Department of Health and Human Services (USDHHS). (1983). ''Dietary Intake Source Data: United States, 1976–1980.'' U.S. Government Printing Office, Washington, DC.

U.S. Department of Health and Human Services (USDHHS). (1990). ''Health, United States.'' U.S. Department of Health and Human Services. Washington, DC.

van Liere, E. J., and Northup, D. W. (1941). The emptying time of the stomach of old people. *Am. J. Physiol*. **134**, 719–722.

Wegener, M., Borsch, G., Schaffstein, J., Luth, I., Rickels, R., and Ricken, D. (1988). Effect of aging on the gastro-intestinal transit of a lactulose-supplemented mixed solid-liquid meal in humans. *Digestion* **39**, 40–46.

Weiffenbach, J. M., Baum, B. J., and Burghauser, R. (1982). Taste thresholds: Quality specific variation with human aging. *J. Gerontol*. **37**, 372–377.

Williamson, D. F. (1993). National Institutes of Health Technology Assessment Conference: Descriptive epidemiology of body weight and weight change in U.S. adults. *Ann. Intern. Med*. **119**, 646–649.

Wolden-Hanson, T., Marck, B. T., Smith, L., and Matsumoto, A. M. (1999). Cross-sectional and longitudinal analysis of age-associated changes in body composition of male brown Norway rats: Association of serum leptin levels with peripheral adiposity. *J. Gerontol.* **54**, B99–B107.

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature (London)* **372**, 425–432.

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59

Thermoregulation during Aging

Old mammals exhibit alterations in their thermoregulatory system that compromise their ability to maintain homeothermy when encountering hot or cold environments. Despite the difficulties in obtaining consistent data on humans, there are now numerous examples of age-related changes in both behavioral and physiological responses, the latter involving heat production as well as heat conservation/dissipation. Studies on old laboratory rodents provide further evidence for blunted thermoregulatory effectiveness with age, for gender differences in cold tolerance, for individual variation (even in in bred strains), and for the concept that the mechanisms underlying age-related changes in thermal responses do not occur in a linear manner. This chapter discusses studies evaluating the effects of age on specific thermal responses/effectors in humans as well as studies on laboratory rodents where mechanistic questions have been addressed.

I. Introduction

The maintenance of homeothermy depends on the balance between heat loss/heat conservation and metabolic heat production (Fig. 59.1). Behavioral strategies are a first line of defense in maintaining core temperature. In humans this includes adding or removing clothing or seeking alternate thermal environments. In other mammals, behavioral responses to cold exposure include group huddling, tucking of tail and paws, and seeking warmer shelter where possible; responses to warm include increasing surface area by adopting a more prone position, spreading saliva for evaporative cooling, and seeking cooler temperatures. Physiological mechanisms are elicited as a second line of defense when behavioral approaches are insufficient to maintain appropriate core temperature. Mammals, in general, respond to temperatures outside their thermal neutral zone with three types of physiological adjustments: (i) peripheral vasoconstriction to minimize heat loss (cold exposure) or vasodilation, panting, and sweating to increase heat loss (heat exposure); (ii) shivering thermogenesis in skeletal muscle (cold exposure), and (iii) nonshivering thermogenesis in sites such as brown adipose tissue (cold exposure).

There is substantial evidence indicating that the ability to maintain homeothermy in the face of either cold or warm temperatures is blunted in older mammals. However, the mechanisms that underlie this attenuation are not well defined. This chapter reviews the evidence for age-related changes in the thermoregulatory responses of older humans to heat and to cold. In addition, because most of the mechanistic studies dealing with cold-induced responses have utilized rodents as model systems, it also includes a discussion of the effects of age on thermoregulatory responses to cold in laboratory rats and mice.

II. Thermoregulation in Elderly Humans

A. Heat-Induced Thermal Responses in the Elderly

1. Overview

Although there are numerous reports that heat-induced failure of thermal homeostasis in old individuals increases morbidity and mortality to a greater extent than it does in younger adult populations (Levine, 1969; Lye and Kamal, 1977; Mirchandani *et al*., 1996), the degree to which aging itself contributes to this dysfunction is difficult to quantify because uncontrolled external factors, including socioeconomic status, disease, physical fitness, and lack of prior heat acclimatization, can confound the results. For example, hyperthermia-related deaths of aged individuals reported in Philadelphia during 1993 more closely reflected preexisting natural diseases in persons who lived alone without air conditioning in upstairs bedrooms with windows shut than it did age per se (Mirchandani *et al*., 1996). Imprecise measurement and definition of core temperature, hydration state of the individual, and a reliance on cross-sectional analysis (i.e., measurement of variables in different groups of individuals at two or more ages) rather than longitudinal investigations (repeated measurements of variables on the same individuals as they age) also introduce significant confounding variables. Nonetheless, taken as a whole, several studies have provided considerable insight into the effects of chronological aging on specific physiological variables (e.g., core temperature, sweat rate, skin blood flow) and on cardiovascular adjustments that are critical to the maintenance of heat tolerance in the elderly.

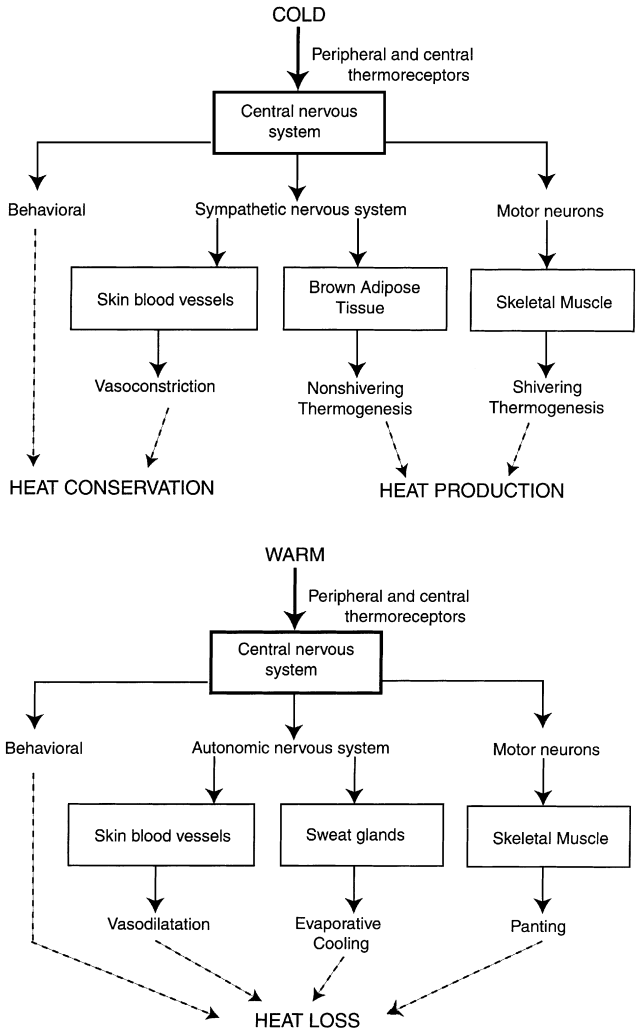

FIG. 59.1. Diagram of the central and peripheral components of the thermoregulatory system that are regulated when mammals are exposed to cold or to warm temperatures.

2. Aging, Heat Tolerance, and Sweat Gland Function

Early investigations comparing young (16–30 years of age) to older (greater than 65 years of age) individuals reported significantly higher aural and tympanic temperatures at the onset of sweating in older compared to younger age cohorts (Fennell and Moore, 1973; Crowe and Moore, 1974). However, there is no indication in these reports that young and old subjects were matched for heat acclimation, body composition, or physical fitness. Yousef *et al*. (1984) noted that younger and older men and women (ages 17–88) that were matched for level of heat acclimation had similar rectal and skin temperatures, heart rate, and total sweat loss after a 1 hr desert walk (40–44°C) at a work intensity of 40% VO_{2max}. These investigators concluded that heat acclimation influences the ability for heat tolerance in the elderly much more so than does age. This conclusion is consistent with several other investigations that have observed similar responses to heat exposure in younger and older subjects (Kenney, 1997). That is, when younger and older individuals were matched for fitness levels, body composition, prior acclimation, and other morphological factors, fewer differences in their response to passive heat exposure were observed.

Sweat rates of older men and women during passive heat exposure (i.e., during exposure to warm temperatures) are significantly less than those of younger individuals (Fennell and Moore, 1973; Crowe and Moore, 1974; Shoenfeld *et al*., 1978; Inoue *et al*., 1995). Age-related declines in sweat rate can reflect several anatomical and physiological factors but have been evaluated primarily from the perspective of sweat gland density and/or sweat rate per gland. Most investigations that have used localized injections of methylcholine, a cholinergic analog, to induce sweating in older men and women, have found that age-related reductions in sweat rate reflect lower output per gland rather than decreased sweat gland density. For example, in cross-sectional analysis comparing three age groups, 22–24, 33–40, and 58–67 years of age, methylcholine injection (10 μM) into the thighs of men produced the greatest localized sweat rate (65 nl/sweat gland/2 min) in the youngest group and the least in the oldest (22 nl/sweat gland/2 min); the sweat rate (41 nl/sweat gland/2 min) of the middle aged group, 33–40 years, was intermediate to the youngest and oldest groups (Kenney and Fowler, 1988). Sweat gland density was not affected by age in this investigation. Results from other studies evaluating sweat gland density and sweat rate as a function of age are consistent with these results (Yousef *et al*., 1984; Tankersley *et al*., 1991; Ferrer *et al*., 1995). However, Inoue (1996) reported significant variation in both density and rate that was dependent on the anatomical site sampled. Altered heat-activated sweat rates and sweat gland densities at five different anatomical locations were determined longitudinally in men who were 65–75 years of age at the first test period. Five years later, these men were retested. Although they had no changes in physical characteristics such as percentage body fat or VO_{2max}, their total body sweat output was significantly less. This decrease reflected significantly lower sweat rates per gland on sites sampled on the back, but no such differences on the thighs. In contrast, sweat gland density did not differ with age on the back, but was significantly less on the thigh. Thus, depending on the site, both density and output/gland appears to be blunted with age.

3. Thermoregulatory Cardiovascular Responses to Heat in the Elderly

The dissipation of heat during active (exercise-induced) or passive (non-exertional-induced) warming is accomplished primarily through an increase in the amount of cardiac output to the skin plus the degree of vasodilatation. Age-related reductions in either variable could result in insufficient heat dissipation and an inappropriate rise in core temperature (hyperthermia). There is general consensus that skin blood flow during passive and active heat stress is significantly less in the elderly compared to younger subjects matched for body weight and VO_{2max} (Kenney *et al*., 1990, 1991, 1997; Armstrong and Kenney, 1993; Kenney and Zappe, 1994; Kenney and Ho, 1995; Kenney and Armstrong, 1996). In a series of investigations performed by Kenney and colleagues, skin blood flow was evaluated in men and women (ages 50–75 and 20–30 years) during exercise in a hot environment. In each of the investigations, the subjects were matched for

VO_{2max}. Although there were differences in the protocols for each experiment, the results showed that the increase in skin blood flow at a given core temperature was less in the older than in the younger subjects (see review by Kenney, 1997). A subsequent investigation further suggested that this blunted heat-induced increase in skin blood flow in the elderly reflected altered cutaneous vasodilatation (Martin *et al*., 1995). In this study, forearm blood flow was measured by venous occlusion plethysmography and by laser Doppler flowmetry in subjects ranging in age from 5 to 85 years. A continual spray of fine water heated to 42°C elevated skin temperature, and resistance to flow was calculated as maximal forearm blood flow/mean arterial pressure. This resistance was significantly greater in the older individuals, resulting in a significant reduction in maximal vasodilatation (Martin *et al*., 1995).

The mechanisms underlying the blunted heat-induced vasodilatation remain to be elucidated. One hypothesis is that increased sympathetic nervous system activity, a finding often reported to occur in aged humans, may enhance vasoconstrictor tone, making a given thermal signal less effective in stimulating dilation. Although this hypothesis is attractive, direct testing of the hypothesis has not been supportive. That is, skin blood flow of younger and older individuals during 1 hr of moderate cycle exercise at 36°C increased similarly before and after administration of prazosin, an α_1-adrenergic antagonist which should inhibit sympathetic-induced vasoconstriction (Kenney *et al*., 1991). Along similar lines, bretylium tosylate, an agent that blocks release of norepinephrine from sympathetic nerve terminals, did not prevent the attenuated increase in skin blood flow in older (66 years) vs younger (22 years) male subjects (Martin *et al*., 1995) exercising at 50% VO_{2max} in a 36°C environment. Based on the results of these two investigations, Kenney and his colleagues have suggested that the age-related decrease in heat-induced vascular conductance reflects alterations in the active vasodilator (rather than constrictor) system. That this reduction in vasodilatation suggested by the skin blood flow studies involves age-related alterations in the structure of the vasculature is supported by reports of age-related increases in vascular wall stiffness (Lakatta, 1995). Such increases would decrease the distensibility of the vessels which, in turn, would result in enhanced resistance to dilation. While the mechanisms underlying increased vessel wall stiffness are unknown, decreased elastin and increased concentrations of collagen cross-links are common findings in vessels collected from aging humans.

Another potential contributor to the age-related attenuation of heat-induced increases in skin blood flow is decreased cardiac output. Here, the data are inconsistent. Sawka (1988) reported that healthy unacclimated elderly men (61–73 years) had cardiac outputs, heart rates, and total sweat outputs during whole body passive heat at 40°C and 40% relative humidity that were comparable to those of younger adult males (21–39 years). Similar results (i.e., no difference in cardiac output or heart rate) were seen in older and younger men and women (17–88 years) who walked for 1 hr in desert heat of 40–44°C (Yousef *et al*., 1984). In contrast, Kenney *et al*. (1997) reported that older (61–73 years) vs younger (21–33 years) men had significantly lower cardiac output and heart rate at all work intensities tested (up to about 60% VO_{2max}) during incremental cycle exercise in the heat (36°C). While stroke volume was higher in the older men, it was not enough to increase cardiac output to the same level as in younger individuals.

Attenuated heat-induced redistribution of cardiac output from splanchnic and renal beds to the skin has also been evaluated as a potential contributor to reduced heat loss in the elderly. Renal blood flow and renal vascular conductance were estimated during exercise (50% VO_{2max}) in a warm environment (30°C) in fit older and younger men matched for body size and VO_{2max} (Kenney and Zappe, 1994). The decrease in renal blood flow induced by heat/exercise was significantly greater in the young men (down 45%) compared to that in the older individuals (down 12%). Decreased renal vascular conductance was also significantly greater in the young (-52%) than in the old (-15%) men. Subsequent work by Kenney *et al*. (1997) has demonstrated that a similar age-related attenuated redistribution of flow occurs in the splanchnic vascular bed.

The extensive studies by Kenney and colleagues support the hypothesis that the blunted heat/exercise-induced skin blood flow of older vs younger men reflects reduced active vasodilatation within the skin and attenuated redistribution of blood flow from renal and splanchnic vascular beds. However, the inclusion of exercise as a modality to increase core temperature could be a confounding variable. That is, the age-related effects on the physiological adjustments to acute and chronic exercise, independent of ambient temperature, are not well understood, raising the concern that the outcomes reported by Kenney and colleagues reflect responses to exercise per se rather than simply alterations in heat dissipation mechanisms. To deal with these concerns, these investigators evaluated the central and peripheral hemodynamic responses of young (19–28 years) and old (64–81 years) men during direct passive heating (Minson *et al*., 1998). Cardiac output in young individuals was significantly greater than that observed in old men at their limit of thermal tolerance (11.1 ± 0.7 and 7.4 ± 0.2 liters/min for young vs old men, respectively; the limit of thermal tolerance was defined as the time at which the subject was unable to continue, esophageal temperature reached 39.5°C, or the subject was unable to control hyperventilation). Furthermore, the older men redistributed less blood flow from the combined splanchnic and renal beds at the limit of thermal tolerance (960 ± 80 vs 720 ± 100 ml/min in young and old subjects, respectively). These data indicate that less blood flow is indeed directed to the skin during non-exertional heating in older vs younger individuals.

4. Summary

Clinical evidence suggests that elderly individuals are at greater risk for the development of hyperthermia than are younger cohorts. Several investigations have shown that in many cases, hyperthermia and the associated increased mortality rate observed in the elderly are more closely correlated with lower socioeconomic status, preexisting disease, low physical fitness, and lack of prior heat acclimatization than with aging per se. Nonetheless, experimental evidence has described specific age-related alterations in thermal homeostatic mechanisms. Localized sweat rates (nl/min/sweat gland) appear to decline with age, but the changes are not uniform at all anato-

mical sites, with some sites displaying no age-related changes. The data describing age-related cardiovascular adjustments to heat exposure are inconsistent. Several investigations using older and younger individuals matched for body composition and fitness levels find reduced heat-induced blood flow to skin, but no reduction in cardiac output, heart rate, or oxygen consumption. However, a series of investigations by Kenney and colleagues suggest that the reduction in heat-induced blood flow to skin of older vs younger individuals reflects an attenuated increase in cardiac output, significantly less redistribution of blood to skin from the renal and splanchnic vascular beds, and resistance to dilation of the skin vasculature.

B. Cold-Induced Thermal Responses in the Elderly

1. Overview

Older humans (as well as laboratory rodents) have been reported to regulate core temperature more poorly than younger adults in cold environments. This impairment may account for the higher than normal mortality rate among elderly humans during winter months (e.g., Collins, 1987; Macey and Schneider, 1993) and for the decreased survival of older laboratory rodents maintained in cold environments for days (e.g., Grad and Kral, 1957; Trujillo *et al*., 1962; Kiang-Ulrich and Horvath, 1979, 1985a; Owen *et al*., 1991). However, unlike data obtained on laboratory rodents, seasonal survivability statistics for old humans may be confounded by many of the same factors that influence heat tolerance in the elderly (i.e., socioeconomic status, disease, physical fitness, body composition, and lack of prior temperature (in this case cold) acclimatization). Nonetheless, as was the case with thermal tolerance to heat, several lines of investigation in humans suggest that the elderly have lower thermogenic capacity, changes in sensory perception, and altered vascular characteristics that could influence heat conservation. Examples of each of these changes are discussed briefly below, followed by an extensive consideration of information regarding underlying mechanisms derived from studies of cold-exposed laboratory rodents. [For a more detailed discussion of some of the difficulties in interpreting the data from humans studies, see the review by Young (1991).]

2. Aging, Thermogenesis, and Thermal Perception

As depicted in Fig. 59.1, thermoregulatory heat production can occur via shivering and nonshivering mechanisms. The major site for the latter is brown adipose tissue, the amount of which is negligible in older humans (Heaton, 1972; Ito *et al*., 1991). Thus, shivering in skeletal muscle is the major contributor to cold-induced thermogenesis in the elderly, and there are several studies which indicate that the magnitude of shivering (cold-induced thermogenesis) is less in old vs young individuals (e.g., Horvath *et al*., 1955; Wagner *et al*., 1974). In addition, although the elderly were shown to maintain the ability to shiver, their bursts of muscle contraction did not peak as high as in the young (Collins *et al*., 1981a; Collins, 1987). This resulted in lower shivering intensity. Collins and his colleagues (1981a; Collins, 1987) also noted that there was a long latent period before maximal shivering occurred in the older cold-exposed subjects. This was in contrast to the rapid onset of maximal shivering in younger individuals. Hence, the characteristics of shivering may change with age. Moreover, since many elderly individuals have lost significant skeletal muscle mass and motor units, their capacity to generate heat is diminished. It should be noted, however, that not all cross-sectional studies have shown significant differences in cold-induced heat production between old and young subjects (e.g., Wagner and Horvath, 1985a), emphasizing the difficulty in drawing general conclusions from selected populations that may not be well matched in terms of variables other than age.

It has also been reported that the ability to discern cold is compromised in many of the elderly. In a behavioral experiment, Collins *et al*. (1981b) measured the preciseness, over a 2.5 hr period, with which comparably dressed male subjects manually controlled the temperature of the room in which they were seated (initial temperature was 19°C). The mean preferred temperatures of these 13 young (18–39 years) and 17 old subjects (70 years and above) did not differ significantly ($23 \pm 2.3°$ vs 22.7 ± 1.2°C, respectively). However, on average, the older subjects showed poorer control of the room temperature than did the young males as indicated by greater peak temperatures and a greater range of temperatures. The young subjects initially allowed the temperature to vary by 3°C but by the end of the experimental period, this variation averaged near 1°C. In contrast, the old subjects increased the amplitude of oscillations from about 3°C initially to 4.5°C by the end of the experimental period (Collins and Exton-Smith, 1986). When hand-skin temperature discrimination was measured in these subjects, it was found that all of the young males could discriminate temperature differences of about 1°C, 12 of the elderly could discriminate temperatures of 2°C or less, but 5 of the elderly men were unable to discriminate differences greater than 2°C. Although no differences were observed between the latter 5 and the 12 other old subjects with respect to preferred temperature or comfort rating, the 5 poor temperature discriminators had the lowest frequency of temperature change/h as well as the highest temperature range (Collins and Exton-Smith, 1986). These data are consistent with the earlier work of Watts (1972) who reported that some (but not all) elderly women (74–86 years old) experienced the sensation of cold only at considerably low ambient temperatures. Thus, it appears that at least some old people perceive ambient temperature changes less accurately than do the young, contributing to their greater vulnerability.

3. Thermoregulatory Cardiovascular Responses to Cold

Heat conserving mechanisms invoked during cold exposure include peripheral vasoconstriction and decreased skin blood flow to the skin. Several investigators have reported age-related alterations in these cold-induced changes. For example, in a longitudinal study of elderly males, Collins and colleagues found that the proportion of individuals who showed little or no vasoconstrictor response to acute cold ($\sim$15°C) increased with age. In their initial measurements, 37 of 43 old subjects (greater than 69 years of age) showed cold-induced decreases in hand blood flow (as compared to 39 of 41 subjects younger than 45 years old). Four years later, only 29 of the 43 old subjects exhibited a vasoconstrictor response and 8 years later, the

number was further reduced (Collins *et al*., 1977, as cited by Young, 1991). Whether this diminished responsiveness reflects changes in the sensitivity of the vasculature or changes in the neural regulation associated with vasoconstriction has not yet been determined.

4. Gender Differences

Epidemiological statistics indicate that the majority of cold-related deaths among the elderly are in men (e.g., Macey and Schneider, 1993). Other investigations have shown that during acute cold exposure, older females are able to maintain core temperature as well as younger females and do so better than older males (e.g., Wagner and Horvath, 1985a). The gender effect in humans may involve differences in body size and composition [i.e., a smaller body surface area relative to mass and more subcutaneous body fat in older females vs older males (Wagner and Horvath, 1985a,b)]. There may also be differences in the peripheral vasoconstrictor response to cold, as indicated by the moderately higher skin temperature of older males vs older females exposed to 10°C for 2 hr (Wagner and Horvath, 1985a) although gender differences in finger or forearm blood flow during acute cold exposure have not always been seen (e.g., Wagner and Horvath, 1985b). Differences in metabolic heat production may also play a role. For example, Wagner and Horvath (1985a) reported that older women have a larger early metabolic increase than do older males and younger females upon exposure to cold (2 hr at 10°C).

5. Summary

Although the sometimes contradictory results reported by various investigators render it difficult to make sweeping generalizations about the effects of age on thermoregulatory ability of cold-exposed humans, there are numerous examples of age-related alterations in both behavioral and physiological responses. The fact that within a given age cohort, there is often considerable variability in individual responses to thermal perturbations emphasizes the dichotomy between chronological age and ''functional'' or biological age and the need to be alert to individual differences as well as group responses. This is further illustrated by the age of onset of senescence in F344 rats (section IV).

III. Cold-Induced Thermoregulatory Responses in Laboratory Rodents

A. Overview

Numerous investigators have examined cold-induced responses in laboratory rodents. As is the case with humans, old mice and rats have a greater mortality rate when exposed to cold than do their younger counterparts. Age-related decrements in cold tolerance have been reported in female C57BL mice (Grad and Kral, 1957), female RF mice (Trujillo *et al*., 1962), male Sprague–Dawley rats (Kiang-Ulrich and Horvath, 1979), and male F344 rats (Kiang-Ulrich and Horvath, 1985a; Owen *et al*., 1991). While there are some strain differences with respect to the degree of cold tolerance in these animals, in all cases, heat production was insufficient to overcome heat loss and subsequent hypothermia.

B. Age-Related Changes in Thermoregulation in Rodents

Various strains of laboratory mice and rats have been used for aging studies of thermoregulation. The majority of these studies have been cross-sectional where age comparisons are made using different groups of animals usually at two or three different ages. Typically, they include an age group that is sexually mature and past the exponential growth phase (young adults) and one that approximates or exceeds the median survival age for the respective population (old). In contrast, longitudinal studies (e.g., Balmagiya and Rozovski, 1983; Reynolds *et al*., 1985; Talan *et al*., 1985) make repeated measurements on the same rats over a given age span.

A summary of laboratory studies (mainly cross-sectional) on changes in body temperature during acute cold exposure in mice and rats is presented in Table 59.1. Those demonstrating that aging negatively alters the ability to regulate core temperature far exceed those finding no aging effect. There are, however, several factors which influence these results—these include strain, gender, age, and severity of cold (exposure time and temperature). Other confounding factors are health status, body size and composition, feeding conditions (fed vs fasted), surgical procedures or anesthetics used, disturbance/handling of the animal during the cold test (e.g., blood collections, insertion of colonic temperature probe, etc.), and physical restraint.

1. Cold Tolerance in Mice

The mouse, with its high body surface area-to-mass ratio that favors rapid heat exchange with the environment (Dawson, 1967; Schmidt-Nielsen, 1984), is especially prone to body temperature imbalances when exposed to low ambient temperatures. Even moderate impairments in physiological and/or behavioral mechanisms that reduce heat loss or increase heat production can result in rapid drops in core temperature to levels that threaten survival. A significant number of aging studies on cold-induced thermoregulation in mice have used males from the C57BL/6J genotype of *Mus musculus*, a relatively short-lived rodent species that does not undergo torpor (an adaptive physiological phenomenon in which core temperature is lowered to less than 34°C for 2 hr or longer (Duffy *et al*., 1987) when there is a need for energy conservation (e.g., during food restriction)). The mean life span of the *ad libitum*-fed C57BL/6J mouse strain has been estimated to be 26–27 months (Goodrick, 1975).

In an early study by Finch *et al*. (1969), young (10-month-old) and old (30-month-old) male C57BL/6J mice were placed individually into 2-quart glass jars and exposed to 3 hr of cold (9–10°C). Rectal temperatures of all the old mice decreased in a steady and progressive manner while those for the young mice changed only slightly. In addition, there was a much higher degree of variability in temperature responses of old vs young mice (range of cold-induced hypothermia values of 3–13°C vs 0–1.5°C, respectively). Similarly, Hoffman-Goetz and Keir (1984) found that partially restrained old (19- to 20 month-old) male C57BL/6J mice had significantly lower rectal

TABLE 59.1 Comparison of Body Temperature Changes in Young and Old Mice and Rats Acutely Exposed to Cold

	Subjects				Exposure conditions		Temperature response to cold				
			(Age months)						ΔTc (°C)		
Study	Strain	Sex	Young	Old	°C	hr	ΔTc	ΔTsk	Young	Old	Comments
					A. Studies on mice						
Finch *et al*., 1969	C57BL/6J	M	10	30	9–10	3	↑	NA	0 to −1.5	−3 to −13	20 hr fast
German and Hoffman-Goetz, 1986	C57BL/6J	M	NA	18–20	15	3	NA	NA		−2.1 to −2.4	Fasted (?); tether-stock restraint; 70% relative humidity; nonexercised
										−0.94	Exercise trained
										−0.01	Intermittent cold acclimation
Talan and Engel, 1984	C57BL/6J	M	10	30	10	3	ø	NA	(−3)	(−3)	20 hr fast; nonrestraint
							↑		(−1)	(−11.5)	20 hr fast; restraint
							↑		(−1)	(−11.5)	Nonfasted; restraint
Talan *et al*., 1984	C57BL/6J	M	9.5	29.5	10	3	↑	NA			Restraint Test (Talan and Engel, 1984)
Talan *et al*., 1985	C57BL/6J	M	8	30	10	3	↑	NA	(−7.4)	(−11.5)	Restraint Test (Talan and Engel, 1984); first cold exposure test (Test 1)
			13				↑		(−8.5)		
			15				↓		(−14.8)		
			22				↑		(−10.4)		
			8	30	10	3	↑		(−3.8)*	(−11.0)	Third cold exposure test (Test 3); (*) significant improvement from Test 1
			13				↑		(−3.8)*		
			15				↑		(−9.9)*		
			22				↑		(−8.1)*		
Talan and Ingram, 1986a	A/J	M	6	25	10	3	↑↑↑	NA	(−7.5)	(−23.4)	Restraint Test (Talan and Engel, 1984); different strains of *Mus musculus*; results of first cold exposure test
	C57BL/6J						↑↑		(−9.5)	(−18.0)	
	$B6AF_1$/J						↑		(−7.1)	(−14.9)	
	Wild type						ø		(−2.6)	(−5.0)	
Tatelman and Talan, 1990a,b, 1993	C57BL/6J	M	9–14	29–31	6	3	↑	NA			Restraint Test (Talan and Engel, 1984)
					B. Studies on Rats						
Hügin and Verzár, 1957	?	M	3 to 31 ~3 months intervals		−4.5 to 7.5	1	↑	NA	0 to −1.2	−1.2 to −3.6	See text
Verzár, 1958	?	M	?	?	−11	1	↑	NA	−2 to −3	−8 to −10	
Jakubczak, 1966	Sprague–Dawley	M	7	28	2	3	ø	NA	(−9)	(−13)	Statistics indicated age had no significant effect on Tc in cold
			12				ø		(−11)		
Huang *et al*., 1980	Long–Evans	M	6–8	20–24	4	6	↑	NA	−1.5	−2.6	
Cox *et al*., 1981	Sprague–Dawley	M	2	12	4	2	ø	ø	(−1)	(−1)	
				18			↑	ø		(−3)	
				24			↑↑	ø		(−3)	
Algeri *et al*., 1982	Sprague–Dawley	M	4	29	4	2	↑	NA			
						4	↑				
						24	↑				
Balmagiya and Rozovski, 1983	Sprague–Dawley	M	3 to 24 (longitudinal)		18	1.5	↑	↑			Tc and Tsk are comparison of 3–9 months vs 24 months
Kiang-Ulrich and Horvath, 1985b	F344	M	3	24	−10	3	↑	NA	−6.7	−11.2	Non-cold-acclimated
			12				↑		−6.7		
			3	24	−10	3	↑	NA	−3.1	−6.7	Cold-acclimated
			12	24	−10	3	↑	NA	−3.1		

continues

TABLE 59.1 *Continued*

	Subjects				Exposure conditions		Temperature response to cold				
			(Age months)						ΔTc (°C)		
Study	Strain	Sex	Young	Old	°C	hr	ΔTc	ΔTsk	Young	Old	Comments
Martin *et al*., 1985	EMD:Wi-AF/Han	F	8–9	37–40	5	4	↑	NA	−0.06	−1.98	
	Iva:WIWU	F	8	33			↑		−0.20	−1.18	
Lee and Wang, 1985	Sprague–Dawley	M	6–9	23–26	−10	2	↑	NA	−3.6	−7.5	Cold exposed under HeO_2 (79% He–21% O_2); food rationed to maintain body weights at 400 g
Wang *et al*., 1992	Sprague–Dawley	M	3–6	26–30	−10	2	↑	NA	−6.1	−8.3	Cold exposed under HeO_2 (79% He–21% O_2); food rationed to maintain body weights at 400 g
Paré, 1989	F344	F	3	24	10.5	3	ø	NA	27%	29%	Test 1: % drop in Tc during restraint-cold stress (all four limbs tied to a plastic base board)
			11				ø		29%		
			3	24	10.5	3	↑	NA	12%	22%	Test 4: rats had three prior cold tests at 1-week intervals
			11	24	10.5	3	↑	NA	12%		
McDonald *et al*., 1987	Sprague–Dawley	F	5	26	6	6	↑	NA	−0.25	−0.7	
McDonald *et al*., 1988b	F344	M	12	24	6	6	↑	NA	(−2)	−5.1	Non-exercise-trained
							ø		(−0.5)	−1.1	Exercise trained
McDonald *et al*., 1989a	F344	M	5	23	6	6	↑	NA	−0.5	−1.8	Gender: 23 months: M>F
				27			↑			−2.9	Gender: 27 months: M>F
		F	5	23			ø		0.0	+0.2	
				27			↑			−0.7	
McDonald *et al*., 1989b	F344	M	12	24	6	6	↑	NA	(−2)	(−5)	
	Osborne–Mendel	M	12	24			ø		(0)	(0)	
McDonald *et al*., 1993	F344	M	6	26	6	1.5		NA	−1.1	−1.9	
			12						−1.1		
		F	6	26					−0.8	−1.7	
			12						−1.2		
McDonald *et al*., 1994	F344	M	6	26	6	2.75	↑	NA	−0.4	−2.5	Gender: 26 months: M>F
			12				↑		−0.2		
		F	6	26			↑		−0.3	−1.5	
			12				↑		−0.6		
Gabaldón *et al*., 1995	F344	M	6	26	6	4	↑	NA	+0.5	−1.2	
			12				↑		−0.3		
		F	6	26			ø		−0.1	−0.6	
			12				ø		−0.5		
Refinetti *et al*., 1990	Long–Evans	M	2	20–28	0	2	ø	NA	+0.43	−1.11	(A) old rats with strong circadian rhythm of body temperature; (B) old rats with poor circadian rhythm of body temperature
		F		(A)			ø		+0.29	−1.05	
		M	2	20–28			↑	NA	+0.43	−2.86	
		F		(B)			↑		+0.29	−2.12	
Scarpace *et al*., 1994	F344	M	3	24	4	1	↑	NA	+0.1	−1.4	

Note: Data are for first cold exposure and without any intervention protocol (e.g., prior cold exposure, exercise training, drugs) unless indicated otherwise. ΔTc, cold-induced change in core (rectal or colonic) temperature; ΔTsk, cold-induced change in skin temperature. For ΔTc and ΔTsk: ø, no difference between old and young animals; ↑, greater change in old animals; ↓, smaller change in old animals; NA, not available. Cold-induced ΔTc (°C) values in parenthesis were estimated from bar or line graph data; values not in parentheses are based on table/text data. Values shown for Talan and coworkers were calculated from temperature slopes (drop in Tc/min). However, mice in these studies were removed from the cold if core temperature fell below 24°C (thus, declines in Tc greater than ∼11°C did not actually occur).

temperatures (~2.2°C) than did young (6- to 7-month-old) mice after 3 h at 15°C. The restraint system consisted of a flexible polyethylene neck tether which allowed for lateral movement as well as behavioral thermoregulation. The less severe hypothermia developed by old mice in this study compared to that of old mice in the study by Finch *et al*. (1969) can be explained in part by the 10-month age difference of older mice as well as by the less severe cold exposure. Talan and Engel (1984) also noted a significant age-related decline in cold tolerance of male C57BL/6J mice when physical restraint was imposed (in order to minimize behavioral influences on body temperature responses) by placing the animals singly into small (30 mm internal diameter) Plexiglas tubes with perforations for ventilation. This restraint procedure prevented gross physical movement but did not inhibit shivering. The average rectal temperature of the restrained young (10-month-old) and old (30-month-old) mice exposed to 10°C for 3 hr decreased by approximately 3 and 11.5°C, respectively. In contrast, in unrestrained mice, the average loss of rectal temperature was similar in both age groups (~3°C), indicating a strong reliance of old mice on behavioral thermoregulation in maintaining homeothermy. Subsequent investigations by Talan and coworkers have confirmed a poorer cold tolerance of old vs young mice to this same restraint–cold stress test (Table 59.1).

2. Cold Tolerance in Rats

The larger size of the rat compared to the mouse makes it relatively more resistant to passive heat loss because of its lower ratio of body surface area to mass. However, the ability of rats to effectively cope with acute and chronic cold still deteriorates with advanced age. An early investigation by Hügin and Verzár (1957) involving 1 hr exposure to −0.5 to −14°C showed that with increasing age (from 3 to 31 months), groups of male rats exhibited significantly greater drops in rectal temperature. With exposure to colder temperatures, the age difference became even more pronounced (Verzár, 1958). As summarized in Table 59.1, subsequent studies have reported age-related alterations of cold-induced thermoregulation in male Long–Evans male rats, male and female Sprague–Dawley rats, female EMD:Wi-AF/Han rats, female Iva:WIWU rats, and male F344 rats. In contrast, the aging male Osborne–Mendel rat remains cold tolerant up to at least 24 months of age (McDonald *et al*., 1989b), and aging F344 females are more cold-resistant than are males at a comparable age (McDonald *et al*., 1989a).

The fact that different strains of mice and rats often exhibit different degrees of age-related decline in cold-induced thermoregulatory abilities (Kiang-Ulrich and Horvath, 1984a, 1985b; Martin *et al*., 1985; Talan and Ingram, 1986a; McDonald *et al*., 1989b) may be correlated with strain-specific life span, as suggested by Talan and Ingram (1986a), who observed greater age-related loss of cold tolerance in relatively short-lived A/J (mean life span of 22 months) vs longer-lived B6/AF_1/J (mean life span of 29 months) males of an inbred strain of *M. musculus*. Cold-induced thermoregulation may also be influenced by the level of domestication, as indicated by the far better cold tolerance of old mice of a pen-bred strain of *M. musculus* captured from the wild than that seen in old mice of the domesticated strains A/J, C57BL/6J, and B6/AF_1/J (Talan and Ingram, 1986a). In some cases, there are also strain differences in cold-induced thermoregulatory abilities of younger animals. For example, Kiang-Ulrich and Horvath (1984a) reported that 3- to 4-month-old male F344 rats better maintained core temperature during exposure to −10°C for 3 hr than did male Sprague–Dawley rats of the same age; in fact, even non-cold-acclimated older (24-month-old) male F344 rats exhibited greater tolerance to cold than did cold-acclimated young Sprague–Dawley rats (Kiang-Ulrich and Horvath, 1985b). The strain effect in younger rats reflected a blunted cold-induced increase in oxygen consumption in Sprague–Dawley vs F344, indicating that differences in heat production were partly responsible (Kiang-Ulrich and Horvath, 1984a). In other cases, strain differences in cold tolerance do not appear until later age. For example, at 12 months of age, both Osborne-Mendel and F344 male rats maintained colonic temperature during exposure to cold (6 hr at 6°C); at 24 months of age, however, only the Osborne-Mendel rats remained cold-tolerant, whereas the F344 rats developed a hypothermia of ~5°C (McDonald *et al*., 1989b). This hypothermia occurred in spite of thermogenesis equivalent to that of older Osborne-Mendel rats, indicating that the older F344 rats had experienced greater heat loss and had a poorer ability to compensate for it metabolically. The primary cause of heat loss was unclear, but did not appear to be due simply to differences in body size and composition. The F344 rat is much smaller in size than the Osborne-Mendel rat (carcass mass of 311.8 gas in grams vs 460.7 g at 24 months), which suggests a greater body surface area-to-mass ratio and thus an increased potential for passive heat loss. However, the higher percentage carcass fat of F344 vs Osborne-Mendel rats (20.6% vs 15.1% at 24 months) implies a greater relative tissue insulation capacity to offset some of the body size effect. It thus seems that the peripheral vasoconstrictor response to cold, which leads to augmented thermal resistance of the body shell (Kenney and Buskirk, 1995), is attenuated in older F344 vs Osborne-Mendel rats. However, the involvement of other heat conservation mechanisms cannot be excluded. The percentage lean body mass and brown adipose tissue thermogenic capacity also differed with strain—both were significantly higher in Osborne-Mendel vs F344 rats (young and old) (McDonald *et al*., 1989b). These data suggest that Osborne-Mendel rats have a greater capacity for shivering and nonshivering thermogenesis than do F344 rats. The finding that cold-induced oxygen consumption did not differ between the two strains could reflect a greater reliance on shivering in the F344 than in the Osborne-Mendel rats (thermogenesis from brown fat is more effective than is shivering in warming the core of the animal because a greater proportion of the heat generated by shivering is lost to the environment due in part to increased blood flow to peripheral muscles and in part to the generation of heat closer to the animal's surface). It could also indicate that the two strains used their thermogenic capacities to different degrees—submaximally in the Osborne-Mendel rats and maximally (or closer to maximal) in the F344 rats (McDonald *et al*., 1989b).

3. Nonlinearity of the Age-Related Decrement in Thermoregulation

Numerous observations suggest that thermoregulation does not decline at a single linear rate over the entire life span of the rodent. One of the earliest is that by Hügin and Verzár

(1957) who studied groups of male rats between ages 3 and 26 months, with measurements made at ~3-month age intervals. They found a gradual loss of cold-induced thermoregulation between ages 3 and 24 months as reflected in the observation that the average hypothermia of the 24 month-old rats was only about 1°C greater than that of the 3-month-old rats. This was followed by a more rapid loss of regulation between ages 24 to 26 months (development of an approximately 2°C greater cold-induced hypothermia). The physiological basis for this acceleration in the rate of loss of function in later life was unclear. We too have found that older rats separated in age by only a few months can exhibit marked differences in their ability to cope with cold. For example, after 6 hr at 6°C, the drop in core temperature in very old (27-month-old) vs old (23-month-old) F344 rats was 2.9°C vs 1.8°C in males and 0.7°C vs 0°C in females (McDonald *et al*., 1989a). Cold-exposed 5-month-old rats of both genders maintained a constant core temperature. These data support the idea of a time-independent component of thermoregulatory decline that is expressed in later life. This was most evident in F344 females, who exhibited no deficit in thermoregulation until some time between ages 23 and 27 months. This study also demonstrated the influence of gender on thermoregulatory loss—that is, while males developed considerable hypothermia by 23 months of age, females at this age still maintained homeothermy. The rate of cold-induced mass-independent oxygen consumption did not differ significantly between the 23- and the 27-month-old rats (McDonald *et al*., 1989a), suggesting that the blunted ability of very old vs old rats to maintain core temperature in response to cold was due to less effective heat conservation rather than attenuated heat production. Although carcass mass was about 10–13% less in the 27-month-old vs 23-month-old males and females (a difference that did not reach statistical significance), the percentage values for the body composition variables (fat, lean body mass, etc.) remained comparable. Thus, rats in both age groups appear to have had the same relative tissue insulation capacity for minimizing heat loss, implicating other heat conservation mechanisms (peripheral vasoconstriction, behavioral adjustments, fur coat). Although increased heat loss rather than reduced heat production seems to have been the direct cause of the greater hypothermia in the 27-month-old vs 23-month-old rats, the fact that this greater heat loss was not compensated for in the very old rats indicates that they also had problems inducing further increases in thermogenesis.

This nonlinearity in the rate of loss of thermoregulatory ability is also reflected in survival rates during long-term cold exposure. For example, Kiang-Ulrich and Horvath (1985a) reported that only 46% of 25-month-old male F344 rats survived 3 weeks of exposure to 5°C compared to 100% survival of 3-, 12-, and 21-month-old rats maintained for 7 to 9 weeks. The 25-month-old rats (survivors and nonsurvivors) lost considerably more body weight in the cold compared to 21-month-old rats, but this was not due simply to differences in food intake, which increased by about the same magnitude in both age groups (Kiang-Ulrich and Horvath, 1985a). The authors speculated that the chronologically older rats may have had problems utilizing glucose, and thus a poorer ability to provide the fuels for thermogenesis required to survive the cold (Kiang-Ulrich and Horvath, 1985a). However, since heat production and core temperature were not measured in this study, the metabolic state of the 25-month-old vs 21-month-old rats is only speculative.

4. Effects of Gender

As with the human studies, work with rats has shown that older females are often less prone to develop hypothermia during cold exposure than are comparably aged males (e.g., McDonald *et al*., 1989a, 1994; Gabaldón *et al*., 1995). Our understanding of the physiological mechanisms underlying this gender difference has been limited by the fact that few investigations have used females. In several of our studies, we have evaluated the effects of age on both females and males. For example, we have shown that cold exposure of 27-month-old rats to 6°C for 6 hr resulted in a 2.9°C decrease in colonic temperature in males compared to only a 0.7°C decrease in females and no significant decrease in 5-month-old rats regardless of gender (McDonald *et al*., 1989a). The 27-month-old females in this study had a significantly higher percentage carcass fat compared to males (25.7 vs 21.3), and this may have provided greater protection against passive heat loss. There were no gender differences in cold-induced mass-independent oxygen consumption of older rats (McDonald *et al*., 1989a), but females may have derived a greater percentage of total heat production from nonshivering thermogenesis in brown adipose tissue than did males. The latter is suggested by the ~1.5-fold greater total amount of purine nucleotide (GDP) binding to brown fat mitochondria isolated from 2 hr cold-exposed older females than that seen in older males (McDonald *et al*., 1989a), this binding being an *in vitro* index of the thermogenic state of brown fat mitochondria. In a subsequent study, we found that sympathetic stimulation to brown fat (as measured by norepinephrine turnover in the tissue) was not diminished more in older male than in female F344 rats, indicating that diminished cold-induced neural signals could not explain the gender difference in the ability to maintain homeothermy (McDonald *et al*., 1994)

C. Mechanisms Underlying the Hypothermia in Older Rodents

The ability to maintain thermal homeostasis depends upon the functional state of the neural pathway (Fig. 59.2) as well as that of the peripheral effectors (Fig. 59.1). As discussed below, age-related alterations at several steps in this regulatory pathway have been suggested to occur.

1. Heat Conservation Mechanisms

Retention of metabolic heat during exposure to low ambient temperatures is dependent upon the effectiveness of the insulation provided by the body shell and coat. Subcutaneous fat and skeletal muscle both contribute to total tissue insulation (Kenney and Buskirk, 1995). A poorly perfused body shell provides a more effective insulation, and this is accomplished by vasoconstriction of the peripheral vasculature. This is especially important in minimizing heat loss from the poorly insulated and highly vascularized regions of the body (the tail, ears, and paws in rodents).

Age-related changes in body composition of rats show strain differences. For example, the cross-sectional study by

McDonald *et al*. (1987) on female Sprague–Dawley rats ages 5 and 26 months indicated a decrease with age in the percentage of lean carcass mass and a significant increase in the percentage of fat. In contrast, there were no major age-related alterations in the carcass composition of male and female F344 rats between ages 5–7 and 23–27 months (McDonald *et al*., 1988a,b, 1989a,b). There were also no major age-related changes in the body composition of male Osborne-Mendel rats between ages 12 and 24 months (McDonald *et al*., 1989b). Thus, the insulative capacity due to adipose tissue does not appear to be diminished with age.

In studies with rodents, measurement of skin temperature is often used as an index of peripheral circulation. In response to cold, sympathetic nervous activity to peripheral blood vessels increases, resulting in vasoconstriction, reduced skin blood flow, rapid lowering of skin temperature, and decreased heat loss. Although it has been suggested that there may be blunted vasoconstriction in old rodents, several studies suggest that this is not the case, and in fact, that vasoconstriction is greater in older rats and mice (Cox *et al*., 1981; Balmagiya and Rozovski, 1983; Talan, 1997; Shefer and Talan, 1997). For example, Cox *et al*. (1981) showed that the tail temperature of male Sprague–Dawley rats decreased comparably in young and old rats in response to cold and that there were no age differences in the rate at which tail temperature fell. Balmagiya and Rozovski (1983), using male Sprague–Dawley rats, showed that the total drop in tail temperature in response to cold (1.5 h at 18–19°C) was two times greater at 13 and 24 months of age than at 3–9 months, and the time for tail temperature to return to baseline after cold became progressively longer with increasing age (i.e., 50 min at 24 months vs 10 min at 3 months). These age-related changes in tail temperature coincided with the changes in rectal temperature (greater cold-induced decrease in older vs younger rats and slower recovery time). This implies that the older rats maintained a more intense vasoconstriction as compensation for insufficient heat production. These findings support the idea that the vasoconstrictor response is intact and functional in at least some strains of older rodents. However, when exposed to chronic cold (6 weeks at 10°C), older (22-month-old) male F344 rats developed tail pathologies which were speculated to have been caused by intense vasoconstriction resulting in a complete shutdown of blood flow to the tail (Owen *et al*., 1991). Thus, the vasoconstrictor response, although present in older animals, may not be regulated well over the entire time course of cold exposure—sometimes there appears to be excessive responsiveness that is detrimental to tissue health.

2. Heat Production Mechanisms

Lower cold-induced heat production, as measured by oxygen consumption, has been observed in several strains of older vs younger mice [e.g., Grad and Kral, 1957 (female C57BL); Estler, 1971 (female NMRI); Tatelman and Talan, 1990a,b, 1993 (male C57BL/6J); Schaefer *et al*., 1996 (male C57BL/6J)] and rats [e.g., Kiang-Ulrich and Horvath, 1985b (male F344); Lee and Wang, 1985 (male Sprague–Dawley); McDonald *et al*., 1987 (female Sprague–Dawley); McDonald *et al*., 1988a (male F344); Wang *et al*., 1992 (male Sprague–Dawley)]. Not all studies, however, have shown diminution in cold-induced thermogenesis with age in rats [e.g., Balmagiya and Rozovski, 1983 (male Sprague-Dawley); McDonald *et al*., 1988b (male F344); McDonald *et al*., 1989a (male/female F344); McDonald *et al*., 1989b (male F344 and Osborne-Mendel rats)]. Cold-induced heat production occurs in skeletal muscle and in brown fat depots which are located at various sites in rodents. From blood flow studies, it has been estimated that warm-acclimated (28°C) younger rats acutely exposed to cold (6°C) derive as much as 33% of their total heat production from skeletal muscle and 37% from brown adipose tissue (Foster and Frydman, 1979). Nonshivering thermogenesis in brown fat continues to play an important role in thermoregulation in old rats and mice; and some studies have shown that brown fat thermogenic capacity is reduced with aging. There is also evidence that shivering ability may be diminished with aging.

A significant loss of lean body mass (and thus diminished shivering capabilities) sometimes occurs in old mammals that have reduced thermal homeostasis. Reduction of oxidative enzyme activity in skeletal muscle or in the availability/handling of substrates that fuel thermogenesis could also decrease the potential for shivering. Studies which suggest an age-related loss of shivering thermogenesis in rodents include those by Balmagiya and Rozovski (1983), Lee and Wang (1985), and Wang *et al*. (1992). Lee and Wang (1985) reported that older (23- to 26-month-old) Sprague–Dawley male rats treated with the drug aminophylline, a phosphodiesterase inhibitor which enhances intracellular cAMP levels, had greater heat production and smaller drops in core temperature during acute cold exposure compared to controls. In contrast, aminophylline did not enhance norepinephrine-induced nonshivering thermogenesis, i.e., brown fat thermogenesis. These results were interpreted as indicating that the primary disruption in cold-induced heat production in older rats was blunted skeletal muscle shivering thermogenesis resulting from decreased substrate availability (due to impaired mobilization or utilization mechanisms regulated by cAMP). The apparent inability of cold-exposed older rats to raise their cAMP levels in skeletal muscle may be due to enhanced adenosine stimulation (Wang *et al*., 1992). This is based on the observation that the endogenous adenosine deaminase activity in neck muscle, a key site of shivering thermogenesis, was significantly lower in older vs younger rats, and that administration of adenosine deaminase greatly improved thermogenesis and cold tolerance of older rats (Wang *et al*., 1992). The ability to sustain shivering thermogenesis is dependent to a large degree on the availability of substrate, but the glycolytic and oxidative capacities of skeletal muscle tissue determine the rate of substrate utilization, and thus the rate of production of the ATP required for sustaining shivering. Oxidative enzyme activity in hindlimb skeletal muscle is increased significantly by several weeks of moderate intensity physical exercise training in older (and younger) rats (and mice). This may account, at least in part, for the improved cold tolerance that occurs after exercise training in some rodents (e.g., McDonald *et al*., 1988b; Shefer and Talan, 1998), albeit not all (Talan and Ingram, 1986b).

Although brown adipose tissue disappears almost entirely by about the eighth decade of human life (Heaton, 1972; Ito *et al*., 1991), it remains an important component of regulatory thermogenesis throughout the life of rats and mice. Some data sug-

gest that brown fat thermogenic capacity does not decrease with age in male rodents (Kirov, *et al*., 1996; Scarpace, 1997; Talan, 1997). However, many other investigations have shown that cold-, norepinephrine-, and β-adrenergic agonist-induced thermogenesis and/or components of the thermogenic pathway in this tissue are attenuated with age (Balmagiya and Rozovski, 1983; Kiang-Ulrich and Horvath, 1984b; Lee and Wang, 1985; McDonald *et al*., 1988a, 1989a; Scarpace *et al*., 1988, 1992; Hamilton *et al*., 1990), with older males retaining less brown fat thermogenic capacity than older females (McDonald *et al*., 1989a). The proliferative response of brown fat to chronic cold is also attenuated with age, at least in male F344 rats (Florez-Duquet *et al*., 1998). On the other hand, there appears to be no age-related decrease in ability to generate the thermogenic signal (i.e., norepinephrine) to brown fat during acute cold exposure (Kawate *et al*., 1993; McDonald *et al*., 1993; Talan, 1997); and although sensitivity of brown adipocytes to the sympathetic signals could be reduced (Scarpace *et al*., 1996; Scarpace, 1997), we have found that brown adipocytes isolated from old (26-month) vs younger (12- and 6-month) F344 rats (males and females) did not show any decrement in their responsiveness to norepinephrine or CL 316,243 (a β_3 adrenergic agonist) with respect to cAMP generation, lipolysis, or oxygen consumption (Gabaldón *et al*., 1998). Thus, it appears that the blunted brown fat thermogenesis generated by the old F344 rats is more reflective of reduced amounts of brown adipocytes than of changes in the adipocytes themselves (Gabaldón *et al*., 1998).

3. Sympathetic Nervous System Response to Cold Stress

Various techniques have been used to assess the functional state of the sympathetic nervous system in the old rodents. Among these are measurements of circulating levels of norepinephrine (which derives primarily from post-ganglionic sympathetic nerve endings); firing rates of nerves innervating specific tissues; and measurement of norepinephrine turnover in target tissues. Acute cold increases the activity of the sympathetic nervous system, and in rodents the effect is typically more pronounced in older vs younger individuals as evidenced by higher plasma norepinephrine concentrations (Avakian *et al*., 1984; McCarty, 1985; Gabaldón *et al*., 1995). Cold-induced sympathetic outflow to the interscapular brown adipose depot has also been shown to be greatly enhanced rather than attenuated with aging in rodents. Kawate *et al*. (1993) recorded the efferent electrical activity from sympathetic nerves to interscapular brown fat in C57BL/6J male mice and found that artificial lowering of body temperature resulted in a significantly greater increase in firing rate in 30-month-old vs 10-month-old animals. In a separate investigation of a similar design, Talan (1997) showed that sympathetic firing rate to interscapular brown fat was greater in 24- to 26-month-old vs 10- to 12-month-old C57BL/6J male mice during artificial lowering of body temperature. This occurred when the mice were acclimated to room temperature (22°C) and to thermoneutrality (29°C); the cold-induced sympathetic activity in old mice became even stronger when they were acclimated to the cooler temperature, but it was not enhanced significantly in adult mice (Talan, 1997). Sympathetic outflow to brown fat is also higher in cold-exposed older vs younger F344 male rats

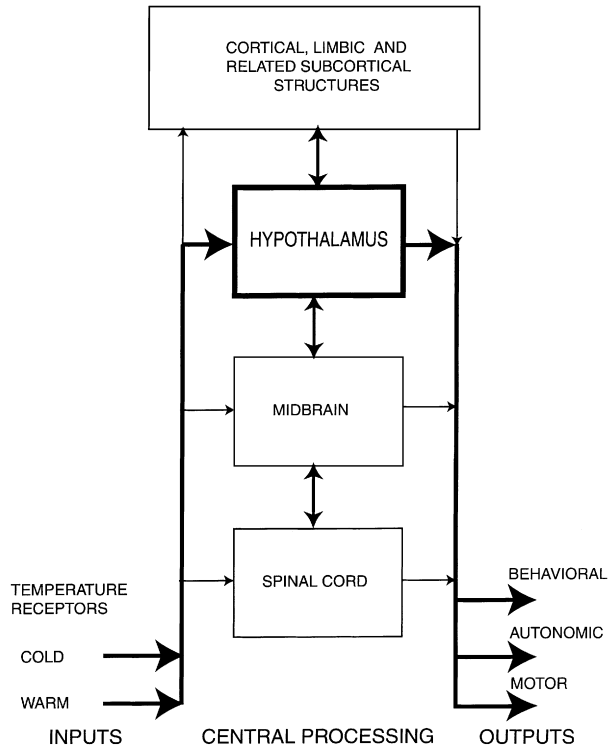

FIG. 59.2. Potential sites of age-dependent neural changes in temperature regulation. The hierarchical control of parallel systems (as indicated by the double arrows) is based on a review by Satinoff (1978) on the neural organization and evolution of thermal regulation in mammals. Warm and cold thermoreceptors in the periphery (Pierau, 1996) and in the central nervous system (Boulant, 1996) send signals to parallel systems within the brain. The dominant role of neural networks in the hypothalamus (Boulant, 1996) controlling autonomic outputs is indicated by darkened lines. Potential sites of age-related thermoregulatory changes can thus occur in a variety of brain areas as well as in the periphery.

(and vs older females) as evidenced by higher norepinephrine turnover rates (McDonald *et al*., 1993). Thus, cold-induced sympathetic neural activity in male rodents appears to be enhanced rather than attenuated with age. In the case of brown fat, this may reflect an attempt to compensate for the diminished amount of thermogenically competent tissue.

4. Central Nervous System

There is evidence to suggest that central nervous control of thermoregulation (Fig. 59.2) is altered with aging in rodents. The findings of Cox *et al*. (1981) suggest that the inability of old rats to respond to temperature changes as effectively as young rats is due, in part, to alterations in concentrations of and/or sensitivity to putative central neurotransmitters involved in hypothalamic thermoregulatory pathways, such as dopamine. These investigators found that intraperitoneal injection of apomorphine (a dopamine agonist) or amphetamine (which causes the release of endogenous dopamine) induced a significant drop in core temperature in young (2-month-old) and old (24-month-old) Sprague–Dawley male rats, but the response was significantly blunted in the old rats. There is also evidence that hypothalamic dopamine content is reduced in old rats (Carfagna *et al*., 1985), and that there

is degeneration of catecholamine terminals in the anterior region of the hypothalamus in old mice (Masuoka *et al*., 1979). Other investigators have also reported age-related alterations in central sensitivity to neurotransmitters/neuromodulators which influence the regulation of body temperature (e.g., Frolkis, 1976; Clark and Lipton, 1981). The possibility of an altered central control mechanism of thermoregulation in older rodents is further supported by the observation that electrical self-stimulation of ''reward'' areas of the hypothalamus improves cold tolerance of old male C57BL/6J mice (Talan *et al*., 1984). The mechanism of this effect is unclear, but it was postulated that electrical stimulation might activate a hypothalamo–hypophyseal regulatory axis which resulted in increased metabolic level which, in turn, facilitated the ability of older mice to increase heat production during cold (Talan *et al*., 1984).

Notwithstanding the above observations, our data showing that 26-month-old male F344 rats respond to acute cold by increasing sympathetic activity to brown fat to a greater extent than do young rats indicate that the circuitry from thermoreceptor to hypothalamic integrator to sympathetic nerves is intact and is functioning appropriately (McDonald *et al*., 1993). Rather, the rate-limiting factor(s) for thermogenesis might instead involve blunted end-organ function and/or reduced substrate availability/handling. This idea is further supported by Tatelman and Talan (1993), who showed that old C57BL/6J male mice were capable of increasing heat production in response to acute cold (3 hr at either 18, 12, or 6°C), but not in proportion to the intensity of the cold stimulus as did young mice. Specifically, maximal thermogenesis in old mice was reached between 18 and 12°C, while maximal thermogenesis in young mice was not reached until 6°C (or lower). The net effect was greater heat production in young vs old mice at 12 and 6°C ($\sim$37 and $\sim$56%, respectively) but comparable heat production at 18°C. This was interpreted as indicating that the lower cold-induced metabolic heat production of old vs young mice reflected reduced capacity for thermogenesis rather than reduced ability to induce it. Thus, while altered central control of thermoregulation may occur with aging, this is probably not the primary mechanism underlying the cold-induced hypothermia in older rodents.

5. Behavioral Thermoregulation

Although age-related alterations in behavioral thermoregulation have been implicated in humans (e.g., Watts, 1972; Collins *et al*., 1981b), the available evidence does not support analogous changes in rodents. In an investigation by Jakubczak (1966), young (7- and 12-month-old) and old (28-month-old) Sprague–Dawley male rats were placed in the cold (16 hr at 2°C) with access to a lever that turned on a heat lamp (250 W, 2 s heat burst/lever press). The young and old rats worked for heat equally well by lever pressing and exhibited comparable amounts of heat loss as measured by rectal temperature. Hence, the mechanisms involved in regulating this heat-seeking behavior are not altered with age. In line with this, Owen *et al*. (1991) found no age-related differences in preferred ambient temperature of F344 male rats placed in a thermocline (7–37°C linear gradient). This was observed regardless of whether the rats were cold-acclimated or not: the preferred ambient temperature of non-cold-acclimated younger and older rats was about 23.7°C; and after 6 weeks at 10°C, rats in both groups selected cooler temperatures (about 7 and 5°C cooler than preacclimation values, respectively).

6. Summary

Old laboratory mice and rats of various strains exhibit an attenuated ability to maintain homeostasis when cold exposed, this decrement being greater in males than in females of the same chronological age. The decrease in cold tolerance does not occur linearly with age but rather, is markedly enhanced in animals as they approach the end of their natural life. Increased heat loss and blunted heat production contribute to the age-related decrease in thermal homeostasis. With respect to heat loss, currently available evidence argues against diminished cold-induced vasoconstriction as a primary cause. Alternative mechanisms such as lower insulative effectiveness of fur have not yet been evaluated. On the heat production side, both shivering and nonshivering thermogenesis appear to be decreased in older rodents, although not to the same degree in the various strains. As in humans, decreased shivering is most apparent in old animals that have lost lean body mass. Similarly, the decrease in nonshivering thermogenesis is most closely related to lower amounts of brown adipose tissue and UCP1, although in some cases there may also be altered β-adrenergic receptor density. Reduced cold-induced sympathetic signaling to brown fat does not appear to play a role in the lower nonshivering thermogenesis of older rodents. Notably, the observation that cold exposure results in increased sympathetic signaling to brown adipose tissue in old presenescent rats (i.e., body weight stable) indicates that the thermoregulatory circuitry is intact and functional. Nonetheless, reports of morphological and neurochemical changes in the brain suggest the possibility of altered hypothalamic regulation of thermoregulation—an area that clearly needs further study.

IV. Senescence and Thermoregulation in Rats

Some older rodents (as well as humans) exhibit spontaneous and relatively rapid weight loss near the end of their life. We have referred to this phase of aging as senescence. It is associated with reduction of food intake (Everitt, 1957, 1958; Blanton *et al*., 1998) as well as a changes in several other physiological variables. As described below, we have found that transition of F344 rats from gradual aging, i.e., chronological aging, to senescence is accompanied by pronounced deterioration of at least two components of the thermoregulatory system. Specifically, senescent rats develop very poor tolerance to cold (McDonald *et al*., 1996) and exhibit weak circadian rhythms of core temperature (McDonald *et al*., 1999).

In the F344 male rats that we obtain from the National Institutes on Aging colony (median life span of $\sim$25 months), senescence, as identified by the period of rapid body weight loss, generally occupies about the last 2–7 weeks of life. Notably, the age of onset of senescence varies from 23 to 30 months of age, indicating that even in an inbred strain such as the F344 rat, chronological age is a poor indicator of this change in functional state. At necropsy following natural death, we have found no consistent pathology that could account for

the rapid loss of body weight (Murtagh-Mark *et al*., 1995); nor were there significant differences in the incidence of leukemia, nephropathy, or pituitary lesions (age-related pathologies characteristic of the F344 rat) between senescent and age-matched presenescent rats (Blanton *et al*., 1998). We also found no evidence of disease-associated cachexia, which is characterized by reduced food intake, increased resting metabolic rate, decreased protein synthesis, and increased whole-body protein degradation. Our rats did not exhibit enhanced resting metabolic rates, higher body temperatures, lower concentrations of serum protein or albumin levels (indices of protein synthesis), or increased amounts of urinary creatinine or urea nitrogen (indices of protein catabolism) (McDonald *et al*., 1996). Thus, senescence appears to be a distinct functional state from chronological aging, but one which cannot be explained by any single pathology.

The senescent F344 rats have severely impaired cold-induced thermoregulation (McDonald *et al*., 1996). When exposed to 6°C for up to 4 hr, they exhibited a rapid decline in core temperature during the first hour of cold (total drop of 2.7°C), followed by a more gradual but significant decline in the hours thereafter; by hour 4, rats completing the cold challenge had dropped their colonic temperature an average of 5.5°C. In contrast, these same rats maintained a more constant core temperature throughout the period of cold exposure before they entered senescence (i.e., when they were body weight stable). This attenuation of thermoregulation in senescent rats was accompanied by 50% lower total amounts of UCP1 in interscapular brown fat depots, indicating decreased potential for nonshivering thermogenesis. To determine if the increased susceptibility to hypothermia resulted from the weight loss per se, we measured the responses of 26-month-old presenescent rats (weight stable) that were food restricted to the same weight loss ($\sim$10%) as the senescent rats. When these food restricted rats were cold exposed under the same conditions as the senescent rats, they did not develop severe hypothermia (McDonald *et al*, 1996). Thus, the loss of body weight of the senescent rats is an indicator that the rats had entered a different functional state—it is not the cause of this state.

The fact that altered circadian rhythms of core temperature (McDonald *et al*., 1999), attenuated cold-induced thermoregulation (McDonald *et al*., 1996), and reduced food intake (McDonald *et al*., 1996, 1999) all occur at approximately the same time suggests that transition from gradual aging to senescence is accompanied by hypothalamic dysfunction. The deterioration of brown fat in senescent rats further supports the possibility of altered neural/hormonal signaling. Brown fat UCP1 levels are highly regulated by the sympathetic nervous system, with modulatory influences from hormones such as triiodothyronine and insulin. The lower UCP1 levels in brown adipose tissue from senescent vs presenescent older rats (McDonald *et al*., 1996) thus suggest possible blunting of neural and/or hormonal signals that regulate its synthesis. We have found significantly lower serum thyroxine concentrations in senescent vs presenescent rats (Blanton *et al*., 1998). This may lessen the capacity of brown adipocytes to generate, via the action of thyroxine 5′-deiodinase, the amount of triiodothyronine necessary to fully enhance norepinephrine-induced UCP1 synthesis. Current studies are examining potential mechanisms for these thermoregulatory changes.

V. Conclusions and Future Directions

Although not all inferences made from studies on thermoregulation in elderly humans have stood the test of time, they have played a valuable role in setting the stage for a series of recent investigations that are more carefully controlled and where data are more conservatively interpreted. It is now recognized that to evaluate the role of aging in the altered thermal responses of the elderly requires consideration of a variety of confounding factors, including physical fitness, socioeconomic status, disease, and prior heat or cold acclimation, if any. Results from longitudinal studies, which allow comparison of changes in a single subject over time, combined with the more variable results from cross-sectional studies, have provided insight into some of the factors contributing to altered thermoregulation in the elderly as well as identification of gender differences. This insight has been greatly advanced by examination of the role of various effectors important in human thermoregulation. These include sweat gland function, vasodilatation, and skin blood flow in warm environments and temperature perception, peripheral vasoconstriction, and skin blood flow in cold environments. However, while these studies have created a framework for describing thermoregulatory changes in elderly humans, a more complete understanding of how age exerts these effects requires further work.

Aging studies on thermoregulatory mechanisms in laboratory rodents have also progressed to a stage where confounding factors have been identified and age-dependent changes in effector mechanisms have been described. Valid comparisons of results in studies on rodents require the recognition that different strains of mice and rats have different life spans, and that chronological age may not represent the same biological age in individuals within the same strain let alone animals of different strains. Nonetheless, studies of laboratory rodents allow analyses that cannot be done in humans. Initial studies on neurotransmitter changes in the central nervous system of aged rats are opening the way for a more complete evaluation of aging and hypothalamic thermoregulation. The rat also is also an excellent model for future neural studies on the hierarchical thermoregulatory system, an area as yet relatively unexplored in aging research; and despite their small size, transgenic mice offer the possibility of testing the role of candidate genes modulating/mediating age-related changes in thermal responses.

Taken as whole, studies on mice, rats, and humans show that age differentially affects various components of the thermoregulatory system. Even though caution must be used in drawing general conclusions across species, studies on different species are contributing to an emerging picture of the mechanisms underlying compromised thermoregulation with age. Additional work is needed to precisely delineate the detailed nature of these mechanisms.

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References

Algeri, S., Calderini, G., Lomuscio, G., Vantini, G., Toffano, G., and Ponzio, F. (1982). Changes with age in rat central monoaminergic system responses to cold stress. *Neurobiol. Aging* **3**, 237–242.

Armstrong, C. G., and Kenney, W. L. (1993). Effects of age and acclimation on responses to passive heat exposure. *J. Appl. Physiol*. **75**, 2162–2167.

Avakian, E. V., Horvath, S. M., and Colburn, R. W. (1984). Influence of age and cold stress on plasma catecholamine levels in rats. *J. Auton. Nerv. Syst*. **10**, 127–133.

Balmagiya, T., and Rozovski, S. J. (1983). Age-related changes in thermoregulation in male albino rats. *Exp. Gerontol*. **18**, 199–210.

Blanton, C. A., Horwitz, B. A., Murtagh-Mark, C., Gietzen, D. W., Griffey, S. M., and McDonald, R. B. (1998). Meal patterns associated with the age-related decline in food intake in the Fischer 344 rat. *Am. J. Physiol*. **275**, R1494–R1502.

Boulant, J. A. (1996). Hypothalamic neurons regulating body temperature. *In* ''Handbook of Physiology'' (M. J. Fregly and C. M. Blatteis, eds.), Sec. 4, Vol. I, pp. 105–126. Oxford University Press, Oxford, UK.

Carfagna, N., Trunzo, F., and Moretti, A. (1985). Brain catecholamine content and turnover in aging rats. *Exp. Gerontol*. **20**, 265–269.

Clark, S. M., and Lipton, J. M. (1981). Hypothermia produced in aged squirrel monkeys by central administration of taurine. *Exp. Aging Res*. **7**, 17–24.

Collins, K. J. (1987). Effects of cold on old people. *Br. J. Hosp. Med*. **38**, 506–508, 510–512.

Collins, K. J., and Exton-Smith, A. N. (1986). Effects of ageing on human homeostasis. *In* ''The Biology of Human Ageing'' (A. H. Bittles and K. J. Collins, eds.), pp. 261–275. Cambridge University Press, Cambridge, UK.

Collins, K. J., Dore, C., Exton-Smith, A. N., Fox, R. H., MacDonald, I. C., and Woodward, P. N. (1977). Accidental hypothermia and impaired temperature homeostasis in the elderly. *Br. Med. J*. **1**, 353–356.

Collins, K. J., Easton, J. C., and Exton-Smith, A. N. (1981a). Shivering thermogenesis and vasomotor responses with convective cooling in the elderly. *J. Physiol. (London)* **320**, 76P.

Collins, K. J., Exton-Smith, A. N., and Doré, C. (1981b). Urban hypothermia: Preferred temperature and thermal perception in old age. *Br. Med. J*. **282**, 175–177.

Cox, B., Lee, T. F., and Parkes, J. (1981). Decreased ability to cope with heat and cold linked to a dysfunction in a central dopaminergic pathway in elderly rats. *Life Sci*. **28**, 2039–2044.

Crowe, J. P., and Moore, R. E. (1974). Proceedings: Physiological and behavioural responses of aged men to passive heating. *J. Physiol. (London)* **236**, 43P–45P.

Dawson, N. J. (1967). The surface area/body weight relationship in mice. *Aust. J. Biol. Sci*. **20**, 687–690.

Duffy, P. H., Feuers, R. J., and Hart, R. W. (1987). Effect of age and torpor on the circadian rhythms of body temperature, activity, and body weight in the mouse (*Peromyscus leucopus*). *Prog. Clin. Biol. Res*. **227B**, 111–120.

Estler, C. (1971). Efficiency of thermoregulation in acutely cold-exposed young and old mice. *Life Sci*. **10**, 1291–1298.

Everitt, A. V. (1957). The senescent loss of body weight in male rats. *J. Gerontol*. **12**, 382–387.

Everitt, A. V. (1958). The change in food and water consumption and in faeces and urine production in ageing male rats. *Gerontologia* **2**, 21–32.

Fennell, W. H., and Moore, R. E. (1973). Responses of aged men to passive heating. *J. Physiol. (London)* **231**, 118P–119P.

Ferrer, T., Ramos, M. J., Pérez-Sales, P., Pérez-Jiménez, A., and Alvarez, E. (1995). Sympathetic sudomotor function and aging. *Muscle Nerve* **18**, 395–401.

Finch, C. E., Foster, J. R., and Mirsky, A. E. (1969). Ageing and the regulation of cell activities during exposure to cold. *J. Gen. Physiol*. **54**, 690–712.

Florez-Duquet, M. L., Horwitz, B. A., and McDonald, R. B. (1998). Cellular proliferation and UCP content in brown adipose tissue of cold-exposed aging Fischer 344 rats. *Am. J. Physiol*. **274**, R196–R203.

Foster, D. O., and Frydman, M. L. (1979). Tissue distribution of cold-induced thermogenesis in conscious warm- or cold-acclimated rats re-evaluated from changes in tissue blood flow: The dominant role of brown adipose tissue in the replacement of shivering by nonshivering thermogenesis. *Can. J. Physiol. Pharmacol*. **57**, 257–270.

Frolkis, V. V. (1976). The hypothalamic mechanisms of aging. *In* ''Hypothalamus, Pituitary, and Aging'' (A. V. Everitt and J. A. Burgess, eds.), pp. 614–633. Thomas, Springfield, IL.

Gabaldón, A. M., Florez-Duquet, M. L., Hamilton, J. S., McDonald, R. B., and Horwitz, B. A. (1995). Effects of age and gender on brown fat and skeletal muscle metabolic responses to cold in F344 rats. *Am. J. Physiol*. **268**, R931–R941.

Gabaldón, A. M., McDonald, R. B., and Horwitz, B. A. (1998). Effects of age, gender, and senescence on β-adrenergic responses of isolated F344 rat brown adipocytes in vitro. *Am. J. Physiol*. **274**, E726–E736.

German, E., and Hoffman-Goetz, L. (1986). The effect of cold acclimation and exercise training on cold tolerance in aged C57BL/6J mice. *J. Gerontol*. **41**, 453–459.

Goodrick, C. L. (1975). Life-span and the inheritance of longevity in inbred mice. *J. Gerontol*. **30**, 257–263.

Grad, B., and Kral, V. A. (1957). The effect of senescence on resistance to stress: Response of young and old mice to cold. *J. Gerontol*. **12**, 172–181.

Hamilton, J. S., Horwitz, B. A., Weidner, W. J., Stern, J. S., and McDonald, R. B. (1990). Effect of age and gender on regional blood flow in young and older cold-exposed rats. *FASEB J*. **4**, A198.

Heaton, J. M. (1972). The distribution of brown adipose tissue in the human. *J. Anat*. **112**, 35–39.

Hoffman-Goetz, L., and Keir, R. (1984). Body temperature responses of aged mice to ambient temperature and humidity stress. *J. Gerontol*. **39**, 547–551.

Horvath, S. M., Radcliffe, C. E., Hutt, B. K., and Spurr, G. B. (1955). Metabolic responses of old people to a cold environment. *J. Appl. Physiol*. **8**, 145–148.

Huang, H. H., Steger, R. W., and Meites, J. (1980). Capacity of old versus young male rats to release thyrotropin (TSH), thyroxine (T_4) and triiodothyronine (T_3) in response to different stimuli. *Exp. Aging Res*. **6**, 3–12.

Hügin, V. F., and Verzár, F. (1957). Versagen der Wärmeregulation bei alten Tieren. *Gerontologia* **1**, 91–106.

Inoue, Y. (1996). Longitudinal effects of age on heat-activated sweat gland density and output in healthy active older men. *Eur. J. Appl. Physiol. Occup. Physiol*. **74**, 72–77.

Inoue, Y., Nakao, M., Okudaira, S., Ueda, H., and Araki, T. (1995). Seasonal variation in sweating responses of older and younger men. *Eur. J. Appl. Physiol. Occup. Physiol*. **70**, 6–12.

Ito, T., Tanuma, Y., Yamada, M., and Yamamoto, M. (1991). Morphological studies on brown adipose tissue in the bat and in humans of various ages. *Arch. Histol. Cytol*. **54**, 1–39.

Jakubczak, L. F. (1966). Behavioral thermoregulation in young and old rats. *J. Appl. Physiol*. **21**, 19–21.

Kawate, R., Talan, M. I., and Engel, B. T. (1993). Aged C57BL/6J mice respond to cold with increased sympathetic nervous activity to interscapular brown adipose tissue. *J. Gerontol*. **48**, B180–B183.

Kenney, W. L. (1997). Thermoregulation at rest and during exercise in healthy older adults. *Exercise Sport Sci. Rev.* **25**, 41–76.

Kenney, W. L., and Armstrong, C. G. (1996). Reflex peripheral vasoconstriction is diminished in older men. *J. Appl. Physiol*. **80**, 512–515.

Kenney, W. L., and Buskirk, E. R. (1995). Functional consequences of sarcopenia: Effects on thermoregulation. *J. Gerontol*. **50A**, 78–85.

Kenney, W. L., and Fowler, S. R. (1988). Methylcholine-activated eccrine sweat gland density and output as a function of age. *J. Appl. Physiol.* **65**, 1082–1086.

Kenney, W. L., and Ho, C. W. (1995). Age alters regional distribution of blood flow during moderate-intensity exercise. *J. Appl. Physiol*. **79**, 1112–1119.

Kenney, W. L., and Zappe, D. H. (1994). Effect of age on renal blood flow during exercise. *Aging* **6**, 293–302.

Kenney, W. L., Tankersley, C. G., Newswanger, D. L., Hyde, D. E., Puhl, S. M., and Turner, N. L. (1990). Age and hypohydration independently influence the peripheral vascular response to heat stress. *J. Appl. Physiol*. **68**, 1902–1908.

Kenney, W. L., Tankersley, C. G., Newswanger, D. L., and Puhl, S. M. (1991). α1-Adrenergic blockade does not alter control of skin blood flow during exercise. *Am. J. Physiol*. **260**, H855–H861.

Kenney, W. L., Morgan, A. L., Farquhar, W. B., Brooks, E. M., Pierzga, J. M., and Derr, J. A. (1997). Decreased active vasodilator sensitivity in aged skin. *Am. J. Physiol*. **272**, H1609–H1614.

Kiang-Ulrich, M., and Horvath, S. M. (1979). Survival of young and old rats in a cold environment. *Age* **2**, 68–69.

Kiang-Ulrich, M., and Horvath, S. M. (1984a). Metabolic responses to tyramine and cold in young male Sprague-Dawley and Fischer 344 rats. *Am. J. Physiol*. **246**, E141–E144.

Kiang-Ulrich, M., and Horvath, S. M. (1984b). Age-related metabolic modifications in male F344 rats. *Exp. Aging Res*. **10**, 89–93.

Kiang-Ulrich, M., and Horvath, S. M. (1985a). Age-related differences in food intake, body weight, and survival of male F344 rats in 5°C cold. *Exp. Gerontol*. **20**, 107–117.

Kiang-Ulrich, M., and Horvath, S. M. (1985b). Age-related differences in response to acute cold challenge (−10°C) in male F344 rats. *Exp. Gerontol*. **20**, 201–209.

Kirov, S. A., Talan, M. I., Kosheleva, N. A., and Engel, B. T. (1996). Nonshivering thermogenesis during acute cold exposure in adult and aged C57BL/6J mice. *Exp. Gerontol*. **31**, 409–419.

Lakatta, E. B. (1995). Cardiovascular system. *In* ''Handbook of Physiology: Aging'' (E. J. Masoro, ed.), pp. 413–474. American Physiological Society, Bethesda, MD.

Lee, T. F., and Wang, L. C. H. (1985). Improving cold tolerance in elderly rats by aminophylline. *Life Sci*. **36**, 2025–2032.

Levine, J. A. (1969). Heat stroke in the aged. *Am. J. Med*. **47**, 251–258.

Lye, M., and Kamal, A. (1977). Effects of a heat wave on mortality-rates in elderly inpatients. *Lancet* **1**, 529–531.

Macey, S. M., and Schneider, D. F. (1993). Deaths from excessive heat and excessive cold among the elderly. *Gerontologist* **33**, 497–500.

Martin, H. L., Loomis, J. L., and Kenney, W. L. (1995). Maximal skin vascular conductance in subjects aged 5-85 yr. *J. Appl. Physiol*. **79**, 297–301.

Martin, J. R., Fuchs, A., and Harting, J. (1985). Senescent rats of two strains exhibit greater cold-induced hypothermia than adult controls. *IRCS Med. Sci*. **13**, 55–56.

Masuoka, D. R., Jonsson, G., and Finch, C. E. (1979). Aging and unusual catecholamine-containing structures in the mouse brain. *Brain Res*. **169**, 335–341.

McCarty, R. (1985). Sympathetic-adrenal medullary and cardiovascular responses to acute cold stress in adult and aged rats. *J. Auton. Nerv. Syst.* **12**, 15–22.

McDonald, R. B., Stern, J. S., and Horwitz, B. A. (1987). Cold-induced metabolism and brown fat GDP binding in young and old rats. *Exp. Gerontol*. **22**, 409–420.

McDonald, R. B., Horwitz, B. A., Hamilton, J. S., and Stern, J. S. (1988a). Cold- and norepinephrine-induced thermogenesis in younger and older Fischer 344 rats. *Am. J. Physiol*. **254**, R457–R462.

McDonald, R. B., Horwitz, B. A., and Stern, J. S. (1988b). Cold-induced thermogenesis in younger and older Fischer 344 rats following exercise training. *Am. J. Physiol*. **254**, R908–R916.

McDonald, R. B., Day, C., Carlson, K., Stern, J. S., and Horwitz, B. A. (1989a). Effect of age and gender on thermoregulation. *Am. J. Physiol*. **257**, R700–R704.

McDonald, R. B., Stern, J. S., and Horwitz, B. A. (1989b). Thermogenic responses of younger and older rats to cold exposure: Comparison of two strains. *J. Gerontol*. **44**, B37–B42.

McDonald, R. B., Hamilton, J. S., and Horwitz, B. A. (1993). Influence of age and gender on brown adipose tissue norepinephrine turnover. *Proc. Soc. Exp. Biol. Med*. **204**, 117–121.

McDonald, R. B., Murtagh, C. M., and Horwitz, B. A. (1994). Age and gender effects on glucose utilization in skeletal muscle and brown adipose tissue of cold-exposed rats. *Proc. Soc. Exp. Biol. Med*. **207**, 102–109.

McDonald, R. B., Florez-Duquet, M., Murtagh-Mark, C., and Horwitz, B. A. (1996). Relationship between cold-induced thermoregulation and spontaneous rapid body weight loss of aging F344 rats. *Am. J. Physiol*. **271**, R1115–R1122.

McDonald, R. B., Hoban-Higgins, T. M., Ruhe, R. C., Fuller, C. A., and Horwitz, B. A. (1999). Alterations in endogenous circadian rhythm of core temperature in senescent Fischer 344 rats. *Am. J. Physiol*. **276**, R824–R830.

Minson, C. T., Wladkowski, S. L., Cardell, A. F., Pawelczyk, J. A., and Kenney, W. L. (1998). Age alters the cardiovascular response to direct passive heating. *J. Appl. Physiol*. **84**, 1323–1332.

Mirchandani, H. G., McDonald, G., Hood, I. C., and Fonseca, C. (1996). Heat-related deaths in Philadelphia—1993. *Am. J. Forensic Med. Pathol*. **17**, 106–108.

Murtagh-Mark, C. M., Reiser, K. M., Harris, R., and McDonald, R. B. (1995). Source of dietary carbohydrate affects life span of Fischer 344 rats independent of caloric restriction. *J. Gerontol*. **50**, B148–B154.

Owen, T. L., Spencer, R. L., and Duckles, S. P. (1991). Effect of age on cold acclimation in rats: Metabolic and behavioral responses. *Am. J. Physiol*. **260**, R284–R289.

Paré, W. P. (1989). Age differences in the body temperature response to restraint-cold stress. *Physiol. Behav.* **45**, 151–154.

Pierau, F.-K. (1996). Peripheral thermosensors. *In* ''Handbook of Physiology'' (M. J. Fregly and C. M. Blatteis, eds.), Sec. 4, Vol. I, pp. 85–104. Oxford University Press, Oxford, UK.

Refinetti, R., Ma, H., and Satinoff, E. (1990). Body temperature rhythms, cold tolerance, and fever in young and old rats of both genders. *Exp. Gerontol*. **25**, 533–543.

Reynolds, M. A., Ingram, D. K., and Talan, M. I. (1985). Relationship of body temperature stability to mortality in aging mice. *Mech. Ageing Dev.* **30**, 143–152.

Satinoff, E. (1978). Neural organization and evolution of thermal regulation in mammals. *Science* **201**, 16–22.

Sawka, M. N. (1988). Physiological responses to acute exercise heat stress. *In* ''Human Performance Physiology and Environmental Medicine at Terrestrial Extremes'' (K. B. Pandolf, M. N. Sawka, and R. R. Gonzalez, eds.), pp. xiv, 637. Benchmark Press, Indianapolis, IN.

Scarpace, P. J. (1997). Thermoregulation with age: Role of beta-adrenergic signal transduction. *Ann. N.Y. Acad. Sci*. **813**, 111–116.

Scarpace, P. J., Mooradian, A. D., and Morley, J. E. (1988). Age-associated decrease in beta-adrenergic receptors and adenylate cyclase activity in rat brown adipose tissue. *J. Gerontol*. **43**, B65–B70.

Scarpace, P. J., Matheny, M. and Borst, S. E. (1992). Thermogenesis and mitochondrial GDP binding with age in response to the novel agonist CGP-12177A. *Am. J. Physiol*. **262**, E185–E190.

Scarpace, P. J., Matheny, M., Borst, S. E., and Tümer, N. (1994). Thermoregulation with age: Role of thermogenesis and uncoupling protein expression in brown adipose tissue. *Proc. Soc. Exp. Biol. Med*. **205**, 154–161.

Scarpace, P. J., Dove, J., and Matheny, M. (1996). Effect of age on beta-adrenergic subtype activation of adenylyl cyclase in brown adipose tissue. *Proc. Soc. Exp. Biol. Med*. **213**, 262–267.

Schaefer, V. I., Talan, M. I., and Shechtman, O. (1996). The effect of exercise training on cold tolerance in adult and old C57BL/6J mice. *J. Gerontol*. **51**, B38–B42.

Schmidt-Nielsen, K. (1984). ''Scaling: Why is Animal Size so Important?'' pp. 77–82. Cambridge University Press, Cambridge, UK.

Shefer, V. I., and Talan, M. I. (1997). Change in heat loss as a part of adaptation to repeated cold exposures in adult and aged male C57BL/6J mice. *Exp. Gerontol.* **32**, 325–332.

Shoenfeld, Y., Udassin, R., Shapiro, Y., Ohri, A., and Sohar, E. (1978). Age and sex difference in response to short exposure to extreme dry heat. *J. Appl. Physiol*. **44**, 1–4.

Talan, M. I. (1997). Age-related changes in thermoregulation of mice. *Ann. N.Y. Acad. Sci*. **813**, 95–100.

Talan, M. I., and Engel, B. T. (1984). An improved cold stress test for aging mice. *Exp. Gerontol*. **19**, 79–86.

Talan, M. I., and Ingram, D. K. (1986a). Age comparisons of body temperature and cold tolerance among different strains of *Mus musculus. Mech. Ageing Dev.* **33**, 247–256.

Talan, M. I., and Ingram, D. K. (1986b). Effects of voluntary and forced exercise on thermoregulation and survival in aged C57BL/6J mice. *Mech. Ageing Dev.* **36**, 269–279.

Talan, M. I., Engel, B. T., and Whitaker, J. R. (1984). Age-related decline in cold tolerance can be retarded by brain stimulation. *Physiol. Behav.* **33**, 969–973.

Talan, M. I., Engel, B. T., and Whitaker, J. R. (1985). A longitudinal study of tolerance to cold stress among C57BL/6J mice. *J. Gerontol*. **40**, 8–14.

Tankersley, C. G., Smolander, J., Kenney, W. L., and Fortney, S. M. (1991). Sweating and skin blood flow during exercise: Effects of age and maximal oxygen uptake. *J. Appl. Physiol*. **71**, 236–242.

Tatelman, H. M., and Talan, M. I. (1990a). Metabolic heat production during repeated cold stress in adult and aged male C57BL/6J mice. *J. Gerontol.* **45**, B215–B219.

Tatelman, H. M., and Talan, M. I. (1990b). Metabolic heat production during repeated testing at 24°C and 6°C in adult and aged male C57BL/6J mice: The effect of physical restraint before cold stress. *Exp. Gerontol*. **25**, 459–467.

Tatelman, H. M., and Talan, M. I. (1993). Metabolic heat production and cold tolerance during cold stress of different intensity of adult and aged male C57BL/6J mice. *Exp. Gerontol*. **28**, 579–584.

Trujillo, T. T., Spalding, J. F., and Langham, W. N. (1962). A study of radiation-induced aging: Response of irradiated and nonirradiated mice to cold stress. *Radiat. Res*. **16**, 144–150.

Verzár, F. (1958). Problems of general biology of aging. *J. Gerontol*. **13**, 6–15.

Wagner, J. A., and Horvath, S. M. (1985a). Influences of age and gender on human thermoregulatory responses to cold exposures. *J. Appl. Physiol*. **58**, 180–186.

Wagner, J. A., and Horvath, S. M. (1985b). Cardiovascular reactions to cold exposure differ with age and gender. *J. Appl. Physiol*. **58**, 187–192.

Wagner, J. A., Robinson, S., and Marino, R. P. (1974). Age and temperature regulation of humans in neutral and cold environments. *J. Appl. Physiol*. **37**, 562–565.

Wang, L. C., Jin, Z. L., and Lee, T. F. (1992). Decrease in cold tolerance of aged rats caused by the enhanced endogenous adenosine activity. *Pharmacol. Biochem. Behav.* **43**, 117–123.

Watts, A. J. (1972). Hypothermia in the aged: A study of the role of cold-sensitivity. *Environ. Res*. **5**, 119–126.

Young, A. J. (1991). Effects of aging on human cold tolerance. *Exp. Aging Res.* **17**, 205–213.

Yousef, M. K., Dill, D. B., Vitez, T. S., Hillyard, S. D., and Goldman, A. S. (1984). Thermoregulatory responses to desert heat: Age, race and sex. *J. Gerontol*. **39**, 406–414.

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Sleep and Hormonal Rhythms in Humans

In the human, sleep and hormonal secretions are under the dual control of circadian rhythmicity and of a homeostatic process relating the depth of sleep to the duration of prior wakefulness. The circadian clock plays a major role in the timings of sleep onset and offset, sleep consolidation, and the distributions of rapid eye movement (REM) sleep. The temporal organization of the secretions of a number of hormones such as melatonin and the hormones of the hypothalamo–pituitary–adrenal axis are also primarily controlled by the circadian pacemaker. On the other hand, the temporal profile of non-REM sleep, in particular slow-wave sleep, and a number of hormones such as prolactin and growth hormone are primarily controlled by the homeostatic process. Normal aging is commonly associated with profound alterations of both the homeostatic process and the circadian pacemaker, which are, however, chronologically dissociated. Alterations of the homeostatic process, as assessed by exponential decreases in slow-wave sleep and in nocturnal growth hormone secretion, are essentially complete by midlife. In contrast, alterations of the circadian pacemaker, as assessed in particular by modifications of REM sleep and of the glucocorticoid secretory profiles, occur essentially from 50 years onward. Strategies for preventing or limiting such alterations should take into account this dissociated chronology of aging. Pharmacological approaches to restore normal sleep could represent an indirect form of hormonal therapy with possible beneficial health effects.

I. Mechanisms Subserving Sleep and Hormonal Rhythms

In the human as in all mammalian species, reproducible changes of essentially all physiological and behavioral variables occur over the course of the 24 hr day, in synchrony with the 24 hr periodicities in the physical environment. However, these daily or diurnal rhythms are not simply a response to the environmental periodicities imposed by celestial mechanics, but instead are generated by an internal time-keeping system, generally referred to as the circadian clock or pacemaker (Turek, 1998). Two small bilaterally paired nuclei in the anterior hypothalamus, called the suprachiasmatic nuclei, function as a master circadian pacemaker (Rusak and Zucker, 1979; Moore-Ede, 1982; Miller *et al*., 1996). In the absence of any environmental time cues, the intrinsic period of this master pacemaker, which is primarily responsible for the generation and entrainment of all circadian rhythms of the body, is rarely exactly 24 hr. In the human, this period is slightly longer than 24 hr. Estimations from early studies of subjects maintained for extended periods of time in temporal isolation, either in underground bunkers or in specially designed units, averaged 24.5–25.0 hr (Wever, 1979; Aschoff, 1981). A recent reevaluation using a different experimental strategy has, however, suggested that the endogenous period of human circadian rhythmicity is actually very close to 24 hr, i.e. 24.18 hr (Czeisler *et al*., 1999). The endogenous pacemaker needs to be synchronized and entrained by environmental signal(s) to ensure adequate adaptation of physiological and behavioral functions to environmental conditions. Otherwise, a clock with a period only a few minutes shorter or longer than 24 hr would soon be totally desynchronized from the environment. The light–dark cycle is the most important synchronizing environmental agent (Turek, 1998). Light–dark information is transmitted from the retina to the suprachiasmatic nuclei and then to the pineal gland, where it regulates melatonin secretion. In turn, melatonin exerts synchronizing effects on the circadian pacemaker as an indirect photic zeitgeber. There is increasing evidence that the rest–activity cycle may also participate in the synchronization of endogenous circadian rhythmicity independently of photic inputs (Turek, 1998).

In the human, sleep is under the dual control of circadian rhythmicity and of a homeostatic process relating the depth of sleep to the duration of prior wakefulness. Thus, circadian rhythmicity plays an important role in the timings of sleep onset and offset, sleep consolidation, and the distribution of rapid eye movement (REM) sleep and sleep spindle activity (Czeisler *et al*., 1980; Dijk and Czeisler, 1995). On the other hand, non-REM sleep, in particular slow-wave sleep, is primarily controlled by the homeostatic process, which is thought to involve a putative neural sleep factor which increases during waking drops and exponentially during sleep (Achermann and Borbély, 1990; Borbély, 1998).

Both sleep and the circadian clock are major regulators of endocrine function. Their relative contributions in the temporal organization of hormonal release differ from one endocrine

axis to another. For most pituitary hormones, the 24 hr profiles result from the interaction of the circadian clock with sleep–wake homeostasis and reflect the superposition of 24 hr periodicities on an ultradian, or pulsatile, pattern of release.

Morphological and neuroanatomical alterations have been evidenced in the suprachiasmatic nuclei of older animals in some, but not all, studies (Wise *et al*., 1987, 1988; Swaab *et al*., 1988; Weiland and Wise, 1990). These alterations could, at least partially, be responsible for age-related changes in 24 hr rhythms which are primarily driven by the circadian pacemaker, such as reduced amplitude and earlier phase. It has been suggested that the phase-advance of circadian rhythmicity in the elderly could result from a shortening of the intrinsic period of the circadian pacemaker (Pittendrigh and Daan, 1974; Weitzman *et al*., 1982; Czeisler *et al*., 1986, 1992). However, a recent study performed under conditions of forced desynchrony—where the subjects are maintained on a sleep–wake and dark–light cycle with a period outside the range of entrainment of the circadian system—found similar durations of intrinsic periods of the circadian pacemaker in healthy young and older individuals (Czeisler *et al*., 1999). It is possible, however, that the relatively short duration of the protocol did not allow for the expression of the endogenous period and of a possible impact of age. Studies in blind subjects who have no photic input to their circadian system and therefore ''free-run'' naturally have indeed provided estimations of the human circadian period around 24.6 hr (Lewy and Newsome, 1983). Alternatively, changes in 24 hr rhythms could reflect age-related modifications in entrainment mechanisms or exposure to entrainment agents, e.g., absence of constraining professional and social schedules and decreased exposure to the synchronizing effects of the dark–light and rest–activity cycles. Indeed, some older individuals maintain well-preserved circadian function into the later stages of adulthood (Monk *et al*., 1995). It has been suggested that individual differences in circadian function in the elderly could reflect individual differences in degree of exposure and responsivity to the synchronizing effects of both photic and nonphotic inputs in the course of aging (Campbell *et al*., 1988; Van Cauter *et al*., 1998). Decreased sleep quality is also a hallmark of aging and is likely to contribute to the development of hormonal alterations in the elderly (Van Cauter *et al*., 1998).

The characteristics and the chronology of age-related changes in sleep duration and quality will be reviewed in the first section of the present chapter. The following sections will be devoted to a review of age-related alterations in a number of hormonal rhythms classically considered to be mainly dependent on sleep–wake homeostasis (growth hormone, prolactin), on circadian rhythmicity (cortisol, melatonin), or on both processes (thyrotropin). Particular emphasis will be put on two hormones for which the chronology of aging has been clearly defined, namely growth hormone and cortisol.

II. Sleep

REM sleep and non-REM sleep both correlate with specific changes in brain activity, muscle tone, and autonomic activity. Non-REM sleep is subdivided, relatively arbitrarily, into four precisely defined stages: stages 1, 2, 3, and 4. Stage 1 sleep is a transitional stage between wakefulness and sleep, and is followed after a few minutes of sleep by stage 2 sleep, which is generally considered to be the onset of true sleep. This is successively followed by stages 3 and 4, which are generally referred to as slow-wave sleep or ''deep sleep.'' During stages 3 and 4, the electroencephalogram is characterized by slow waves in the range 0.5–4.0 Hz, often referred to as ''delta waves.'' REM sleep, also referred to as ''paradoxical sleep,'' is a state in which the brain is highly activated but the body is paralyzed, and is preferentially associated with dreaming and eye movements. Under normal conditions, sleep starts with non-REM stages, followed by the first period of REM sleep. As the night progresses, REM sleep increases in duration with each successive cycle, which lasts approximately 90 min, while slow-wave sleep decreases (Fig. 60.1, top). Thus, in a young normal adult, normal sleep architecture typically consists of four to five alternating non-REM and REM cycles, with REM sleep occupying approximately 25% and non-REM sleep approximately 75% of the total sleep time.

Profound disruptions of the daily sleep–wake cycle are commonly observed in normal aging. Elderly individuals frequently complain of sleep problems, usually reporting shallow, unrefreshing sleep, frequent awakenings during the night, early morning awakenings and unwanted daytime naps (Prinz, 1995). Consistent with this decrease in sleep quality with aging, regular use of sedative and hypnotic medications also increases in elderly.

Numerous studies have demonstrated that these age-related changes in subjective sleep quality reflect marked alterations in polysomnographically defined sleep architecture (Prinz *et al*., 1990; Bliwise, 1993, 1994; Prinz, 1995). While the sleep period (i.e., the interval separating sleep onset from final morning awakening) remains relatively constant across adulthood, significant sleep fragmentation occurs after 50 years of age, resulting in a decrease in total sleep time (i.e., the sleep period minus the total duration of awakenings) and therefore in a reduction of sleep efficiency. Morever, aging exerts differential effects on the different sleep stages (Fig. 60.1). The most spectacular age-related change is the decrease in slow-wave (stages III and IV) sleep and in delta wave activity. This decrease appears to be more pronounced in men than in women. The decline in REM time, while less marked, is accompanied by a redistribution of REM stages across sleep. Indeed, REM stages are shifted toward the early part of the night. This is illustrated in Fig. 60.2, which shows that following sleep onset, the time necessary to accumulate 50% of the total amount of REM sleep is 75 min shorter in normal old men than in young controls. Since the distribution of REM sleep is mainly controlled by the circadian pacemaker, these alterations suggest that a phase advance of the circadian clock could be a hallmark of aging.

The chronology of age-related changes in sleep quality is illustrated in Fig. 60.3. From young adulthood (16–25 years) to midlife (35–50 years), the duration of slow-wave sleep decreases rapidly (almost 30 min per decade) so that by midlife slow-wave sleep represents less than 10% of the sleep period, with practically no more stage IV sleep. This decrease in deep sleep is compensated by an increase in light non-REM sleep (i.e., stages I and II) while the durations of REM sleep and

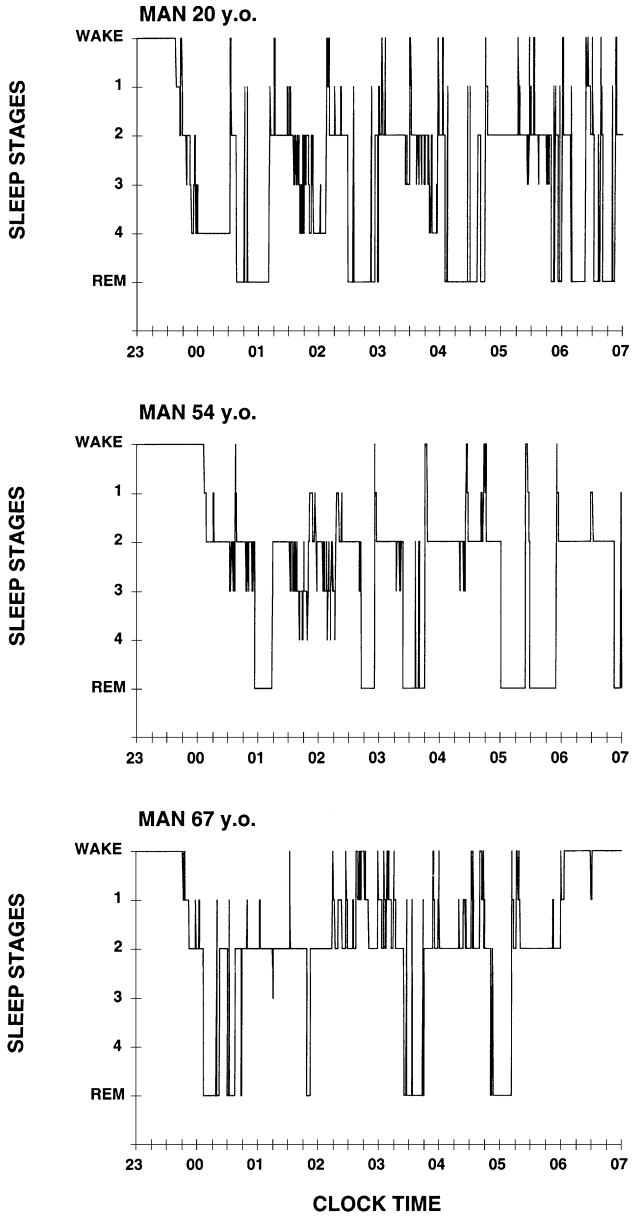

FIG. 60.1. Sleep scores at 30 sec intervals throughout the night in three normal men ages 20 years (top), 54 years (middle), and 67 years (bottom).

of wake and the total sleep time remain stable. In contrast, from midlife to old age (70–83 years), there is no additional decline in slow-wave sleep, but the duration of REM sleep decreases and the duration of wake increases, resulting in a progressive decline in total sleep time.

This differential pattern of age-related changes in slow-wave sleep, primarily controlled by the homeostatic process, and in REM sleep, mainly dependent on the circadian pacemaker, suggests that an alteration in sleep–wake homeostasis may constitute an early biological marker of aging in normal men.

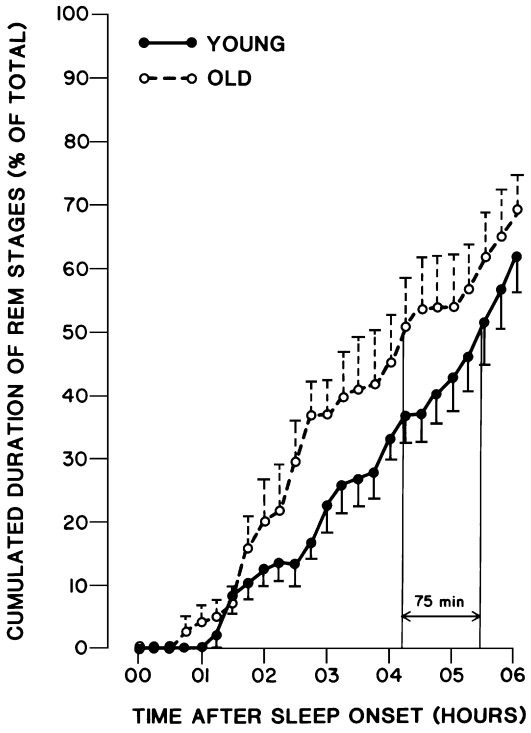

FIG. 60.2. Cumulated distribution of REM stages (mean ± SEM) in normal men ages 20–27 years (solid lines) and 67–84 years (dashed lines). Adapted from van Coevorden *et al.* (1991).

III. Hormones Primarily Controlled by Sleep–Wake Homeostasis: Prolactin and Growth Hormone

A. Prolactin

In normal young adults, the 24 hr profile of circulating prolactin levels is characterized by a major nocturnal elevation starting shortly after sleep onset and culminating around midsleep (Sassin *et al.*, 1972, 1973; Van Cauter *et al.*, 1981). Sleep onset has a stimulatory effect on prolactin release, irrespective of the time of the day, but the amplitude of the prolactin rise associated with daytime sleep may be dampened as compared with nocturnal sleep (Van Cauter and Refetoff, 1985). Conversely, modest elevations of prolactin levels may persist during waking around the time of the usual sleep onset, particularly in women. Thus, prolactin secretion appears to be also modulated by circadian rhythmicity, and maximal stimulation occurs only when sleep and circadian effects are superimposed (Desir *et al.*, 1982; Spiegel *et al.*, 1994; Waldstreicher *et al.*, 1996). The circadian component of prolactin secretion is much more pronounced in women than in men (Waldstreicher *et al.*, 1996). In addition, two studies have indicated that elevations of nocturnal prolactin levels in the absence of sleep could also depend on other factors. In one study, prolactin increased when the subjects were kept awake in total darkness but not in the presence of light (Okatani and Sagara, 1993). Therefore, the authors suggested that the nocturnal elevation of prolactin may be partially dependent on melatonin. Indeed, administration of low doses of melatonin during the daytime results in an

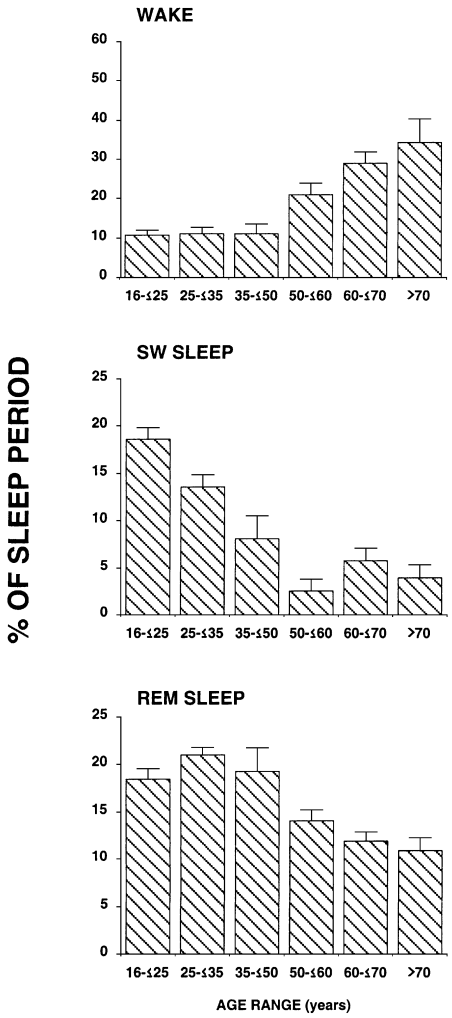

FIG. 60.3. Percentages of the sleep period spent in wake, slow-wave (SW) sleep, and REM sleep as a function of age in normal men (mean ± SEM). Data source: Van Cauter *et al*. (2000).

elevation of plasma prolactin concentrations. In the other study, elevations of prolactin during nocturnal wakefulness occurred only when subjects were in a state of "quiet rest" but not if they expected to be disturbed (Wehr *et al*., 1993).

The relationship between sleep stages and prolactin release has been investigated in a few studies. A close temporal relationship has been evidenced between slow-wave activity (estimated by spectral analysis) and sleep-associated prolactin secretion (Spiegel *et al*., 1995). Conversely, awakenings inhibit nocturnal prolactin release (Spiegel *et al*., 1995). Thus, fragmented sleep will generally be associated with lower nocturnal levels of prolactin.

As illustrated in Fig. 60.4, the night time prolactin rise is generally dampened in elderly subjects (van Coevorden *et al*., 1991). This could be due to increased sleep fragmentation. When considered in relation to the time of the sleep onset, the timings of the nocturnal rise and of the early morning decline are not modified (van Coevorden *et al*., 1991). Daytime levels remain essentially unchanged. No data are available concerning the time course of the occurrence of prolactin alterations with aging.

B. Growth Hormone

It was recognized more than 30 years ago that GH secretion is markedly stimulated during sleep (Quabbe *et al*., 1966; Takahashi *et al*., 1968; Honda *et al*., 1969; Sassin *et al*., 1969). In normal young adults, the 24 hr profile of circulating GH levels consists of stable low concentrations abruptly interrupted by secretory pulses. In normal young adult males, the largest and most reproducible pulse occurs shortly after sleep onset (Takahashi *et al*., 1968; Sassin *et al*., 1969; Van Cauter *et al*., 1992), while in normally cycling young women, this sleep-onset-associated pulse, while also present, does not generally account for the majority of the 24 hr secretion, since daytime pulses are far more frequent and of higher amplitude than in men. The increased daytime GH secretion in women appears to be correlated with circulating free estradiol levels (Ho *et al*., 1987). The close association between sleep onset and GH secretory bursts is still present in subjects submitted

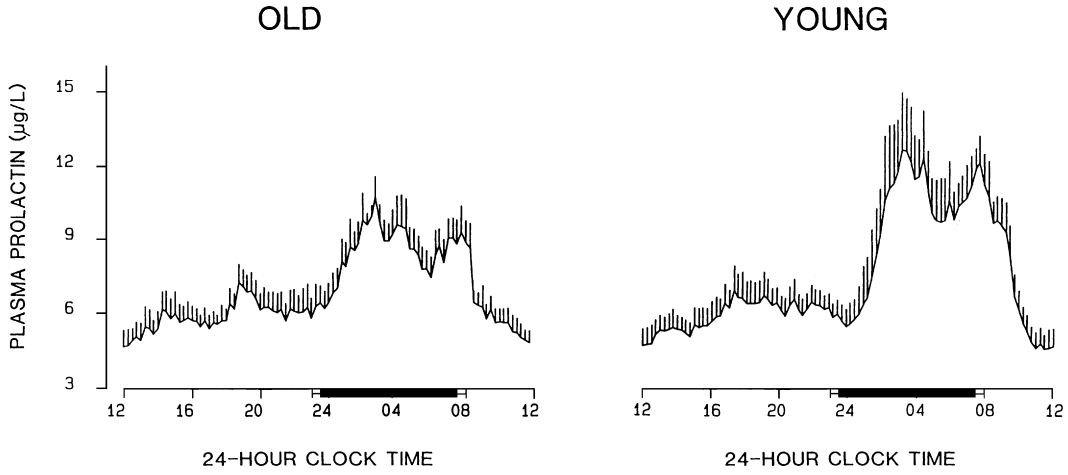

FIG. 60.4. Mean 24 hr (± SEM) profiles of plasma prolactin in normal men ages 67–84 years (left) and 20–27 years (right). Black bars denote the bedtime periods. Adapted from van Coevorden *et al*. (1991).

to various manipulations of the sleep–wake cycle, including recovery sleep following sleep deprivation, sleep interruptions followed by reinitiations of sleep, phase advances, and phase delays (Takahashi *et al*., 1968; Sassin *et al*., 1969; Beck *et al*., 1975; Golstein *et al*., 1983; Davidson *et al*., 1991; Van Cauter *et al*., 1991, 1992; Pietrowsky *et al*., 1994; Weibel *et al*., 1997). Thus, shifts of the sleep–wake cycle are immediately followed by parallel shifts of the GH rhythm. This is observed after transmeridian shifts (Golstein *et al*., 1983), in shift work conditions (Weibel *et al*., 1997), and in subjects living in free-running conditions, i.e., in temporal isolation without any environmental time cues (Weitzman *et al*., 1981; Moline *et al*., 1986). However, modest GH secretory pulses may persist during waking in the late evening and in the early part of the night following abrupt delays of the sleep period, indicating the existence of a modulation of the somatotropic axis by circadian rhythmicity (Aschoff, 1979; Van Cauter *et al*., 1992). It has been shown that during nocturnal sleep, the major GH pulse occurring following sleep onset is caused by a sleep-associated surge of hypothalamic GH-releasing hormone (GHRH) which coincides with a circadian period of relative somatostatin disinhibition (Jaffe *et al*., 1995; Ocampo-Lim *et al*., 1996).

The relationship between sleep stages and nocturnal GHRH surges—resulting in GH secretory bursts—can be investigated using a deconvolution procedure to estimate GH secretory rates. This procedure provides an accurate estimation of the amount of GH released during each secretory pulse and allows to delineate precisely the temporal limits of each pulse. A robust relationship has been evidenced between slow-wave sleep and nocturnal GH secretion (Holl *et al*., 1991; Van Cauter *et al*., 1992). Thus, maximal GH release occurs within minutes of the onset of slow-wave sleep; the longer the slow-wave episode, the more likely it is to be associated with a GH pulse; the amount of GH secreted during pulses occurring during slow-wave sleep is quantitatively correlated with the duration of the slow-wave episode. Similar correlations have been evidenced between GH secretion and concomitant values of spectral power density of the electroencephalogram in the delta range, i.e., 0.5–4.0 Hz (Gronfier *et al*., 1996). However, this relationship between slow-wave sleep and GH secretion, although strong and consistent, is not obligatory, since nocturnal GH secretion may also occur in the absence of slow-wave sleep, and approximately one-third of the slow-wave periods are not associated with detectable GH secretion (Van Cauter *et al*., 1992). These dissociations could reflect variations in the somatostatinergic tone which exerts an inhibitory action on GH secretion (Jaffe *et al*., 1995).

The mechanisms underlying the relationship between slow-wave sleep and GH release are still a matter of speculation. Based on rodent data, it has been suggested that the promotion of slow-wave sleep and the stimulation of GH release are two separate processes which involve GHRH neurons situated in two distinct areas of the hypothalamus (Obál *et al*., 1991; Krueger and Obál, 1993; Bredow *et al*., 1996). Daytime pituitary GH release would primarily involve GHRH neurons in the arcuate nucleus, while promotion of slow-wave sleep would implicate GHRH neurons of other hypothalamic area(s) (Meister and Hökfelt, 1992; Toppila *et al*., 1997). The association between slow-wave sleep and GH release would reflect synchronous activity of GHRH neurons in these distinct regions. However, the existence of a quantitative relationship between various measures of slow-wave activity and the amount of GH secreted (Van Cauter *et al*., 1992, 1997; Gronfier *et al*., 1996) suggests that the GHRH neurons which are implicated in the promotion of slow-wave sleep also participate in the control of nocturnal pituitary GH secretion.

Aging is associated with dramatic decreases in circulating levels of GH and insulin-like growth factor I (IGF-I) (Ho *et al*., 1987; van Coevorden *et al*., 1991; Landin-Wilhelmsen *et al*., 1994). This is illustrated in Fig. 60.5, which shows individual 24 hr GH profiles in young, middle aged, and old adult men. In normal men over 65 years, daily GH secretion is only about one-third of the amount secreted by young adults (Finkelstein *et al*., 1972; Ho *et al*., 1987; Vermeulen, 1987; Iranmanesh *et al*., 1991; van Coevorden *et al*., 1991; Frank

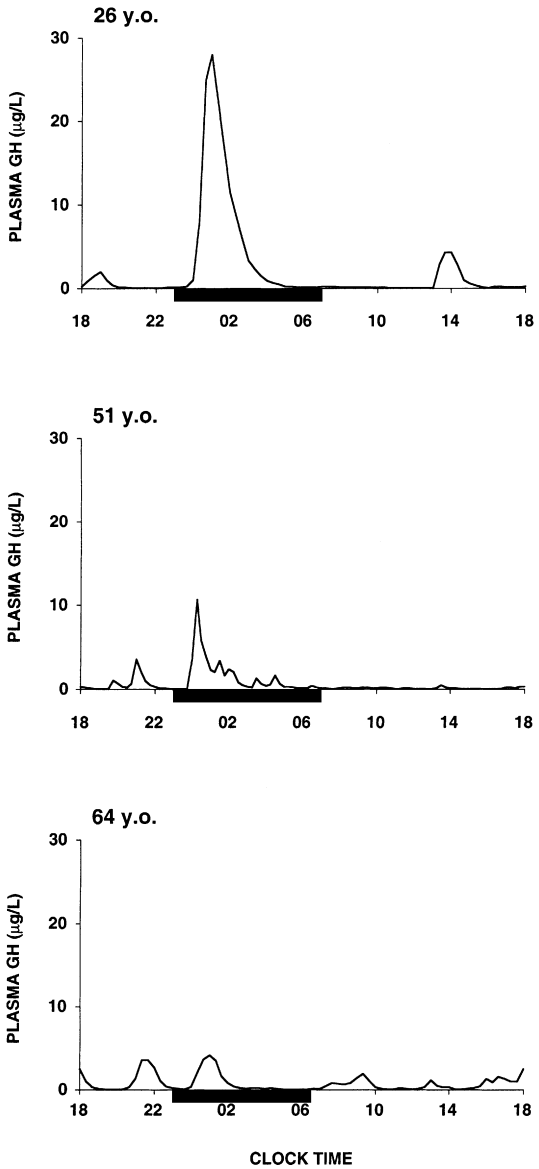

FIG. 60.5. 24 hr profiles of plasma GH in three normal men ages 26 years (top), 51 years (middle), and 64 years (bottom). Black bars denote the bedtime periods.

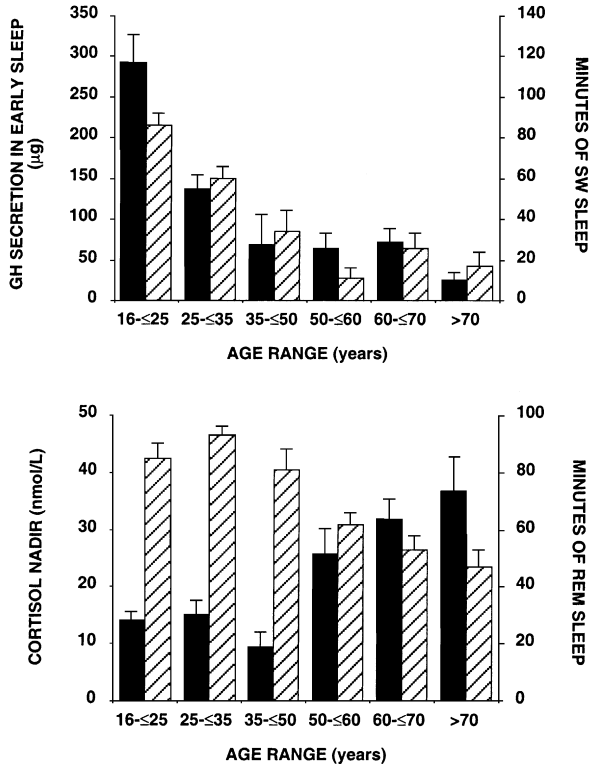

FIG. 60.6. (Top) Growth hormone secretion in early sleep (black bars) and minutes of slow-wave sleep (dashed bars) as a function of age in normal men. (Bottom) Value of cortisol nadir (black bars) and minutes of REM sleep (dashed bars) as a function of age in normal men. All values are mean ± SEM. Data source: Van Cauter *et al*. (2000).

et al., 1995). This reduction is achieved by a decrease in amplitude, rather than in frequency, of GH pulses (Vermeulen, 1987; Veldhuis *et al*., 1995). Moreover, it has recently been suggested that the orderliness of GH secretion decreases with aging (Veldhuis *et al*., 1997). Interestingly, it has been shown that in men aging affects GH release —both during sleep and during wake—with a similar chronology to that observed for slow-wave sleep (Fig. 60.6, top), the age-related GH decrease is exponential, and despite the persistence of high levels of sex steroid hormones, circulating GH concentrations and pulsatile GH secretion rates fall in midlife to less than half the values achieved in young adulthood. This decrease is followed by further more progressive decrements from midlife to old age. In a cross-sectional study performed in 114 normal men (Van Cauter *et al*., 2000), the 24 hr GH secretion for ages 25–35 years and 60–70 years averaged around 50 and 30%, respectively, of the secretion for ages 16–25 years. These age effects are independent of modifications in the body mass index. Decreases in GH secretion rates and circulating levels are followed by a more gradual decline in plasma IGF-I levels (Clemmons and Van Wyk, 1984; Landin-Wilhelmsen *et al*., 1994). In the elderly, IGF-I levels are about 50% of the values in young adults, but there is a wide individual variation. About 20% of normal men beyond 60 years old have IGF-I values within the normal range of young (20–30 years old) men. Conversely, there is a considerable overlap between healthy elderly subjects and patients with GH deficiency due to pituitary disease.

The origin of this decrease in GH-IGF-I axis activity in aging is very probably multifactorial but the underlying mechanisms remain largely speculative. The major primary alteration appears to be an increase of hypothalamic secretion of somatostatin (Muller *et al*., 1995). The ability of the hypothalamus to synthesize and release GHRH and the intrinsic secretory capacity of somatotroph cells do not appear to be altered, but the secretion rate of GHRH is decreased, at least partly, because of the increased somatostatinergic tone (Martin *et al*., 1997). Thus, the reduction of GH secretion would result from increased somatostatin inhibitory activity and decreased GHRH responsiveness (Martin *et al*., 1997). In addition, the decrease in the orderliness of GH pulses suggests that the fine coordination of the GHRH–somatostatin interaction at the pituitary level may be impaired in the elderly and contribute to the blunting of GH secretion (Veldhuis *et al*., 1995). A possible increased negative feedback by GH and/or IGF-I in the elderly has been evoked to explain the increase in somatostatin, but a recent study suggests on the contrary that the negative feedback exerted by IGF-I on GH secretion is blunted in old age (Chapman *et al*., 1997). It has also been suggested that diminished cholinergic tone could be, at least partly, responsible for the somatostatin increase (Martin *et al*., 1997). The parallelism between GH and slow-wave sleep alterations in the elderly suggests the involvement of common mechanisms. Decreased physical activity as well as decreased sex steroid hormone concentrations in older men and women certainly contribute to the reduction in GH secretion (Veldhuis *et al*., 1997).

Normal elderly subjects display a number of features similar to those observed in young adults with GH deficiency due to pituitary disorders: increased body fat, reduced protein synthesis and protein turnover, reduced lean body mass, muscle strength and exercise capacity, decreased bone mass and bone mineral content, reduced renal blood flow and glomerular filtration, reduced cellular immunity (Cuneo *et al*., 1992; Corpas *et al*., 1993; Rosen *et al*., 1993). Since in young adults with GH deficiency, these features can be partly reversed by long-term treatment with GH (Marcus and Hoffman, 1998), it has been suggested that in older healthy subjects, they may result from relative GH-IGF-I deficit and represent a syndrome that has been named somatopause (Hoffman *et al*., 1993). However, the causal link between decreased GH-IGF-I activity and these somatic features in the elderly has not been proven.

Nevertheless, the effects of sustained recombinant human GH therapy in healthy older subjects have been investigated in a limited number of clinical trials, with mixed results (Rudman *et al*., 1991; Holloway *et al*., 1994; Papadakis *et al*., 1996). Though circulating IGF-I values were restored within the normal range for young adults and despite significant increases in lean body mass, no consistent improvement in functional capacities, as assessed by grip strength and endurance, could be demonstrated. Similarly, there was no consistent improvement in cognitive performances. Only slight beneficial effects on bone mineral density were evidenced. Moreover, there was a high prevalence of side effects, mainly edema, carpal tunnel syndrome, gynaecomastia, and hyperglycemia, possibly because the recombinant human GH dosages used were excessive.

Treatment with rhGH, which needs daily—or at least three weekly—subcutaneous injections, does not actually restore a

physiological GH-IGF-I profile, even if circulating IGF-I levels are carefully monitored and rhGH doses adjusted accordingly. Indeed, because of the very broad range of normal IGF-I values, the goal to be reached in each individual cannot be defined with precision. Morever, it is impossible to preserve the normal pulsatile pattern of GH secretion. In addition, GH injections inhibit GHRH secretion and are therefore likely to have detrimental effects on slow-wave sleep, since GHRH is involved in the generation of slow-wave sleep (Kerkhofs *et al*., 1993).

Preliminary investigations suggest that orally active pharmacological GH secretagogues might potentially be used during the somatopause to both stimulate GH secretion and enhance slow-wave sleep. Prolonged oral administration of an experimental GH secretagogue, MK-0677, was found to stimulate pulsatile GH release and to increase IGF-I levels in normal young and old subjects (Chapman *et al*., 1996; Copinschi *et al*., 1996). Moreover, it was also found to have beneficial effects on sleep, with significant increases in the duration of stage IV and of REM sleep (Copinschi *et al*., 1997). Interestingly, in normal young subjects, the rise in GH levels appeared to be less important than the enhancement of IGF-I values (Copinschi *et al*., 1996), possibly because elevated IGF-I levels exert a negative feedback effect on GH secretion. This relative dissociation between increases in GH and IGF-I levels might reduce the occurrence of negative side effects of the treatment, although MK-0677 had an adverse effect on glucose tolerance in older subjects (Chapman *et al*., 1996).

Two recent studies indicate that compounds known to stimulate slow-wave sleep could represent a novel class of GH secretagogues and be potentially useful for the treatment of somatopause. Reliable stimulation of both slow-wave sleep and GH secretion has been obtained in normal young subjects with oral administration of ritanserin, a selective serotonin 2 receptor antagonist (Gronfier *et al*., 1996), as well as with low doses of γ-hydroxybutyrate (Van Cauter *et al*., 1997), a simple four-carbon fatty acid which is used as an investigational drug for the treatment of narcolepsy.

Thus, experimental approaches which might mimic natural GH secretory patterns and improve sleep quality would appear more promising than recombinant human GH administration. The question still remains, however, as to whether the aim of restoring GH and IGF-I levels to the normal young range is legitimate. If that was the case, such interventions should target early midlife rather than subjects over 60 years of age, in whom peripheral tissues have been continuously exposed to very low GH and IGF-I levels for at least two decades. Indeed, initiating in subjects over 60 years old a treatment aiming at restoring GH secretion to the normal young range of may be compared to starting an estrogen replacement therapy in postmenopausal women when they are 70 years old.

IV. Thyrotropin: A Hormone Controlled by Both Sleep–Wake Homeostasis and Circadian Timing

The 24 hr pattern of circulating thyrotropin levels appears to be generated by frequency as well as amplitude modulation of secretory pulses (Veldhuis *et al*., 1990). Daytime levels are low and relatively stable. In normal young adults, the nocturnal rise starts in the early evening and the maximum occurs around the beginning of the sleep period. This presleep elevation is interrupted by sleep onset and levels progressively decline throughout the sleep period toward low daytime values (Brabant *et al*., 1990). Because the onset of the nocturnal rise occurs well before sleep onset, it is considered to reflect a circadian effect. Conversely, the decline in thyrotropin levels observed following sleep onset is thought to reflect an inhibitory influence of sleep on thyrotropin secretion (Parker *et al*., 1976). Indeed, during sleep deprivation, the nocturnal decline does not occur and thyrotropin levels continue to increase until the middle of the usual sleep period, as illustrated in Fig. 60.7, left). The sleep-related thyrotropin inhibition appears to be associated with slow-wave stages (Goichot *et al*., 1992). Conversely, awakenings are frequently associated with thyrotropin increments (Hirschfeld *et al*., 1996). Interestingly, thyrotropin levels are not suppressed significantly below normal daytime levels when sleep occurs during daytime hours (Hirschfeld *et al*., 1996). Thus, the inhibitory action of sleep on thyrotropin secretion appears to be operative only when the circadian elevation has occurred. While the onset of the thyrotropin nocturnal rise may be considered as a robust marker of the circadian clock, the diurnal thyrotropin profile appears to be a good illustration of the interaction between sleep and circadian rhythmicity.

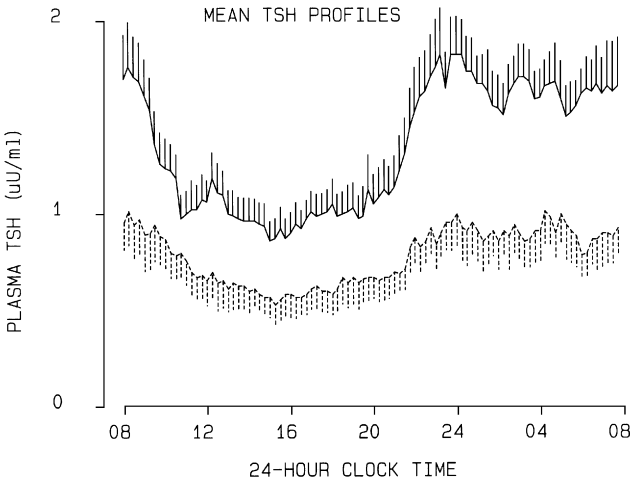

FIG. 60.7. Mean ($\pm$ SEM) thyrotropin profiles in normal young men ages 22–27 years and normal old men ages 59–72 years during a 52 hr period including 8 hr of nocturnal sleep, 28 hr of sleep deprivation, and 8 hr of daytime sleep. Black bars denote the bedtime periods.

Aging is associated with a progressive decrease in overall thyrotropin secretion (which is achieved by a decrease in amplitude, rather than in frequency, of secretory pulses) and in circulating thyrotropin levels, and with a dampening of the amplitude of the circadian variation (van Coevorden *et al*., 1991). This is illustrated in Fig. 60.7 (right) and 60.8. Figure 60.7 shows a comparison of thyrotropin profiles obtained during a 53 hr study period including 8 hr of nocturnal sleep, 28 hr of sleep deprivation, and 8 hr of daytime sleep in normal young men ages 22–27 years and normal old men ages 59–72 years, while Fig. 60.8 shows a comparison of 24 hr thyrotropin profiles in normal young men ages 20–27

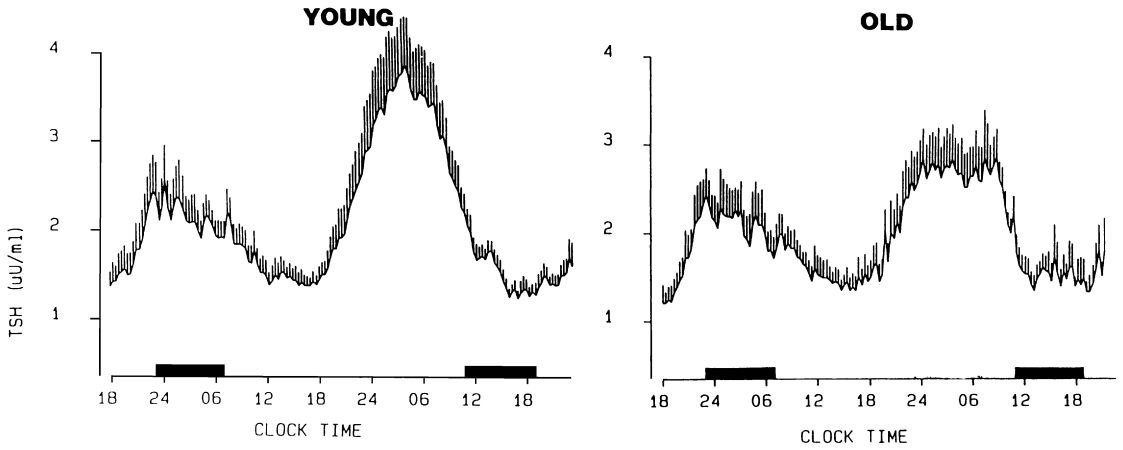

FIG. 60.8. Mean (± SEM) 24 hr thyrotropin profiles in normal young men ages 20–27 years (solid lines) and normal old men ages 67–84 years (dashed lines). From van Coevorden *et al.* (1991).

years and normal old men ages 67–83 years. In the older elderly (Fig. 60.8), thyrotropin levels are clearly lower than in young adults thoughout the 24 hr span, although the difference is more marked during sleep than during the daytime period. In the younger elderly (Fig. 60.7), age-related decreases in thyrotropin levels are apparent only during the period of nocturnal sleep deprivation. Thus, it appears that the thyrotropin secretory capacity declines progressively with aging.

The timing of the onset of the nocturnal rise was found to be advanced by approximately 1.5 hr in a group of normal old men ages 67–84 years, as compared to subjects under 20 years of age (van Coevorden *et al.*, 1991). This finding is consistent with a phase advance of the circadian clock in the elderly.

V. Hormones Primarily Controlled by the Circadian Clock

A. Melatonin

Not surprisingly, in view of the neuroanatomical connection between the hypothalamic suprachiasmatic nuclei and the pineal gland, the 24 hr profile of plasma melatonin is a robust marker of the human circadian clock (Rosenthal, 1991). During daytime, circulating levels are low and stable. In normal young adults, the circadian rise starts in the evening, between 21:00 and 23:00 hr, and the maximum occurs around the middle of the sleep period. Thereafter, melatonin levels progressively decrease to return to low daytime values in the morning, between 08:00 and 09:00 hr. Although a recent study indicated that high-intensity exercise may have acute stimulatory effects on melatonin secretion when applied during the night (Buxton *et al.*, 1997), melatonin rhythmicity is otherwise primarily dependent on the circadian pacemaker and does not appear to be directly affected by sleep and nonphotic stimuli (Geoffriau *et al.*, 1998). In contrast, exposure to light of sufficient intensity (>200–500 lux) exerts immediate direct inhibitory effects on the melatonin secretion, resulting in a dose-dependent suppression of nocturnal melatonin levels (Lewy *et al.*, 1980).

Daytime levels of melatonin are similar in young and old normal adults as illustrated in Fig. 60.9. The nocturnal elevation is markedly dampened in the elderly (van Coevorden *et al.*, 1991), although some old people may have melatonin peak values within the normal range for young adults. This is illustrated in Fig. 60.8. Moreover, as shown in Fig. 60.10, the circadian rise occurs almost 1.5 hr earlier in older than in young adults (van Coevorden *et al.*, 1991).

B. Cortisol

Diurnal profiles of plasma cortisol reflect the circadian pattern of adrenocorticotropic activity (which, in turn, results from periodic changes in level of pituitary stimulation by corticotropin-releasing hormone). The 24 hr rhythm of plasma cortisol is a good model for estimating the circadian temporal organization of the corticotropic axis because of its reproducibility and large amplitude. Mathematical procedures (Cleveland, 1979; Van Cauter, 1979) have been used to quantify the 24 hour profile of plasma cortisol and to determine times of occurrence and values of the maximum (acrophase) and the minimum (nadir) of the circadian rhythm as well as its amplitude. In young normal adults, plasma cortisol profiles show an early morning maximum around 07:00–08:00 hr, declining levels during the daytime, followed by a prolonged period of minimal levels (sometimes referred to as the quiescent period) centered around midnight and an abrupt elevation (referred to as the circadian rise) during the later part of the night (Fig. 60.11) (Van Cauter and Spiegel, 1999). This pattern is primarily controlled by the circadian pacemaker and is produced by modulation of the height of successive secretory pulses (Veldhuis *et al.*, 1989). However, modulatory effects are also exerted by sleep–wake homeostasis. Indeed, sleep onset is consistently associated with a short-term inhibition of cortisol secretion (Weitzman *et al.*, 1983; Born *et al.*, 1988; Van Cauter *et al.*, 1991; Bierwolf *et al.*, 1997). This inhibitory effect of sleep appears to be related to slow-wave stages (Follenius *et al.*, 1992). Conversely, during the second part of the night, awakenings—and particularly the final morning awakening—are consistently followed by bursts of cortisol secretion (Van Cauter *et al.*, 1990, 1991; Spath-Schwalbe *et al.*, 1991; Pruessner *et al.*, 1997). Nevertheless, adaptation of the 24 hr pattern of cortisol to abrupt shifts of the sleep–

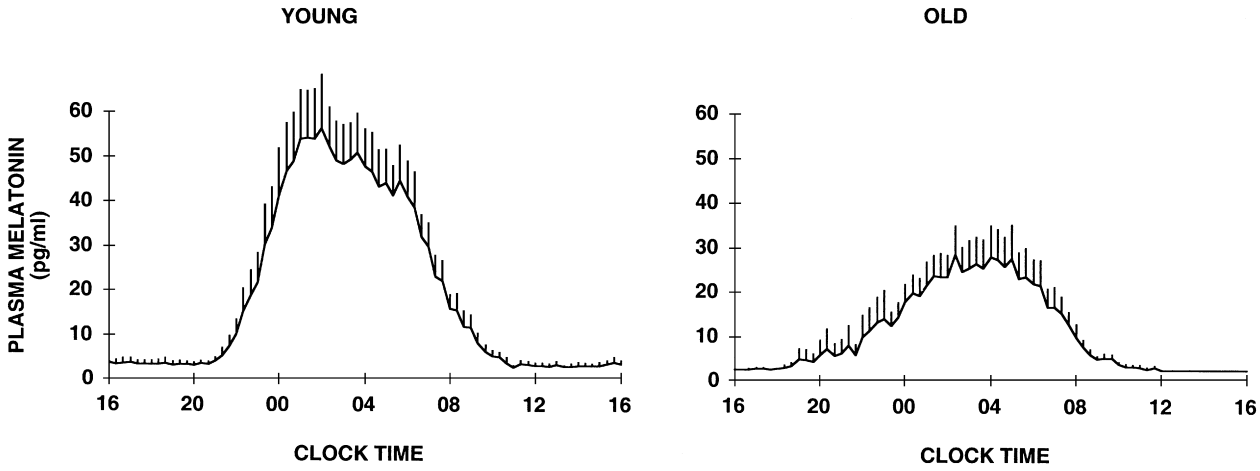

FIG. 60.9. Mean (± SEM) 24 hr profiles of plasma melatonin in normal men ages 20–27 years (left) and 67–84 years (right). Adapted from van Coevorden *et al.* (1991).

wake cycle needs several days to be complete and therefore the 24 hr profile of plasma cortisol, especially the timing of the onset of the early morning circadian rise, may be considered as a robust marker of circadian timing (Van Cauter and Turek, 1995). This morning rise represents a response to an endogenous stimulatory signal timed by the circadian clock. On the other hand, the subsequent decline of cortisol levels and the occurrence and the maintenance of the quiescent period all partially reflect the recovery of the corticotropic axis from this endogenous challenge.

Aging is associated with marked gender-specific effects on the levels and diurnal variation of plasma cortisol. This was examined in a retrospective analysis of 24 hr cortisol profiles recorded in 90 normal men and 87 normal women, with ages spanning seven decades, from 18 to 83 years (Van Cauter *et al.*, 1996). This study indicates that although diurnal rhythmicity is preserved in old age, alterations of the cortisol profiles, which essentially develop from 50 years of age onward, are present in the elderly, as illustrated for men in Fig. 60.11. A modest but significant increase in 24 hr cortisol levels from young adulthood to old age is observed for both sexes. Between 20 and 80 years of age, basal cortisol levels increase by 20–50%, more in women than in men, so that cortisol levels, which are slightly higher in young men than in young women, become

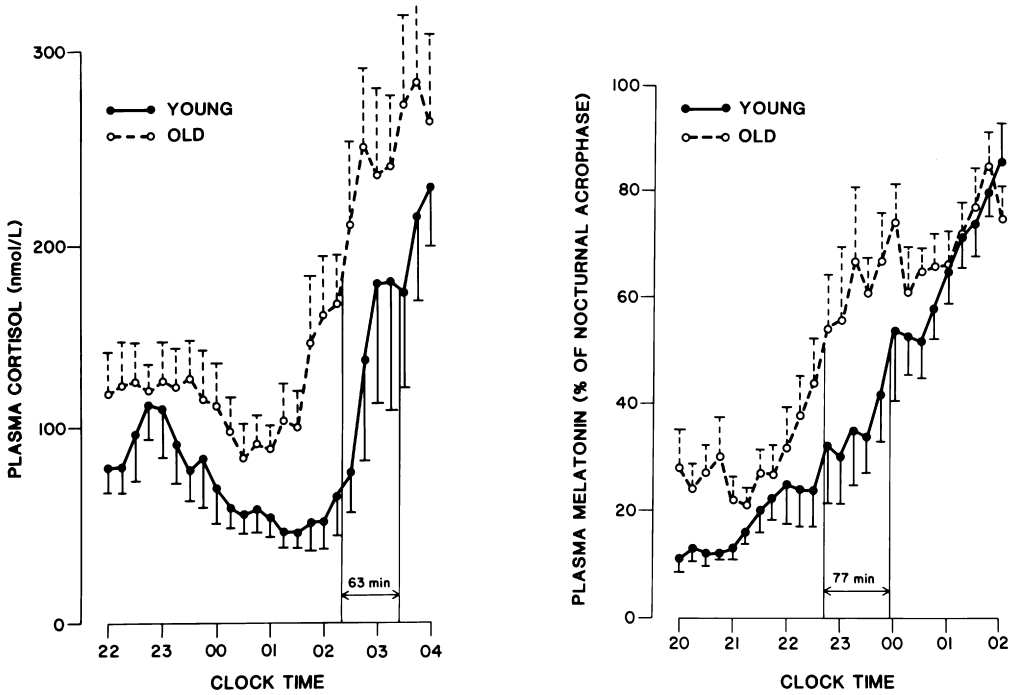

FIG. 60.10. Mean (± SEM) profiles of the circadian rises of cortisol (left) and melatonin (right) in normal men ages 20–27 years (solid lines) and 67–84 years (dashed lines). Adapted from van Coevorden *et al.* (1991).

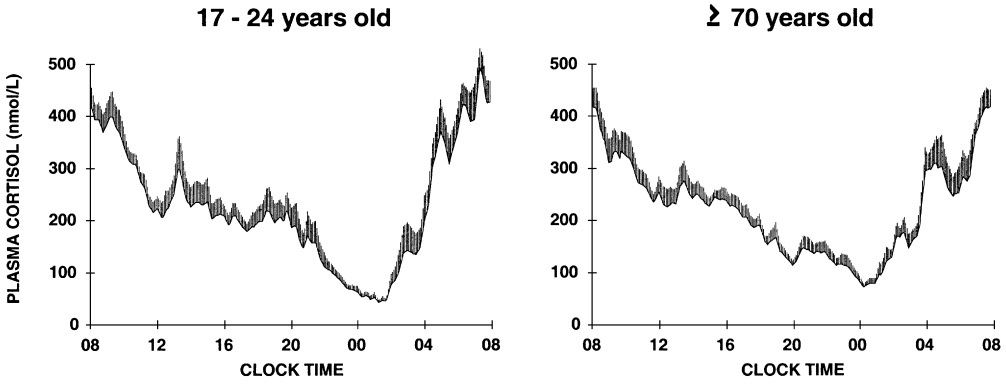

FIG. 60.11. Mean (± SEM) 24 hr profiles of plasma cortisol in normal men ages 17–24 years (left) and over 70 years (right).

similar in older men and women. With advancing age, both evening and morning cortisol levels increase in women, while only evening levels rise in men. Typically, the cortisol nadir in a subject over 70 years of age is three- to four-fold higher than in a young adult. As a result, a dampening of the amplitude of the circadian variation is observed with aging in both sexes. While there is no effect of age on the timing of the morning acrophase, the timing of the nadir and the timing of the onset of the circadian rise advance markedly with aging, similarly in men and in women. Between 20 and 80 years of age, the timing of the onset of the circadian rise—a marker of the circadian phase—advances 1.5 to 3 hr, as illustrated in Fig. 60.11. The quiescent period starts later and ends earlier in older subjects than in young adults. As a result, the duration of the quiescent period is markedly shortened and increasingly fragmented, as illustrated in Fig. 60.12. Between 25 and 65 years of age, the reduction in the duration of the quiescent period averages almost 3 hr in men and approximately 4.5 hr in women, so that the quiescent period, which is somewhat longer in young women than in young men, has a similar duration in older men and women.

These data confirm that in late adulthood, aging is associated with an advance of circadian phase and a dampening of the amplitude of the circadian variations which suggests that the strength of the signal originating from the hypothalamic pacemaker decreases with advancing age. The major and

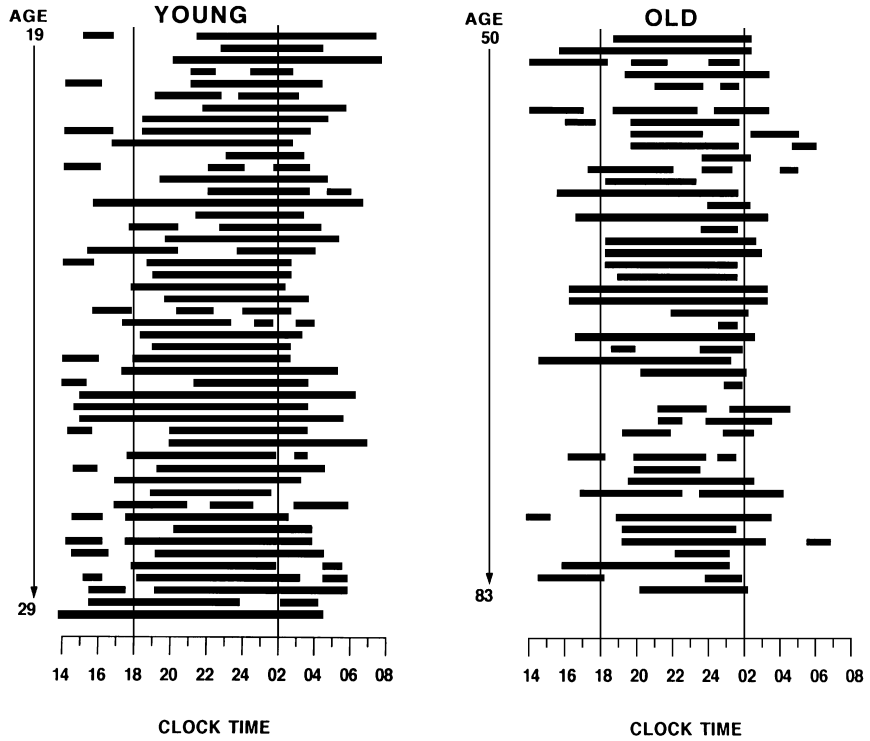

FIG. 60.12. Individual quiescent periods (represented by black bars) in young (ages 19–29 years; left) and old (ages 50–83 years; right) normal men. Vertical lines at 18:00 and 02:00 hr serve to facilitate the visual estimation of onsets and offsets of quiescent periods. Note the later onset, the earlier offset, and the increased fragmentation of quiescent periods in older subjects. Adapted from Van Cauter *et al*. (1996).

most consistent age-related alteration in cortisol levels is an elevation of the evening nadir. Both animal and human studies have indicated that deleterious effects of hyperactivity of the hypothalamo–pituitary–adrenal axis, especially at the hippocampal level, are more pronounced at the time of the nadir of the rhythm than at the time of the peak (Dallman *et al*., 1993; Plat *et al*., 1999). Thus, even modest elevations in evening cortisol levels could facilitate the development of central and peripheral disturbances associated with glucocorticoid excess, such as insulin resistance and memory deficits (McEwen and Stellar, 1993; McEwen, 1998a). The age-related increase in evening cortisol levels is likely to reflect a loss of resiliency of the hypothalamo–pituitary–adrenal axis (i.e. ability to recover from challenge), consistent with the concept of ''wear and tear'' of lifelong exposure to stress, and is likely to reflect neuronal loss in the hippocampus (McEwen, 1998b). Such hippocampal defects may underlie some of the memory deficits that occur in many older adults. In addition, the loss of resiliency could also contribute to the increased sleep fragmentation in the elderly, since elevated cortisol levels promote awakenings.

Interestingly, REM sleep is characterized by highly synchonized electroencephalographic activity in the 4- to 10-Hz ''theta frequency'' range in the hippocampus (Siegel, 1994). Whether hippocampal theta waves during REM sleep reflect a restorative action on neuronal mechanisms underlying the negative feedback regulation of glucocorticoid secretion is not known, but this hypothesis would be consistent with the fact that age-related alterations in REM sleep and in evening cortisol levels occur in a mirror image, as illustrated in Fig. 60.6 (bottom). Thus, decreased sleep quality could contribute to the allostatic load, i.e. to the wear and tear resulting from overactivity of stress-responsive systems (McEwen, 1998b).

VI. Conclusion

The transition from early adulthood to midlife is associated with profound alterations of sleep–wake homeostasis, resulting in particular in exponential decreases in slow-wave sleep and in GH secretion. Statistical analysis of the data shown in Fig. 60.6 indicates that the reduction in GH secretion was significantly associated with the reduction in amount of slow-wave sleep, independently of age. In contrast, alterations of the circadian pacemaker exert their effects more progressively, essentially during the transition from midlife to old age, resulting in particular in modifications of REM sleep and of the glucocorticoid profiles. Reduced amounts of REM sleep could be related to increased evening cortisol levels. Strategies for preventing or limiting such alterations should take into account this dissociated chronology. Pharmacological approaches to restore normal sleep could represent an indirect form of hormonal therapy with possible beneficial health effects.

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References

Achermann, P., and Borbély, A. A. (1990). Simulation of human sleep: Ultradian dynamics of electroencephalographic slow-wave activity. *J. Biol. Rhythms* **5**, 141–157.

Aschoff, J. (1979). Circadian rhythms: General features and endocrinological aspects. *In* ''Endocrine Rhythms'' (D. T. Krieger, ed.), pp. 1–61. Raven Press, New York.

Aschoff, J. (1981). A survey on biological rhythms. *In* ''Biological Rhythms'' (J. Aschoff, ed.), pp. 3–10. Plenum Press, New York.

Beck, U., Brezinova, V., Hunter, W. M., and Oswald, I. (1975). Plasma growth hormone and slow wave sleep increase after interruption of sleep. *J. Clin. Endocrinol. Metab.* **40**, 812–815.

Bierwolf, C., Struve, K., Marshall, L., and Fehm, H. L. (1997). Slow wave sleep drives inhibition of pituitary-adrenal secretion in humans. *J. Neuroendocrinol.* **9**, 479–484.

Bliwise, D. L. (1993). Sleep in normal aging and dementia. *Sleep* **16**, 40–81.

Bliwise, D. L. (1994). Normal aging. *In* ''Principles and Practice of Sleep Medicine'' (M. H. Kryger, T. Roth, and W. C. Dement, eds.), pp. 26–39. Saunders, Philadelphia.

Borbély, A. A. (1998). Processes underlying sleep regulation. *Horm. Res.* **49**, 114–117.

Born, J., Muth, S., and Fehm, H. L. (1988). The significance of sleep onset and slow wave sleep for nocturnal release of growth hormone (GH) and cortisol. *Psychoneuroendocrinology* **13**, 233–243.

Brabant, G., Prank, K., Ranft, U., Schuermeyer, T., Wagner, T. O. F., Hauser, H., Kummer, B., Feistner, H., Hesch, R. D., and von zür Mühlen, A. (1990). Physiological regulation of circadian and pulsatile thyrotropin secretion in normal man and woman. *J. Clin. Endocrinol. Metab.* **70**, 403–409.

Bredow, S., Taishi, P., Obál, F. J., Guha-Thakurta, N., and Krueger, J. M. (1996). Hypothalamic growth hormone-releasing hormone mRNA varies across the day in rats. *NeuroReport* **7**, 2501–2505.

Buxton, O. M., L'Hermite-Baleriaux, M., Hirshfeld, U., and Van Cauter, E. (1997). Acute and delayed effects of exercise on human melatonin secretion. *J. Biol. Rhythms* **12**, 568–574.

Campbell, S. S., Kripke, D. F., Gillin, J. C., and Hrubovcak, J. C. (1988). Exposure to light in healthy elderly subjects and Alzheimer's patients. *Physiol. Behav.* **42**, 141–144.

Chapman, I. A., Bach, M. A., Van Cauter, E., Farmer, M., Krupa, D., Taylor, A. M., Schilling, L. M., Cole, K. Y., Skyles, E. H., Pezzoli, S. S., Hartman, M. L., Veldhuis, J. D., Gormley, G. J., and Thorner, M. O. (1996). Stimulation of the growth hormone (GH)-insulin-like growth factor-I axis by daily oral administration of a GH secretagogue (MK-677) in healthy elderly subjects. *J. Clin. Endocrinol. Metab.* **81**, 4249–4257.

Chapman, I. M., Hartman, M. L., Pezzoli, S. S., Harrell, F. E., Jr., Hintz, R. L., Alberti, K. G., and Thorner, M. O. (1997). Effect of aging on the sensitivity of growth hormone secretion to insulin-like growth factor-I negative feedback. *J. Clin. Endocrinol. Metab.* **82**, 2996–3004.

Clemmons, D. R., and Van Wyk, J. J. (1984). Factors controlling blood concentration of somatomedin C. *Clin. Endocrinol. Metab.* **13**, 113–143.

Cleveland, W. S. (1979). Robust locally weighted regression and smoothing scatterplots. *J. Am. Stat. Assoc.* **74**, 829–836.

Copinschi, G., Van Onderbergen, A., L'Hermite-Balériaux, M., Mendel, C. M., Caufriez, A., Leproult, R., Bolognese, J. A., De Smet, M., Thorner, M. O., and Van Cauter, E. (1996). Effects of a 7-day treatment with a novel orally active nonpeptide growth hormone secretagogue, MK-677, on 24-hour growth hormone profiles, insulin-like growth factor-I and

adrenocortical function in normal young men. *J. Clin. Endocrinol. Metab.* **81**, 2776–2782.

Copinschi, G., Leproult, R., Van Onderbergen, A., Caufriez, A., Cole, K. Y., Mendel, C. M., De Lepeleire, I., Bolognese, J. A., and Van Cauter, E. (1997). Prolonged oral treatment with MK-677, a novel growth hormone secretagogue, improves sleep quality in man. *Neuroendocrinology* **66**, 278–286.

Corpas, E., Harman, S. M., and Blackman, M. R. (1993). Human growth hormone and human aging. *Endocr. Rev.* **14**, 20–39.

Cuneo, R. C., Salomon, F., McGauley, G. A., and Sonksen, P. H. (1992). The growth hormone deficiency syndrome in adults. *Clin. Endocrinol. (Oxford)* **37**, 387–397.

Czeisler, C. A., Weitzman, E. D., Moore-Ede, M. C., Zimmerman, J. C., and Knauer, R. S. (1980). Human sleep: Its duration and organization depends on its circadian phase. *Science* **210**, 1264–1267.

Czeisler, C. A., Allan, J. S., Strogatz, S. H., Ronda, J. M., Sanchez, R., Rios, C. D., Freitag, W. O., Richardson, G. S., and Kronauer, R. E. (1986). Bright light resets the human circadian pacemaker independent of the timing of the sleep-wake cycle. *Science* **233**, 667–671.

Czeisler, C. A., Dumont, M., Duffy, J. F., Steinberg, J. D., Richardson, G. S., Brown, E. N., Sanchez, R., Rios, D. C., and Ronda, J. M. (1992). Association of sleep-wake habits in older people with changes in output of circadian pacemaker. *Lancet* **340**, 933–936.

Czeisler, C. A., Duffy, J. F., Shanahan, T. L., Brown, E. N., Mitchell, J. F., Rimmer, D. W., Ronda, J. M., Silva, E. J., Allan, J. S., Emens, J. S., Dijk, D.-J., and Kronauer, R. E. (1999). Stability, precision, and near-24-hour period of the human circadian pacemaker. *Science* **284**, 2177–2181.

Dallman, M. F., Strack, A. L., Akana, S. F., Bradbury, M. J., Hanson, E. S., Scribner, K. A., and Smith, M. (1993). Feast and famine: Critical role of glucocorticoids with insulin in daily energy flow. *Front. Neuroendocrinol*. **14**, 303–347.

Davidson, J. R., Moldofsky, H., and Lue, F. A. (1991). Growth hormone and cortisol secretion in relation to sleep and wakefulness. *J. Psychiatr. Neurosci*. **16**, 96–102.

Desir, D., Van Cauter, E., L'Hermite, M., Refetoff, S., Jadot, C., Caufriez, A., Copinschi, G., and Robyn, C. (1982). Effects of ''jet lag'' on hormonal patterns. III. Demonstration of an intrinsic circadian rhythmicity in plasma prolactin. *J. Clin. Endocrinol. Metab.* **55**, 849–857.

Dijk, D. J., and Czeisler, C. A. (1995). Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity in humans. *J. Neurosci*. **15**, 3526–3538.

Finkelstein, J. W., Roffwarg, H. P., Boyar, R. M., Kream, J., and Hellman, L. (1972). Age-related change in the twenty-four-hour spontaneous secretion of growth hormone. *J. Clin. Endocrinol. Metab.* **35**, 665–670.

Follenius, M., Brandenberger, G., Bardasept, J., Libert, J., and Ehrhart, J. (1992). Nocturnal cortisol release in relation to sleep structure. *Sleep* **15**, 21–27.

Frank, S., Roland, D. C., Sturis, J., Byrne, M. M., Spire, J. P., Refetoff, S., Polonsky, K. S., and Van Cauter, E. (1995). Effects of aging on glucose regulation during wakefulness and sleep. *Am. J. Physiol*. **269**, E1006–E1016.

Geoffriau, M., Brun, J., Chazot, G., and Claustrat, B. (1998). The physiology and pharmacology of melatonin in humans. *Horm. Res*. **49**, 136–141.

Goichot, B., Brandenberger, G., Saini, J., Wittersheim, G., and Follenius, M. (1992). Nocturnal plasma thyrotropin variations are related to slow-wave sleep. *J. Sleep Res*. **1**, 186–190.

Golstein, J., Van Cauter, E., Desir, D., Noel, P., Spire, J., Refetoff, S., and Copinschi, G. (1983). Effects of ''jet lag'' on hormonal patterns. IV. Time shifts increase growth hormone release. *J. Clin. Endocrinol. Metab.* **56**, 433–440.

Gronfier, C., Luthringer, R., Follenius, M., Schaltenbrand, N., Macher, J. P., Muzet, A., and Brandenberger, G. (1996). A quantitative evaluation of the relationships between growth hormone secretion and delta wave electroencephalographic activity during normal sleep and after enrichment in delta waves. *Sleep* **19**, 817–824.

Hirschfeld, U., Moreno-Reyes, R., Akseki, E., L'Hermite-Balériaux, M., Leproult, R., Copinschi, G., and Van Cauter, E. (1996). Progressive elevation of plasma thyrotropin during adaptation to simulated jet lag: Effects of treatment with bright light or zolpidem. *J. Clin. Endocrinol. Metab.* **81**, 3270–3277.

Ho, K. Y., Evans, W. S., Blizzard, R. M., Veldhuis, J. D., Merriam, G. R., Samojlik, E., Furlanetto, R., Rogol, A. D., Kaiser, D. L., and Thorner, M. O. (1987). Effects of sex and age on the 24-hour profile of growth hormone secretion in man: Importance of endogenous estradiol concentrations. *J. Clin. Endocrinol. Metab.* **64**, 51–58.

Hoffman, A. R., Pyka, G., Lieberman, S. A., Ceda, G. P., and Marcus, R. (1993). The somatopause. *In* ''Growth Hormone and Somatomedins During Life Span'' (E. E. Muller, D. Cocchi, and V. Locatelli, eds.), pp. 265–274. Springer-Verlag, Berlin.

Holl, R. W., Hartmann, M. L., Veldhuis, J. D., Taylor, W. M., and Thorner, M. O. (1991). Thirty-second sampling of plasma growth hormone in man: Correlation with sleep stages. *J. Clin. Endocrinol. Metab.* **72**, 854–861.

Holloway, L., Butterfield, G., Hintz, R. L., Gesundheit, N., and Marcus, R. (1994). Effects of recombinant human growth hormone on metabolic indices, body composition, and bone turnover in healthy elderly women. *J. Clin. Endocrinol. Metab.* **79**, 470–479.

Honda, Y., Takahashi, K., Takahashi, S., Azumi, K., Irie, M., Skuma, M., Tsushima, T., and Shizume, K. (1969). Growth hormone secretion during nocturnal sleep in normal subjects. *J. Clin. Endocrinol. Metab.* **29**, 20–29.

Iranmanesh, A., Lizarralde, G., and Veldhuis, J. D. (1991). Age and relative adiposity are specific negative determinants of the frequency and amplitude of growth hormone (GH) secretory bursts and the half-life of endogenous GH in healthy men. *J. Clin. Endocrinol. Metab.* **73**, 1081–1088.

Jaffe, C., Turgeon, D., DeMott Friberg, R., Watkins, P., and Barkan, A. (1995). Nocturnal augmentation of growth hormone (GH) secretion is preserved during repetitive bolus administration of GH-releasing hormone: Potential involvement of endogenous somatostatin - A clinical research center study. *J. Clin. Endocrinol. Metab.* **80**, 3321–3326.

Kerkhofs, M., Van Cauter, E., Van Onderbergen, A., Caufriez, A., Thorner, M. O., and Copinschi, G. (1993). Sleep-promoting effects of growth hormone-releasing hormone in normal men. *Am. J. Physiol*. **264**, E594–E598.

Krueger, J., and Obál, F. J. (1993). Growth hormone-releasing hormone and interleukin-1 in sleep regulation. *FASEB J*. **7**, 645–652.

Landin-Wilhelmsen, K., Wilhelmsen, L., Lappas, G., Rosen, T., Lindstedt, G., Lundberg, P., and Bengtsson, B. (1994). Serum insulin-like growth factor I in a random population sample of men and women: Relation to age, sex, smoking habits, coffee consumption and physical activity, blood pressure and concentrations of plasma lipids, fibrinogen, parathyroid hormone and osteocalcin. *Clin. Endocrinol. (Oxford)* **41**, 351–357.

Lewy, A. J., Wehr, T. A., Goodwin, F. K., Newsome, D. A., and Markey, S. P. (1980). Light suppresses melatonin secretion in humans. *Science* **210**, 1267–1269.

Lewy, F. J., and Newsome, D. A. (1983). Different types of melatonin circadian secretory rhythms in some blind subjects. *J. Clin. Endocrinol. Metab.* **56**, 1103–1107.

Marcus, M., and Hoffman, A. R. (1998). Growth hormone as therapy for older men and women. *Annu. Rev. Pharmacol. Toxicol*. **38**, 45–61.

Martin, F. C., Yeo, A. L., and Sonksen, P. H. (1997). Growth hormone secretion in the elderly: Ageing and the somatopause. *Baillières Clin. Endocrinol. Metab*. **11**, 223–250.

McEwen, B. S. (1998a). Protective and damaging effects of stress mediators. *N. Engl. J. Med*. **338**, 171–179.

McEwen, B. S. (1998b). Stress, adaptation, and disease. Allostasis and allostatic load. *Ann. N. Y. Acad. Sci*. **840**, 33–44.

McEwen, B. S., and Stellar, E. (1993). Stress and the individual. *Arch. Intern. Med*. **153**, 2093–2101.

Meister, B., and Hökfelt, T. (1992). The somatostatin and growth hormone-releasing factor systems. *In* "Neuroendocrinology" (C. B. Nemeroff, ed.), pp. 219–278. CRC Press, Boca Raton, FL.

Miller, J. D., Morin, L. P., Schwartz, W. J., and Moore, R. Y. (1996). New insights ito the mammalian circadian clock. *Sleep* **19**, 641–667.

Moline, M., Monk, T., Wagner, D., Pollak, C., Kream, J., Fookson, J., Weitzman, E., and Czeisler, C. (1986). Human growth hormone release is decreased during sleep in temporal isolation (free-running). *Chronobiologia* **13**, 13–19.

Monk, T. H., Buysse, D. J., Reynolds, C. F. I., Kupfer, D. J., and Houck, P. (1995). Circadian temperature rhythms of older people. *Exp. Gerontol*. **30**, 455–474.

Moore-Ede, M. C. (1982)." The Clocks that Time Us." Harvard University Press, Cambridge, MA.

Muller, E. E., Cella, S. G., Parenti, M., Deghenghi, R., Locatelli, V., De Gennaro Colonna, V., Torsello, A., and Cocchi, D. (1995). Somatotropic dysregulation in old mammals. *Horm. Res*. **43**, 39–45.

Obál, F. J., Payne, L., Kapás, L., Opp, M., Alföldi, P., and Krueger, J. M. (1991). Growth hormone releasing hormone (GHRH) in sleep regulation. *Sleep Res*. **20A**, 192.

Ocampo-Lim, B., Guo, W., DeMott Friberg, R., Barkan, A. L., and Jaffe, C. A. (1996). Nocturnal growth hormone (GH) secretion is eliminated by infusion of GH- releasing hormone antagonist. *J. Clin. Endocrinol. Metab.* **81**, 4396–4399.

Okatani, Y., and Sagara, Y. (1993). Role of melatonin in nocturnal prolactin secretion in women with normoprolactinemia and mild hyperprolactinemia. *Am. J. Obstet. Gynecol*. **168**, 854–861.

Papadakis, M. A., Grady, D., Black, D., Tierney, M. J., Gooding, G. A., Schambelan, M., and Grunfeld, C. (1996). Growth hormone replacement in healthy older men improves body composition but not functional ability. *Ann. Intern. Med*. **124**, 708–716.

Parker, D. C., Pekary, A. E., and Hershman, J. M. (1976). Effect of normal and reversed sleep-wake cycles upon nyctohemeral rhythmicity of thyrotropin (TSH): Evidence suggestive of an inhitory influence in sleep. *J. Clin. Endocrinol. Metab*. **43**, 315–322.

Pietrowsky, R., Meyrer, R., Kern, W., Born, J., and Fehm, H. (1994). Effects of diurnal sleep on secretion of cortisol, luteinizing hormone, and growth hormone in man. *J. Clin. Endocrinol. Metab.* **78**, 683–687.

Pittendrigh, C. S., and Daan, S. (1974). Circadian oscillations in rodents: A systematic increase of their frequency with age. *Science* **186**, 548–550.

Plat, L., Féry, F., L'Hermite-Balériaux, M., Mockel, J., and Van Cauter, E. (1999). Metabolic effects of short-term physiological elevations of plasma cortisol are more pronounced in the evening than in the morning. *J. Clin. Endocrinol. Metab*. **84**, 3082–3092.

Prinz, P. N. (1995). Sleep and sleep disorders in older adults. *J. Clin. Neurophysiol*. **12**, 139–146.

Prinz, P. N., Vitiello, M. V., Raskind, M. A., and Thorpy, M. J. (1990). Geriatrics: Sleep disorders and aging. *N. Engl. J. Med*. **323**, 520–526.

Pruessner, J. C., Wolf, O. T., Hellhammer, D. H., Buske-Kirschbaum, A., von Auer, K., Jobst, S., Kaspers, F., and Kirschbaum, C. (1997). Free cortisol levels after awakening: A reliable biological marker for the assessment of adrenocortical activity. *Life Sci*. **61**, 2539–2549.

Quabbe, H., Schilling, E., and Helge, H. (1966). Pattern of growth hormone secretion during a 24-hour fast in normal adults. *J. Clin. Endocrinol. Metab.* **26**, 1173–1177.

Rosen, T., Hansson, T., Granhed, H., Szucs, J., and Bengtsson, B. A. (1993). Reduced bone mineral content in adult patients with growth hormone deficiency. *Acta Endocrinol. (Copenhagen)* **129**, 201–206.

Rosenthal, N. E. (1991). Plasma melatonin as a measure of the human clock. *J. Clin. Endocrinol. Metab.* **73**, 225–226.

Rudman, D., Feller, A. G., Cohn, L., Shetty, K. R., Rudman, I. W., and Draper, M. W. (1991). Effects of human growth hormone on body composition in elderly men. *Horm. Res*. **36** (Suppl. 1), 73–81.

Rusak, B., and Zucker, I. (1979). Neural regulation of circadian rhythms. *Physiol. Rev.* **59**, 449–526.

Sassin, J. F., Frantz, A. G., Weitzman, E. D., and Kapen, S. (1972). Human prolactin: 24-hour pattern with increased release during sleep. *Science* **177**, 1205–1207.

Sassin, J. F., Frantz, A. G., Kapen, S., and Weitzman, E. D. (1973). The nocturnal rise of human prolactin is dependent on sleep. *J. Clin. Endocrinol. Metab.* **37**, 436–440.

Sassin, J. F., Parker, D. C., Mace, J. W., Gotlin, R. W., Johnson, L. C., and Rossman, L. G. (1969). Human growth hormone release: Relation to slow-wave sleep and sleep-waking cycles. *Science* **165**, 513–515.

Siegel, J. M. (1994). Brainstem mechanisms generating REM sleep. *In* "Principles and Practice of Sleep Medicine" (M. H. Kryger, T. Roth, and W. C. Dement, eds.), pp. 125–144. Saunders, Philadelphia.

Spath-Schwalbe, E., Gofferje, M., Kern, W., Born, J., and Fehm, H. L. (1991). Sleep disruption alters nocturnal ACTH and cortisol secretory patterns. *Biol. Psychiatry* **29**, 575–584.

Spiegel, K., Follenius, M., Simon, C., Saini, J., Ehrhart, J., and Brandenberger, G. (1994). Prolactin secretion and sleep. *Sleep* **17**, 20–27.

Spiegel, K., Luthringer, R., Follenius, M., Schaltenbrandt, N., Macher, J. P., Muzet, A., and Brandenberger, G. (1995). Temporal relationship between prolactin secretion and slow-wave electroencephalographic activity during sleep. *Sleep* **18**, 543–548.

Swaab, D. F., Fisser, B., Kamphorst, W., and Troost, D. (1988). The human suprachiasmatic nucleus; neuropeptide changes in senium and Alzheimer's disease. *Basic Appl. Histochem*. **32**, 43–54.

Takahashi, Y., Kipnis, D. M., and Daughaday, W. H. (1968). Growth hormone secretion during sleep. *J. Clin. Invest*. **47**, 2079–2090.

Toppila, J., Alanko, L., Asikainen, M., Tobler, I., Stenberg, D., and PorkKa-Heiskanen, T. (1997). Sleep deprivation increases somatostatin and growth hormone-releasing hormone messenger RNA in the rat hypothalamus. *J. Sleep Res*. **6**, 171–178.

Turek, F. W. (1998). Circadian rhythms. *Horm. Res*. **49**, 103–113.

Van Cauter, E. (1979). Method for characterization of 24-h temporal variation of blood constituents. *Am. J. Physiol*. **237**, E255–E264.

Van Cauter, E., and Refetoff, S. (1985). Multifactorial control of the 24-hour secretory profiles of pituitary hormones. *J. Endocrinol. Invest*. **8**, 381–391.

Van Cauter, E., and Spiegel, K. (1999). Circadian and sleep control of endocrine secretions. In "Regulation of Sleep and Circadian Rhythms" (F. W. Turek and P. C. Zee, eds.), pp. 397–426. Marcel Dekker, Inc., New York.

Van Cauter, E., and Turek, F. W. (1995). Endocrine and other biological rhythms. *In* "Endocrinology" (L. J. DeGroot, ed.), pp. 2487–2548. Saunders, Philadelphia.

Van Cauter, E., L'Hermite, M., Copinschi, G., Refetoff, S., Desir, D., and Robyn, C. (1981). Quantitative analysis of spontaneous variations of plasma prolactin in normal man. *Am. J. Physiol.* **241**, E355–E363.

Van Cauter, E., van Coevorden, A., and Blackman, J. D. (1990). Modulation of neuroendocrine release by sleep and circadian rhythmicity. *In* "Advances in Neuroendocrine Regulation of Reproduction" (S. Yen and W. Vale, eds.), pp. 113–122. Serono Symposia USA, Norwell, MA.

Van Cauter, E., Blackman, J. D., Roland, D., Spire, J. P., Refetoff, S., and Polonsky, K. S. (1991). Modulation of glucose regulation and insulin secretion by circadian rhythmicity and sleep. *J. Clin. Invest*. **88**, 934–942.

Van Cauter, E., Kerkhofs, M., Caufriez, A., Van Onderbergen, A., Thorner, M. O., and Copinschi, G. (1992). A quantitative estimation of GH secretion in normal man: Reproducibility and relation to sleep and time of day. *J. Clin. Endocrinol. Metab.* **74**, 1441–1450.

Van Cauter, E., Leproult, R., and Kupfer, D. J. (1996). Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. *J. Clin. Endocrinol. Metab*. **81**, 2468–2473.

Van Cauter, E., Plat, L., Scharf, M., Leproult, R., Cespedes, S., L'Hermite-Balériaux, M., and Copinschi, G. (1997). Simultaneous stimulation of slow-wave sleep and growth hormone secretion by gamma-hydroxybutyrate in normal young men. *J. Clin. Invest*. **100**, 745–753.

Van Cauter, E., Plat, L., Leproult, R., and Copinschi, G. (1998). Alterations of circadian rhythmicity and sleep in aging: Endocrine consequences. *Horm. Res*. **49**, 147–152.

Van Cauter, E., Leproult, R., and Plat, L. (2000). Age-related changes in slow-wave sleep and REM sleep and relationship with growth hormone and cortison levels in healthy men. *JAMA, J. Am. Med. Assoc*. **284**, 861–868.

van Coevorden, A., Mockel, J., Laurent, E., Kerkhofs, M., L'Hermite-Balériaux, M., Decoster, C., Nève, P., and Van Cauter, E. (1991). Neuroendocrine rhythms and sleep in aging men. *Am. J. Physiol*. **260**, E651–E661.

Veldhuis, J. D., Iranmanesh, A., Johnson, M. L., and Lizarralde, G. (1989). Amplitude, but not frequency, modulation of adrenocorticotropin secretory bursts gives rise to the nyctohemeral rhythm of the corticotropic axis in man. *J. Clin. Endocrinol. Metab.* **71**, 452–463.

Veldhuis, J. D., Iranmanesh, A., Johnson, M. L., and Lizarralde, G. (1990). Twenty-four-hour rhythms in plasma concentrations of adenohypophyseal hormones are generated by distinct amplitude and/or frequency modulation of underlying pituitary secretory bursts. *J. Clin. Endocrinol. Metab.* **71**, 1616–1623.

Veldhuis, J., Liem, A., South, S., Weltman, A., Weltman, J., Clemmons, D., Abbott, R., Mulligan, T., Johnson, M., Pincus, S., Straume, M., and Iranmanesh, A. (1995). Differential impact of age, sex steroid hormones, and obesity on basal versus pulsatile growth hormone secretion in men as assessed in an ultrasensitive chemiluminescence assay. *J. Clin. Endocrinol. Metab.* **80**, 3209–3222.

Veldhuis, J. D., Iranmanesh, A., and Weltman, A. (1997). Elements in the pathophysiology of diminished growth hormone (GH) secretion in aging humans. *Endocrine* **7**, 41–48.

Vermeulen, A. (1987). Nyctohemeral growth hormone profiles in young and aged men: Correlation with somatomedin-C levels. *J. Clin. Endocrinol. Metab.* **64**, 884–888.

Waldstreicher, J., Duffy, J. F., Brown, E. N., Rogacz, S., Allan, J. S., and Czeisler, C. A. (1996). Gender differences in the temporal organization of prolactin (PRL) secretion: Evidence fort a sleep-independent circadian rhythm of circulating PRL levels—A Clinical Research Center study. *J. Clin. Endocrinol. Metab*. **81**, 1483–1487.

Wehr, T., Moul, D., Barbato, G., Giesen, H., Seidel, J., Barker, C., and Bender, C. (1993). Conservation of photoperiod-responsive mechanisms in humans. *Am. J. Physiol*. **265**, R846–R857.

Weibel, L., Follenius, M., Spiegel, K., Gronfier, C., and Brandenberger, G. (1997). Growth hormone secretion in night workers. *Chronobiol. Int*. **14**, 49–60.

Weiland, N. G., and Wise, P. M. (1990). Aging progressively decreases the densities and alters the diurnal rhythms of alpha-1 adrenergic receptors in selected hypothalamic regions. *Endocrinology (Baltimore)* **126**, 2392–2397.

Weitzman, E. D., Czeisler, C. A., Zimmerman, J. C., and Ronda, J. M. (1981). The sleep-wake pattern of cortisol and growth hormone secretion during non-entrained (free-running) conditions in man. *In* "Human Pituitary Hormones. Circadian and Episodic Variations" (E. Van Cauter and G. Copinschi, eds.), pp. 29–41. Martinus Nijhoff, The Hague.

Weitzman, E. D., Moline, M. L., Czeisler, C. A., and Zimmerman, J. C. (1982). Chronobiology of aging: Temperature, sleep-wake cycle rhythms and entrainment. *Neurobiol. Aging* **3**, 299–309.

Weitzman, E. D., Zimmerman, J. C., Czeisler, C. A., and Ronda, J. M. (1983). Cortisol secretion is inhibited during sleep in normal man. *J. Clin. Endocrinol. Metab.* **56**, 352–358.

Wever, R. A. (1979). "The Circadian System of Man: Results of Experiments Under Temporal Isolation." Springer-Verlag, New York.

Wise, P. M., Walovitch, R. C., Cohen, I. R., Weiland, N. G., and London, D. E. (1987). Diurnal rhythmicity and hypothalamic deficits in glucose utilization in aged ovariectomized rats. *J. Neurosci*. **7**, 3469–3473.

Wise, P. M., Cohen, I. R., Weiland, N. G., and London, D. E. (1988). Aging alters the circadian rhythm of glucose utilization in the suprachiasmatic nucleus. *Proc. Natl. Acad. Sci. U. S. A*. **85**, 5305–5309.

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Circadian Rhythms and Sleep in Aging Rodents

Circadian rhythms are found many aspects of animal physiology. The efficacy of input pathways to and output pathways from central nervous system circadian pacemakers change with advancing age, as does the functioning of the central pacemaker itself. While the timing of sleep is partly under the control of the circadian clock, other brain regions are involved in the timing of sleep and the homeostatic sleep mechanism. Many sleep parameters also undergo age-related changes. The scientific literature on the aging of the circadian timing and sleep systems is reviewed here, along with data from experiments that suggest some of the mechanisms underlying these changes and interventions that may attenuate their severity. © 2001 Academic Press.

I. General Introduction

One of the fundamental assumptions underlying the study of physiology is that organisms are driven to keep their internal milieu as constant as possible. Thus, under the classical model of the negative feedback loop, a perturbation to a physiological system will be detected and the organism's effector systems will be mobilized to return the system to a set point. While this model is generally valid, it is complicated by the presence of a circadian clock. That is, physiological systems do not in fact keep their internal milieu constant, but rather vary it daily in a rhythmic fashion.

Why should an organism complicate its physiology so? Circadian clocks are thought to have evolved for several purposes. First, they regulate an organism's behavioral state. By keeping activity confined to one portion of the day an animal can forage under optimal conditions and avoid predation. Animals whose circadian pacemakers have been disrupted are more susceptible to predation in the field (DeCoursey *et al*., 1997; DeCoursey and Krulas, 1998). Second, circadian clocks allow an animal the ability to go beyond simply responding to periodic changes in the environment and instead predict such changes and begin to alter physiology and behavior before the changes even occur.

Finally, circadian clocks provide a degree of internal synchronicity; that is, the master circadian pacemaker can keep the rhythms of various internal systems in the proper phase relationships with one another. The rest–activity state is by no means the only physiological parameter under the control of the circadian clock. In fact, variables such as heart rate, blood pressure, acid production, and body temperature are all under circadian clock control (Zee and Turek, 1999). By keeping many oscillating systems coordinated, the circadian clock promotes a healthy internal environment. In fact, maintaining organisms as diverse as tomatoes (Hillman, 1956), blowflies (Saint Paul and Aschoff, 1978), *Drosophila* (Pittendrigh and Minis, 1972), rats (Kort and Weijma, 1982; Kort *et al*., 1986; Nelson and Halberg, 1986), hamsters (Penev *et al*., 1998b), and humans (Kawachi *et al*., 1995) in altered light:dark cycles can result in health disturbance or even decreased longevity. Clearly the circadian clock is crucial in maintaining an appropriate internal physiological environment.

Sleep, too, is under the control of the circadian clock, as well as the influence of a homeostatic mechanism and at least one ultradian pacemaker (Zee and Turek, 1999). Recent evidence, which will be discussed further herein, suggests that the homeostatic components of the sleep mechanism are also influenced by the circadian clock. Thus any problem in the circadian system could have consequences for a wide number of physiological systems, including sleep and the restorative role it is thought to play.

Aging itself is one source of such disruption. There are profound changes in both sleep and circadian rhythms in advanced age. These changes are seen both in humans (Carrier *et al*., 1996) and in a large number of laboratory animals (van Gool and Mirmiran, 1983; Welsh *et al*., 1986; Van Reeth *et al*., 1992; Whealin *et al*., 1993; Turek *et al*., 1995a; Valentinuzzi *et al*., 1997; Naylor *et al*., 1998a). Using animal models of rhythms, sleep, and aging, we can begin to understand the mechanisms of how these systems age. In mammals, the master circadian clock is housed in the suprachiasmatic nuclei of the anterior hypothalamus (Klein *et al*., 1991). No single sleep center has been found, but certain areas including the pons, and the cholinergic basal forebrain are implicated in the control of

sleep. By understanding how sleep and circadian rhythms change in advancing age, and by finding the anatomical and biochemical alterations underlying such changes, we can also learn the importance of the components of these physiological systems to their normal functions.

II. Effects of Aging on Circadian Rhythmicity

A. Introduction

In 1974, Pittendrigh and Daan first described systematic age-related changes in the circadian timing system of rodents. They observed that old animals' circadian clocks run at a different speed than when those same animals were young. When animals are placed in an environment devoid of time cues (e.g., constant darkness), the daily changes once associated with the light:dark cycle, for example the increase in wheel-running in the early evening by nocturnal rodents, persist. This suggests that the animals have an internal time-keeping mechanism. Those rhythms that persist in the absence of time cues with a period of approximately 24 hr are referred to as circadian rhythms; the period with which they persist is referred to as τ. Pittendrigh and Daan (1974) showed that golden hamsters and two species of mice have shorter τ, or faster-running clocks, when they are old. While any oscillating system is composed of inputs to the oscillator, which synchronize it with external cues, the central pacemaker, and outputs, τ is a fundamental property of the central oscillator. Thus the consistent shortening of circadian period in old hamsters and deer mice suggested that the central oscillator's function changes with age (Pittendrigh and Daan, 1974). This was the first piece of evidence that the circadian system changes with age and the picture has become considerably more complex as more data have been collected. These changes include, but are not limited to, a change in the timing of the active phase under a light:dark cycle, changes in the phase-resetting properties of both photic and nonphotic stimuli, an increase in rhythm fragmentation, and a decrease in the amplitude of overt rhythms (Morin, 1988; Rosenberg *et al*., 1991; Zee *et al*., 1992; Van Reeth *et al*., 1993; Mrosovsky and Biello, 1994; Penev *et al*., 1995, 1998a; Benloucif *et al*., 1997a; Valentinuzzi *et al*., 1997). While some of these changes can be partly explained by changes in the inputs and outputs of the circadian timing system, it also appears that the central pacemaker itself changes with advanced age. The recent discovery of a molecular mechanism of the circadian clock in mammals (Dunlap, 1999) and similar mechanisms in *Drosophila* (Young, 1998) and *Neurospora* (Bell-Pedersen *et al*., 1996; Dunlap, 1996) as well as information gleaned from the complex relationships between inputs, outputs, and the central pacemaker offer hints as to how clocks might be breaking in old age and what we can do to fix them.

Since Pittendrigh and Daan (1974) first described the change in τ with advancing age a large body of data has been collected on age-related changes in circadian behavior in laboratory animals, primarily in rats, mice, and hamsters. First we will examine the evidence from experimental animals that circadian rhythms change with age. We will explore the possibility that changes in either the inputs to or the outputs from the clock can explain the phenomena observed. We will examine why these explanations are inadequate and the evidence for a change in the central pacemaking mechanism. Finally, we present evidence that it may be possible to intervene to delay the onset or reduce the severity of these changes.

B. Changes in Inputs to the Circadian Clock

1. Photic Inputs

The rotation of the earth on its axis provides a dynamic yet predictable environment. This environmental light:dark cycle is the major entraining cue, or *zeitgeber*, to the circadian timing system. Shifts in the light:dark cycle lead to gradual reentrainment, as can occur when people move rapidly across time zones. It is thought that the mechanism by which entrainment occurs is through daily adjustments of the circadian clock, whose endogenous period is rarely exactly 24 hr. By delaying this internal clock slightly each evening or advancing it slightly each morning, the environmental light:dark cycle keeps the animal's physiology and behavior synchronized to Earth's 24 hr rotation.

Information about the light:dark cycle has access to the circadian system by two routes. Retinal ganglion cells project directly to the suprachiasmatic nuclei via the retino-hypothalamic tract and indirectly through the thalamic intergeniculate leaflet (Morin *et al*., 1992). Light changes the phase of the circadian clock by a cascade of events within suprachiasmatic nucleus cells, including the activation of the *mPer1* gene (Akiyama *et al*., 1999) and immediate–early genes (Kornhauser *et al*., 1990), the phosphorylation of the cyclic AMP response element binding protein (Zhang *et al*., 1996), and ultimately *de novo* protein synthesis (Inouye *et al*., 1988; Khalsa *et al*., 1992). Old animals are less sensitive to changes in the light:dark cycle, as measured by either biochemical or behavioral means. Old rats fail to show a shift in the rhythm of pineal *N*-acetyl transferase, an enzyme in the melatonin synthesis pathway under strict suprachiasmatic nucleus control, 4 days after an advance of the light:dark cycle, whereas young adult rats show a clear phase shift (Buresová *et al*., 1990). Old hamsters (Rosenberg *et al*., 1991; Zhang *et al*., 1996), rats (Sutin *et al*., 1993), and mice (Benloucif *et al*., 1997a) all show smaller phase shifts to light pulses than young animals, indicating that either the visual transduction system or the suprachiasmatic nucleus's ability to respond to such a signal has been modulated by age. It is important to note that this phenomenon is true only at relatively dim light intensities, i.e., those that produce a submaximal shift in the phase of the circadian rhythm of locomotor activity in young animals (Rosenberg *et al*., 1991; Zhang *et al*., 1996). Old hamsters also have reduced photic induction of c-*fos*, a gene that is necessary for photic phase shifting in young rodents, and CREB phosphorylation (Zhang *et al*., 1996). Old mice show a reduced induction of both *c-fos* and *Jun*-B mRNA after phase-shifting light pulses (Benloucif *et al*., 1997a). Together, these studies show that the deficit in shifting the phase of the circadian rhythm of locomotor activity is not simply a failure of outputs from the circadian system, but rather a failure of the circadian system to respond to the light pulse. While the aging process can compromise visual function through disease processes such as cataracts, these phenomena do not fully account for

the loss of sensitivity to photic *zeitgebers* that accompanies aging (Zhang *et al*., 1996, 1998). Compared to young hamsters, old hamsters' lenses transmit only about 75% of 500-nm light (the wavelength involved in photic phase-shifting of the circadian clock). However, this fails to fully explain the fact that the phase shifts induced by light at this wavelength in old animals are only 5% as large as in young animals (Zhang *et al*., 1998). Additionally, tract-tracing studies indicate that there is no effect of age on either the size of or degree of retinal innervation of the suprachiasmatic nucleus (Zhang *et al*., 1998). Therefore, there appears to be a change in either the efficacy of the retina to transmit photic information or a change in the suprachiasmatic nucleus's response to this information.

2. Nonphotic Inputs

While light is the major *zeitgeber*, it is not the only stimulus that can entrain the circadian clock. For example, social interaction, food availability, and even maternal signals to a fetus can entrain the circadian clock. Stimuli that induce a large amount of activity (including, but not limited to, the benzodiazapine triazolam, pulses of darkness on a background of constant light, and access to a novel running wheel) induce robust phase advances in hamsters when administered during the subjective day (Van Reeth and Turek, 1989; Mrosovsky, 1996). Activity, or its neural correlate, is necessary for these phase shifts (Van Reeth and Turek, 1989). Like photic stimuli, nonphotic *zeitgebers* also lose their efficacy to induce phase shifts in old rodents. Even though these stimuli induce a similar amount of activity in old hamsters, the subsequent phase shifts are attenuated (Van Reeth *et al*., 1992, 1993; Mrosovsky and Biello, 1994). Activity-inducing stimuli, such as triazolam, are thought to induce phase advances by stimulating the intergeniculate leaflet to release neuropeptide Y (NPY) from terminals in the suprachiasmatic nucleus (Huhman and Albers, 1994; Wickland and Turek, 1994). A change in the efficacy of the intergeniculate leaflet in either integrating nonphotic information or transmitting such information to the suprachiasmatic nucleus, or in the suprachiasmatic nucleus' efficacy in interpreting these signals could result in a decreased response to all these stimuli. These data do not permit us to distinguish the effect of aging on the input pathways from the effect of aging on the central pacemaker (the suprachiasmatic nucleus); both may be compromised in old age.

The serotonergic system may also mediate nonphotic phase shifts. When injected peripherally during the subjective day, the serotonin receptor agonist 8-OH-DPAT induces large phase shifts in young hamsters and smaller phase shifts in old hamsters (Penev *et al*., 1995). 8-OH-DPAT most likely exerts its effects by activating both the dorsal and medial raphe nuclei, which send projections to the intergeniculate leaflet and the suprachiasmatic nucleus, respectively (MeyerBernstein *et al*., 1997). Old hamsters show smaller phase advances in response to injections of 8-OH-DPAT and a decreased ability of light to attenuate 8-OH-DPAT-induced phase shifts (Penev *et al*., 1995, 1997). The effects of age on the circadian system, including changes in τ and decreased sensitivity to triazolam injections and subsaturating light pulses, are mimicked by monoamine depletion in young hamsters, suggesting that a loss of serotonergic input to the suprachiasmatic nucleus and/or the intergeniculate leaflet is responsible for some of the age-related changes in circadian rhythmicity (Penev *et al*., 1993, 1994).

Clearly physiological inputs cannot convey information to the circadian clock in old animals as they can in young animals. While the inputs to the circadian clock are by definition separate from the central oscillating mechanism, they do comprise an important part of the overall physiological system. Proper function of the input mechanisms is necessary for the organism to synchronize its internal timekeeping mechanism with the appropriate environmental cues. Thus the age-related changes observed in input efficacy may partly underlie the age-related changes in circadian physiology. However, the changes in circadian timing system with age are likely to also occur at the level of the central pacemaker as well.

C. Changes in the Central Pacemaker

Many of the changes observed in circadian rhythms with advancing age can be attributed to changes in the function of the central pacemaker. Current evidence points to a change in the amplitude of the circadian oscillator as an important component of the aging circadian phenotype. Here we review the literature supporting the hypothesis that the circadian pacemaker itself is altered in advanced age and fails to operate as robustly as in young adults.

Perhaps the most consistent age-related change in circadian rhythmicity is the one first noticed by Pittendrigh and Daan (1974), the change in τ. While their paper reported a consistent decrease in τ, data gathered over the past 25 years show that there can be both increases and decreases in τ, and the direction of the change seems to be species-dependent. Some laboratories have failed to find a change in τ across the life span in golden hamsters (Davis and Viswanathan, 1998), but many other studies have shown an age-related shortening of the free-running period of the locomotor activity and body temperature rhythms in rats (Witting *et al*., 1994), hamsters (Rosenberg *et al*., 1991; Morin, 1993, 1988; Penev *et al*., 1995, 1997), and field mice (*Mus booduga*) (Sharma and Chandrashekaran, 1998). In contrast, C57BL/6J, DBA/2J, and SWR mice (*Mus musculus*) show increases in τ with advanced age, as determined from the circadian rhythm of locomotor activity (Mayeda *et al*., 1997; Valentinuzzi *et al*., 1997). Whereas the direction of the change is species-specific, a change in τ in constant darkness is one of the most consistent effects of age on the circadian clock. Frequency is a fundamental property of any oscillating system, and a change in frequency most likely indicates that the oscillator itself is changing. That such changes are seen in advancing age by several laboratories working with several species suggests that these changes are a fundamental part of circadian aging. Figure 61.1 presents actograms from young and old hamsters and mice. Note that the free-running period sometimes, but not always, changes with advancing age.

Behavioral data on the phase-shifting effects of light suggest that aging changes the amplitude of the pacemaker. While Zhang *et al*. (1996) found that subsaturating light pulses induce smaller phase shifts in older animals, Rosenberg *et al*. (1991) reported that old animals in fact show larger phase shifts to saturating (i.e., when an increase in either intensity or duration of light fails to further increase the magnitude of

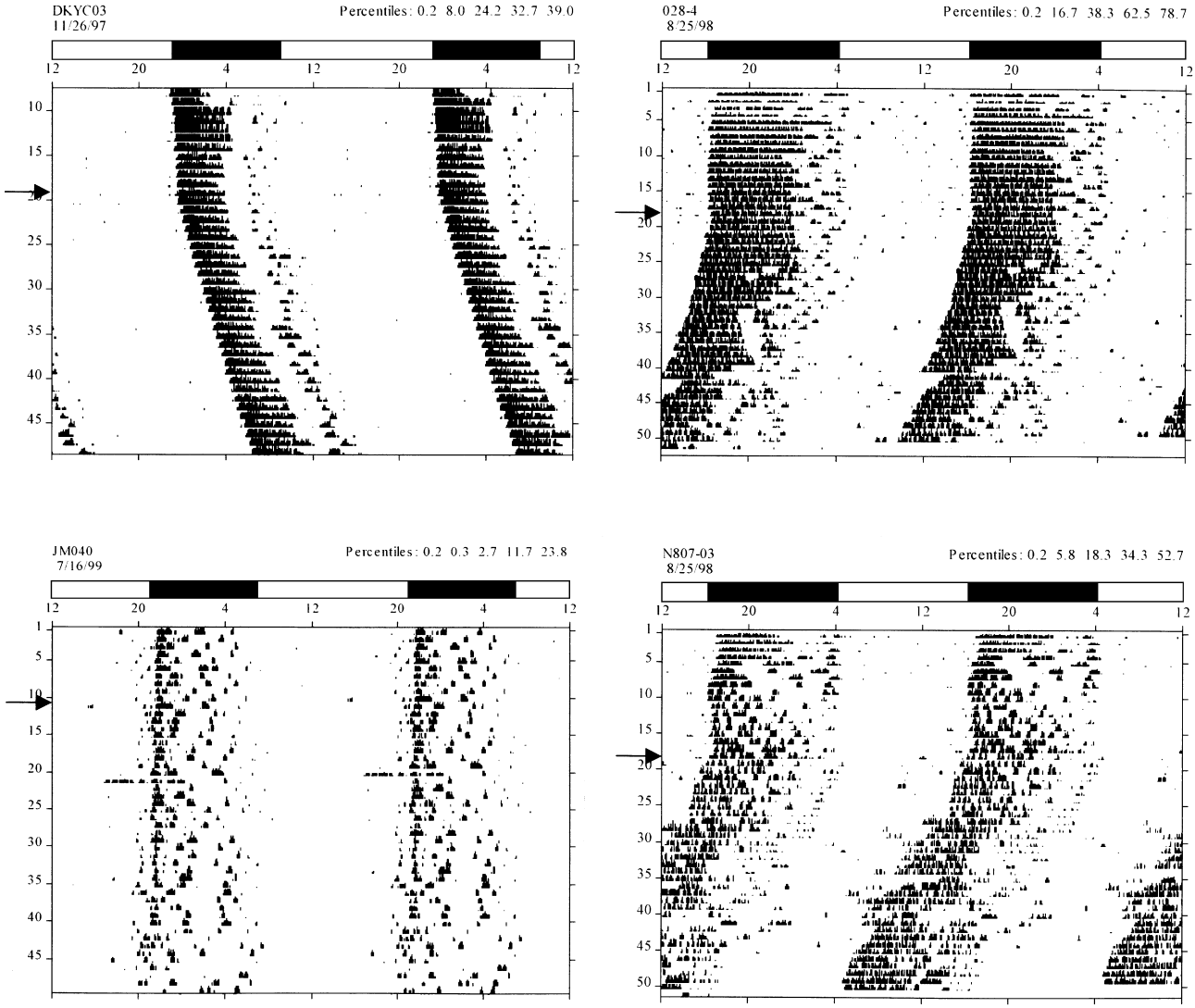

FIG. 61.1. Actograms of representative young (top) and old (bottom) golden hamsters (left) and mice (right). Tick marks indicate periods of wheel-running activity. Forty-eight hours are plotted across the horizontal axis to ease visualization. Successive days are plotted beneath one another. The dark bars above each activity record indicate times of darkness when the animals were maintained in a light:dark cycle. The arrows indicate the time of transfer to constant darkness. Note the increase in fragmentation of the activity pattern in both species and the shortening of the free-running period in hamsters.

the phase shifts in young animals) light pulses, a seemingly contradictory result. However, this is true only when the light pulses are administered around circadian time 16[1]; at this time old animals show phase shifts three times as large as those seen in young animals (Rosenberg *et al*., 1991). These large phase shifts have two possible explanations. Either light has a greater salience in old animals and is therefore able to exert a larger effect (i.e., an age-related change in potency) or the oscillator's amplitude is decreased such that the same stimulus that perturbs the system slightly in young animals drastically perturbs it in old animals (Rosenberg *et al*., 1991). However, the former is not likely since subsaturating light pulses have an attenuated effect on phase shifting, gene induction, and protein phosphorylation in the suprachiasmatic nucleus of old hamsters, and this is not due to decreased lens transmittance alone (Zhang *et al*., 1996, 1998). Clearly light does not have an increased salience in old hamsters; thus the large phase shifts seen with saturating light pulses are more likely due to reduced oscillator amplitude.

The rhythms generated by the central circadian pacemaker are often observed to damp out in old age. For the circadian rhythm of locomotor activity, the most commonly measured overt rhythm in rodents, this damping in the rhythm is seen as a reduced difference between the amount of activity during the subjective day and the subjective night. It is also visible as an increase in fragmentation of the pattern of activity. Penev *et al*. (1998b) report quantitative results showing that old hamsters have significantly more bouts of activity per day than

[1]Circadian time 12 is defined in a nocturnal animal as the time of the onset of the activity rhythm in the absence of time cues, such as when the animal is placed in constant darkness or constant light.

young hamsters. This confirms visual inspection of old hamsters' actograms, which clearly show more bouts of activity in the night and the day. Similar qualitative and quantitative results have also been reported in C57BL/6J mice (Valentinuzzi *et al*., 1997). While the increase in the number of bouts of activity during the night might be explained by factors independent of the circadian timing system, including decreased muscle tone and respiratory problems, an age-related increase in the number of bouts of activity during the day indicates an inappropriately timed signal to become active and/or difficulty in maintaining sleep or rest states.

Results from experiments looking at suprachiasmatic nucleus function more directly support the hypothesis that the amplitude of the circadian pacemaker is decreased in old age. While each suprachiasmatic nucleus is composed of approximately 8000 neurons (van den Pol, 1991), it appears that each neuron within the nucleus is capable of generating circadian rhythms independently; the synaptic connections between the individual neurons probably serve to coordinate the pacemakers into a unified entity (Welsh *et al*., 1995; Liu *et al*., 1997). In young rodents there is a robust circadian rhythm of the suprachiasmatic nucleus' firing rate *in vitro*, with a peak in firing rate during the subjective day. Compared to those of young hamsters, the suprachiasmatic nuclei of old hamsters have a significantly lower firing rate during the subjective day and a decreased difference between the firing rate during the day and the night (Watanabe *et al*., 1995). In old rats, the amplitude of the firing rhythm is damped, without a change in mean firing rate (Satinoff *et al*., 1993). The authors of this study suggest that this is not due to a disappearance of cells capable of firing at fast rates, but rather can be attributed to either a change in the individual oscillators' ability to generate circadian rhythms or a decrease in the coupling strength between these oscillators (Satinoff *et al*., 1993). Interestingly, there is not a decrease in the number of neurons within the suprachiasmatic nucleus (Roozendaal *et al*., 1987; Woods *et al*., 1993; Madeira *et al*., 1995), so the age-related changes in circadian rhythms cannot be explained by cell death. Rather they appear to be due to changes in either the individual oscillators that comprise the circadian clock or the connections between them. In addition, old rats have a change in the rhythm of glucose use in the suprachiasmatic nucleus without a change in overall glucose use (Wise *et al*., 1988), again suggesting a change in the function of the oscillators or the coupling between them. These are all examples of decreases in the amplitude of the circadian pacemaker's oscillation. They are best explained by a decrease in the coupling between individual oscillators or by a decreased function of many of these oscillators while their synaptic couplings remain intact.

Gene expression plays an important role in the generation of circadian rhythms. Many neurons in the suprachiasmatic nucleus are thought to be capable of generating and maintaining circadian rhythms individually through an autoregulatory feedback loop. This loop contains both positive elements that start transcription and negative elements that inhibit it (see Dunlap, 1999, for a review of the molecular machinery comprising the circadian clock). In the suprachiasmatic nucleus, many genes, both within and outside of this loop, are transcribed on a circadian basis and can serve as indicators of the clock's function. In aging, the rhythmic expression of some of these genes in the suprachiasmatic nucleus is altered. Two independent research groups have shown that old rats have a reduced rhythm of vasoactive intestinal peptide in the suprachiasmatic nucleus; in fact this rhythm is absent by the time the animals are middle-aged (Kawakami *et al*., 1997; Krajnak *et al*., 1998). While there is no change in the rhythm of argenine-vasopressin expression on old rats, there is a decrease in the number of argenine-vassopressin-producing cells in the suprachiasmatic nucleus (Roozendaal *et al*., 1987; Krajnak *et al*., 1998). As argenine-vasopressin may serve as a diffusible output of the circadian clock (Silver *et al*., 1996), this finding suggests that the signal from the suprachiasmatic nucleus to the rest of the body may be attenuated in old age. However, the decrease in the number of cells producing an output signal without a change in its rhythmicity would not explain all the changes observed in overt circadian rhythmicity, both behavioral and biochemical (e.g., electrophysiological firing rhythms in the suprachiasmatic nucleus *in vitro*). Production of the immediate–early gene product Fos, which is produced in response to photic stimuli that phase shift the circadian clock (Kornhauser *et al*., 1990; Wollnik *et al*., 1995), is attenuated in middle-aged and old rats (Cai *et al*., 1997a) and old hamsters (Zhang *et al*., 1996). The amount of Jun-B protein induced by photic stimuli is attenuated in old rats (Cai and Wise, 1996). Several of the genes that are responsible for either generation or phase-shifting of circadian rhythms cease to function properly in old rodents.

Transplant studies also suggest that *in vivo* the aging of circadian rhythms are due in part to changes within the suprachiasmatic nucleus itself. Transplanting fetal suprachiasmatic nucleus tissue into old host hamsters restores the sensitivity to the phase-shifting effects of triazolam; this effect is not obtained with control (cerebellar) fetal tissue, indicating that it is an suprachiasmatic nucleus specific effect (Van Reeth *et al*., 1994). Suprachiasmatic nucleus transplants have also been reported to increase the amount of wheel-running activity and the length of survival of the hosts compared to those animals that received control transplants (Hurd and Ralph, 1998). In rats, transplanting fetal suprachiasmatic nucleus tissue restores the diurnal pattern of Fos expression in the host suprachiasmatic nucleus (Cai *et al*., 1997a,b) as well as the ability of light to induce Jun-B protein production (Cai and Wise, 1996). Other studies have shown that fetal suprachiasmatic nucleus transplants into old animals can restore the diurnal rhythms of corticotropin-releasing hormone and proopiomelanocortin gene expression in nuclei driven by the suprachiasmatic nucleus clock (Cai *et al*., 1997b). Together these data suggest that the age-related changes in overt circadian rhythms and the underlying changes in gene expression are in part due to changes within the suprachiasmatic nucleus tissue itself; changes that alter the ability of the suprachiasmatic nucleus to generate rhythms and to respond to external stimuli.

D. Changes in Function of Effector Systems

In addition to the changes in the efficacy of the inputs to the circadian clock and the changes in the central pacemaker, aging also alters the function of at least some of the outputs of the clock. Locomotor activity is one of the most commonly observed circadian outputs of laboratory animals. Old hamsters

(Penev *et al*., 1995, 1997; Davis and Viswanathan, 1998), and mice (Wax, 1975; Valentinuzzi *et al*., 1997) both run fewer revolutions in their running wheels than young animals. A weakening of the muscles could easily explain this difference. Similarly, aging reduces pineal (Reiter *et al*., 1980) and serum (Pang and Tang, 1983) melatonin content in hamsters and sometimes reduces mean body temperature in rats (Satinoff, 1998). The point is that many effector systems *do* break down in old age; the decrease in mean levels of either pineal melatonin or body temperature need not be explained by changes in the circadian system, rather the outputs of the circadian system could have begun to fail in their own right. Care should be taken not to confuse breakdowns of the central pacemaker with breakdowns of the organ that produces the observed rhythm. In order to infer the health of the pacemaker it is important to not only observe the mean level of any driven rhythm, but also parameters specific to its timing: the period, phase, and amplitude of the rhythm, as well as its degree of fragmentation.

Some of the systems downstream of the circadian system have feedback effects on the clock and therefore the age-related changes in these systems have the potential to further alter circadian clock function. For example, the fact that old hamsters run less than their young counterparts (Penev *et al*., 1995, 1997; Davis and Viswanathan, 1998) could reduce the efficacy of activity feedback as a synchronizing agent.[2] Similarly, the age-related decrease in pineal (Reiter *et al*., 1980) and serum (Pang and Tang, 1983) melatonin levels might alter animals' sensitivity to either melatonin or other *zeitgebers*. While one study found no effect of age on the phase-shifting effects of melatonin (Benloucif *et al*., 1997b), other species have not been studied in this manner. Since melatonin can act as both an input to and an output of the circadian clock, its age-related decline may be important in modulating responses to other phase-shifting agents. Whereas the outputs of the clock are often thought to merely reflect the position of the hands (i.e., the phase of the clock contained within the suprachiasmatic nuclei), they do in fact have effects on the clock itself. Thus the effects of age on the circadian system can be magnified by changes in feedback of the outputs back onto the clock.

E. Can Age-Related Changes in Circadian Rhythms Be Attenuated or Reversed?

Since the clock is less responsive to inputs, one way to enhance its oscillations might be to provide it with stronger inputs. As light is the major *zeitgeber*, an obvious remedy would be to increase the intensity of the light:dark cycle. Two such experiments have shown promising results. First, Witting *et al*. (1993) increased the light intensity from 3.5 to 445 lux during the light phase of the light:dark cycle. They found that the brighter intensities were able to reverse some of the age-related changes in rats' sleep–wake cycle (Witting *et al*., 1993). For example, the bright light intensities increased the day:night differences in the amount of quiet sleep; viewed in circadian terms the bright light increased the amplitude of the diurnal rhythm (Witting *et al*., 1993). Interestingly all of the improvement in rhythm amplitude was due to changes in behavioral states during the light phase (Witting *et al*., 1993). This suggests that the bright light can suppress inappropriate timing of activity in this nocturnal species.

In a second study, Labyak *et al*. (1998) found that after increasing the light intensity from 300 to 1500 lux, middle-aged hamsters, which already show many of the signs of aging discussed above, showed fewer daily bouts of activity during the light phase and an increase in the daily number of wheel revolutions. However, the same increases in light intensity failed to significantly affect old hamsters' rhythms, suggesting that interventions to the aging of the circadian system must be made during middle age. There are two possible explanations for the failure of light to reverse the effects of aging in the oldest hamsters. First, Labyak *et al*. (1998) used 300 lux as their lowest light intensity; this is almost as bright as the Witting *et al*. (1993) brightest intensity. The lack of significant improvement in diurnal rhythmicity by old hamsters exposed to bright light in the Labyak *et al*. study could be due to a ceiling effect. Second, the two experiments examined different outputs of the circadian timing system. The coupling strength of the circadian clock could be more strongly coupled to the sleep–wake system than to the networks underlying locomotor activity.

While there are not yet convincing data on an age-related change in melatonin sensitivity (see previous section), the age-related decline in melatonin levels are well-documented (Reiter *et al*., 1980). Experiments with melatonin and its analogs, indicating that melatonin can act as a *zeitgeber* (Kumar *et al*., 1997), raise the possibility that increasing the melatonin signal might reverse some of the effects of aging on the circadian system. It has been suggested that melatonin may be useful in treating a number of human circadian disorders, including age-related insomnia (Arendt and Deacon, 1997). The melatonin agonist S20242 significantly increased a measure of rhythm stability, the χ^2 value of the periodogram, when administered to middle-aged and old rats (Koster-van Hoffen *et al*., 1993). This treatment also increased the amplitude of the circadian rhythm of body temperature in middle-aged, but not old rats (Koster-van Hoffen *et al*., 1993). Daily feeding of a different melatonin agonist (S20098) to old hamsters restored their sensitivity to a dark pulse; when the treated food was removed, dark pulses once again failed to phase-shift the old hamsters (Van Reeth *et al*., 1997). Together, these two studies suggest that exogenous melatonin can restore some of the youthful properties to the circadian systems of old animals.

If the age-related changes in circadian rhythmicity are due to changes in the function or expression patterns of circadian clock genes, then genetic interventions might be able to modulate the onset or severity of these changes. Senescence-accelerated mice are a series of inbred strains selected for short longevity and rapid onset of biomarkers of aging. Senescence-resistant mice serve as genetic controls. A brief study investigating the role of the senescence-accelerated mouse genotype on the aging of the circadian timing system examined only two strains, one senescence-accelerated mouse and one senescence-resistant mouse. The authors found that there was no difference in the overall pattern of circadian activity, free-running period, or time to reentrain following a 6 hr delay

[2]Recall that the decreased efficacy of non-photic phase-shifting agents in old animals is independent of the amount of activity induced (Van Reeth *et al*., 1992, 1993).

of the light:dark cycle (Sanchez-Barcelo *et al*., 1997). There was no difference in either the overall morphology or number of vasopressin-producing neurons in the suprachiasmatic nucleus (Sanchez-Barcelo *et al*., 1997). However, this failure to find changes related to senescence-accelerated mouse genotype is not conclusive, as only one strain from each of the selected lines was tested. In theory, the exact genetic makeup of each of the selected senescence-accelerated mouse lines could differ. More thorough experimentation involving multiple senescence-accelerated mouse and senescence-resistant mouse lines are necessary to determine the role of genetic background on the acceleration of circadian aging. Additionally, classical genetic techniques such as quantitative trait loci analysis could help to elucidate genetic components to the aging of the circadian timing system.

In summary, with advanced age there are changes in several components of the circadian timing system and each may play a role in the age-related decline in efficacy of the system as a whole. While a decreased strength of input signals to the circadian system may explain some of the age-related decline in sensitivity to both photic and pharmacological stimuli, concurrent changes in the function of the central pacemaking mechanism itself may account for some changes in sensitivity. Changes in the suprachiasmatic nucleus itself may occur at the level of the intracellular loop of transcription and translation that has been recently elucidated, although the experiments that would test this hypothesis have yet to be performed. In addition, some of the decline in overt circadian rhythmicity may be due to deteriorating function of the body's aging effector systems.

III. Effects of Aging on Sleep

A. Introduction

Age-related changes in the sleep patterns of animals have not been as well-studied as the effects of age on circadian rhythmicity. Only a few studies on each of several species have been carried out, almost always with differing methodologies, experimental protocols, and analysis techniques. Indeed, the experiments are often sufficiently different from one another that the results, which can seem contradictory, cannot always be meaningfully compared. Combined with the rapidly changing technology for the collection and analysis of electroencephalographic (EEG) waveforms, the results reported in one paper cannot necessarily be compared to those reported earlier. Both the mechanisms underlying the sleep process and its functional significance are not nearly so well understood as those underlying circadian rhythmicity. Nevertheless, there are some trends which emerge from the literature on age-related changes in rodent sleep that may help us understand analogous changes in humans.

Since the physiological sleep mechanism is poorly understood, our discussion of the age-related changes in sleep are placed within the framework of a theoretical model. Borbély's two-process model provides a theoretical backdrop upon which to understand both the buildup and diminution of sleep need (Borbély, 1982, 1998). He posits two processes, one circadian (termed process C) and the other homeostatic (termed process S). As an animal stays awake, process S accumulates in a logarithmic fashion with a slow time constant. When an animal sleeps, process S decreases exponentially with a much faster time constant until it reaches a certain point, determined by the interaction of the endogenous setpoint the sinusoidal nature of process C. The drive, or pressure, to sleep is determined by the difference between the level of process S and that of process C. Thus the duration of sleep is determined by both the amount of time previously spent awake and the phase of the circadian cycle at which sleep occurs; the model predicts that a sleep-deprived subject will sleep less if he begins to sleep in the afternoon than if he begins to sleep in the evening after an equal amount of sleep deprivation (Borbély, 1982). Changes in the duration of sleep can be caused by changes in the set point (i.e., the level to which process S must decrease before the animal awakes), the phase of process C, which varies in a circadian fashion, changes in the rate of accumulation of S, or changes in the kinetics with which S decreases. This model makes certain predictions that have been borne out by experiments in both humans and animals [see Borbély (1998) for a review of agreement between predictions of the model and empirical data].

Because the duration of sleep is dependent on the interaction between these two processes, the amount of sleep that an animal exhibits does not necessarily tell us anything about process S. As will be described in the following sections, many species of animals show age-related changes in the duration of sleep, and some experiments have shown age-related changes in the fragmentation of sleep. However, it is important to keep in mind that these changes can be explained fully by the well-documented changes in circadian rhythmicity described above. To be able to conclude that age has an effect on process S, the homeostatic sleep mechanism, one must measure it in action (i.e., by studying the intensity of sleep as opposed to the duration). In most species, this is best reflected by the power of electroencephalograph waves in the delta range (Borbély, 1982; Dijk *et al*., 1990; Franken *et al*., 1991) (generally defined as 0.5–4 Hz, but varying slightly by laboratory and species studied). Waveforms of this nature generally occur during deep, non-rapid-eye-movement (non-REM) sleep. Rapid eye movement (REM) or paradoxical sleep is characterized by EEG waves that are similar to those seen during the waking state [see Rechtschaffen and Kales (1968) for a complete description of sleep stages and the corresponding waveforms].

In aging humans, there are clear changes in both circadian rhythmicity (process C) and sleep (process S) (Copinschi and Van Cauter, 1995; Turek *et al*., 1995b; Bundlie, 1998). There are decreases in both slow-wave and REM sleep, with the changes in slow-wave sleep occurring earlier than those in REM sleep (Van Cauter *et al*., 1998). As the changes in sleep in aging humans are described elsewhere in this volume, they will not be detailed here. While the data from experimental animals are often contradictory, there do appear to be some general changes in both the amount and the qualitative nature of sleep in old animals.

B. Changes in Sleep Due to Changes in the Circadian Clock

Since the timing of sleep is under the control of the circadian clock, anything that affects the stability of the pacemak-

ing mechanism may affect sleep. As reviewed earlier in this chapter, old age causes a general loss of function of the circadian clock, which results in increased fragmentation and decreased rhythm amplitude, among other changes. Since sleep is an output of the clock, one would expect to see changes in sleep fragmentation and the amplitude of the sleep–wake cycle in advancing age. This phenomenon has been observed in several species, suggesting that the mechanisms that underlie the age-related fragmentation in other circadian rhythms may also underlie some of the age-related changes in sleep in animals.

1. Rats

While measuring EEGs in young and old animals, some studies detected a change in circadian, but not sleep-related parameters (Rosenberg *et al*., 1979; Li and Satinoff, 1995). For example, Li and Satinoff (1995) measured sleep parameters and the diurnal body temperature rhythm in young and old female Long–Evans rats. They found that some old rats maintained their stable daily temperature rhythms and others had unstable temperature rhythms. In contrast, all young animals had robust temperature rhythms. Those old rats that had unstable body temperature rhythms had significantly less REM sleep during the light phase and significantly more REM sleep during the dark phase (Li and Satinoff, 1995). However, there was not a difference between young and old rats with robust body-temperature rhythms in any of the sleep parameters measured (Li and Satinoff, 1995). This suggests that the effects on REM sleep that they observed were not due to direct effects of age on REM sleep centers in the brain, but could be due to either a breakdown of the circadian timing system[3] or the secondary disruptions of the thermoregulatory system. These results are entirely consistent with a breakdown in the circadian pacemaker driving the timing of sleep.

Van Gool and Mirmiran (1983) found that compared to young adult Wistar rats, 22 month old rats had increased amounts of wake and decreased amounts of REM sleep during the light phase (van Gool and Mirmiran, 1983). The old rats showed a decreased light:dark ratio of wakefulness and of active (REM) sleep (van Gool and Mirmiran, 1983). However this is not necessarily reflective of a change in process S, as it can be explained by the decreased amplitude of the circadian pacemaker detailed above. In fact, the Wistar rats showed no age-related change in the maximum EEG frequency in the delta or theta (5–9 Hz) ranges, nor was there a change in the theta:delta ratio (van Gool and Mirmiran, 1983). These data suggest that sleep is another overt rhythm whose amplitude changes in old age. The age-related changes in circadian sleep parameters (i.e. fragmentation of sleep and decreased day: night differences in the duration of sleep) of male Wistar rats and the absence of such an effect in female Long–Evans rats suggests that there may be an interaction between sex and aging of the circadian control of sleep in rats (van Gool and Mirmiran, 1983; Li and Satinoff, 1995).

Additionally, an early study by Rosenberg *et al*. (1979) measured activity in the delta (2–4 Hz) range in young and old Fischer 344 rats but failed to detect a difference. However, this study did find a decreased amplitude of the diurnal sleep–wake rhythm, which is consistent with a breakdown of the circadian timing system (Rosenberg *et al*., 1979). Old rats slept significantly more than young rats, and did show more REM sleep as a percentage of total sleep time (Rosenberg *et al*., 1979). This suggests that process S is changing in old age, but absent a significant change in EEG, it is not clear whether the differences in sleep architecture are due to changes in process S, process C, or both. However, the increased amount of total sleep time seen in this strain of old rats is similar to the increase recently reported in old hamsters (see below) (Naylor *et al*., 1998a).

2. Mice

A study by Welsh and colleagues (1986) looking at the aging of sleep phenotypes in the mouse found changes similar to those in rats (Rosenberg *et al*., 1979; van Gool and Mirmiran, 1983) and hamsters (Naylor *et al*., 1998a). Compared to young mice, old C57BL/10 and C57BL/6 mice showed an increased fragmentation of sleep and wake over several circadian cycles (Welsh *et al*., 1986). As described above, these differences could be reflective of changes in either process C, process S, or both. The authors did not report data on EEG spectra and thus no information on the effect of age on delta power was obtained.

One particular advantage of mice as laboratory animals is their use in understanding the genetics of physiology and behavior. To this end, Eleftheriou *et al*. (1975) studied the effects of age on sleep in two inbred strains of mice. They found that both C57BL/6J and DBA/2J mice showed significant age-related increases in time spent awake and decreases in time spent in both non-REM and REM sleep (Elefthériou *et al*., 1975). This is in contrast with the age-related increases seen in sleep in hamsters (Naylor *et al*., 1998a) and several strains of rats (Rosenberg *et al*., 1979; van Gool and Mirmiran, 1983; Tani and Ishihara, 1988). Significant age-by-strain interactions were found on the amount of time spent in REM and non-REM sleep as well as REM sleep time as a percentage of total sleep time (Eleftheriou *et al*., 1975), indicating that at least some sleep parameters and age-related changes in sleep patterns are genetically determined. Hopefully, the wealth of genetic information available on mice will enable future research to identify genes involved in the aging of sleep phenotypes.

C. Changes in the Homeostatic Sleep Mechanism

In addition to changes in the circadian clock control of sleep, process S, the homeostatic drive for sleep, also changes in old age. This is best measured by EEG power, and age-related changes in EEG, particularly in the delta range associated with deep, slow-wave sleep, have been observed in a number of species.

1. Rats

While Rosenberg *et al*. (1979) found only changes in the diurnal rhythm of sleep of Fischer 344 rats and not in the amount of activity in the delta frequency range (2–4 Hz), a later study in the same strain found significant age-related dif-

[3]The age-related changes in the circadian timing system of female rats have been described in detail by Wise and her colleagues. See Wise *et al*., (1997) for a review.

ferences in EEG power (Tani and Ishihara, 1988). Tani and Ishihara (1988) measured EEG of both frontal cortex and the dorsal hippocampus. They found that awake older rats showed decreased power of alpha (8.0–12.8 Hz) waves in both brain regions, and also showed higher delta (2.0–3.6 Hz) activity in the hippocampus. This indicates that even while the animals are behaviorally awake, they have intrusions of delta waves, which suggests that they are chronically sleep-deprived. The age-related differences in EEG were more pronounced during sleep. In the first few hours of EEG recording, which the researchers termed the ''drowsy period,'' all old animals showed bursts of activity at 8–9 Hz and 15–16 Hz in the frontal cortex; none of the young animals showed this pattern (Tani and Ishihara, 1988). During sleep, old rats showed significantly more hippocampal theta (4.0–7.6 Hz) and less alpha-2 (10.0–12.8 Hz) activity than young animals. The sleeping old rats also showed significantly less power in the delta range than did young rats (Tani and Ishihara, 1988). This suggests that the old rats are not sleeping as efficiently as the young ones, perhaps reflecting a deficit in process S, and could account for the increase in delta power during wake. Unfortunately, Tani and Ishihara (1988) did not report total sleep time or percentage of time spent in REM and non-REM sleep. Thus, their data preclude any conclusions about the importance of the observed shifts in EEG power spectra to changes in total sleep time and sleep fragmentation previously reported in this strain (Rosenberg *et al*., 1979).

Old Sprague–Dawley rats have significantly less REM sleep and a blunted day:night difference in the amount of time spent sleeping (Stone *et al*., 1989). Compared to young Sprague–Dawleys, old rats have significantly less REM sleep, shorter bouts of REM sleep, and spend less of their time during the light period sleeping (Stone *et al*., 1989). The increase in the number of bouts of REM sleep suggests that process C has caused an increase in fragmentation. However, the change in the amount of REM sleep without a change in total sleep time (Stone *et al*., 1989) suggests that process S has changed, as this is independent of the timing of sleep or the amplitude of the ?tlsb> sleep rhythm. Like hamsters, this strain of rat shows evidence of changes in both components of Borbély's model of sleep.

Fisher rats show no change in total sleep time with age, but the amount time spent in deep non-REM sleep, as defined by amplitude of EEG waveforms greater than the mode of all non-REM waves, was decreased in middle-aged and old rats, when compared to young animals (Mendelson and Bergmann, 1999). However, these animals showed an increase in the lighter stages of non-REM sleep, so total non-REM sleep time was not affected by age (Mendelson and Bergmann, 1999). While there was no effect of age on the day:night differences in sleep and wake time, there was an age-related shortening of the length of each sleep bout; again this difference was apparent by middle age. However, the total number of sleep and waking bouts was not altered by age. Together these data suggest as this strain ages, the homeostatic sleep process (process S) is affected but process C is spared. However, these researchers used a relatively crude measure of circadian amplitude (data were averaged into 12 hr bins); studies specifically designed to examine circadian rhythms, as opposed to sleep, in aging rats have found effects of age (see data presented in the first section of this chapter).

2. Hamsters

Recent data from our laboratory show that as Syrian hamsters age they show an increased amount of non-REM sleep (Naylor *et al*., 1998a). The difference in daily non-REM sleep duration between young and old animals is entirely due to a difference in the amount of non-REM sleep during the dark phase, which is the active phase in this nocturnal species (Naylor *et al*., 1998a). While this could be attributed to a decreased circadian amplitude, EEG power in the delta (0.5–4 Hz) range, during non-REM sleep epochs, was decreased in old hamsters (Naylor *et al*., 1998a). This change is unlikely due to a change in process C and is more likely due to a change in process S. Since delta sleep is thought to be the restorative sleep (i.e., the component of sleep which decreases the level of process S in Borbély's model), by having delta sleep of decreased efficacy, the old animals may not able to break down process S as effectively during the light (inactive) phase and thus must sleep more during the dark (active) phase. This hypothesis is suggested by the observations made in two independent studies of old Fischer 344 rats mentioned above. While neither study reported both EEG power and percentage of time spent in sleep and wake, the data suggest that old rats show both decreased delta power during sleep (Tani and Ishihara, 1988) and increased time spent sleeping (Rosenberg *et al*., 1979). Additionally, old rats showed increased delta power while awake (Tani and Ishihara, 1988), suggesting that they may be trying to sleep more during the light phase to compensate for a decreased efficiency of process S. Thus the decreased delta power and concomitant increased sleep time seen in old hamsters (Naylor *et al*., 1998a) may apply to rodents in general.

D. Potential Mechanisms of the Age-Related Changes in Sleep and Implications for Treatment

1. Humoral and Metabolic

Although the suprachiasmatic nucleus has been identified as the master circadian pacemaker in mammals, no single brain structure has yet been found to have an analogous role for sleep. Thus it is difficult to study the physiological mechanisms behind either sleep or its age-related changes. Nevertheless, some intriguing results have been reported which suggest either potential mechanisms for these age-related changes or ways to prevent these changes from occurring.

The relationships between peripheral glucose regulation, sleep, and cognitive function point to the importance of the former in advancing age. Stone *et al*. (1990) found that old Sprague–Dawley rats with poor tolerance of a 500 mg/kg intraperitoneal bolus injection of glucose had significantly shorter bouts of REM sleep. There was no significant correlation in young animals (Stone *et al*., 1990), suggesting that as rats age, some animals lose their ability to regulate glucose, and those with compromised glucose regulation are subject to more fragmented REM sleep. Low doses of glucose (100 mg/kg) into old rats attenuated the age-related shortening of REM sleep bouts (Stone *et al*., 1992). This effect is age-specific, that is, glucose injections of this magnitude have no effect on REM bout duration in young rats (Stone *et al*., 1992).[4] This

[4]Significantly higher doses (2.5 g/kg) of glucose can alter REM duration in young rats (Sangiah and Caldwell, 1988).

has practical implications for both rat and human health, as other experiments have shown that sleep fragmentation is also associated with impairments on cognitive tasks in both species (Markowska *et al*., 1989) (see Stone *et al*., 1990, for a brief review of the relationship between glucose regulation and cognitive function, especially in diabetics). Since memory scores in middle-aged rats can predict their glucose tolerance, cognitive ability, and REM bout length when they are old (Stone *et al*., 1997), there are opportunities to intervene in youth or middle age to prevent some of the age-related changes. The finding that glucose can diminish the age-related shortening of REM sleep bouts suggests that the age-related changes are not due to permanent structural brain changes, but rather are due to shortages in energy or activity of neurons and could be secondary to problems with peripheral glucose regulation (Stone *et al*., 1992). Although decreased glucose tolerance (often associated with insulin resistance) and decreased amount of REM sleep have been observed in older adult people, the precise relationship between these manifestations and memory and cognitive deficits has not yet been investigated (Frank *et al*., 1995).

Sleep parameters are also correlated with brain neurochemical levels. Cortical serotonin is negatively correlated with total sleep time and non-REM sleep time in old rats; REM sleep time is positively correlated with serotonin in the caudate nucleus (Markowska *et al*., 1989). However, this study only examined aging rats and thus no conclusions can be drawn as to whether these neurotransmitters are involved in the regulation of sleep in young adult animals or in the aging of the sleep systems. Nevertheless, as the serotonergic system may also underlie the aging of the circadian timing system (Penev *et al*., 1993, 1994, 1995; Turek *et al*., 1995b), it is possible that therapies which restore the function of the serotonergic system might influence both the homeostatic sleep process (process S) and the circadian timing system (process C).

Other age-related changes in sleep and cognitive function can be mimicked by lesions of the nucleus basalis magnocellularis in animals (Stone *et al*., 1989). Old Sprague–Dawley rats have significantly less REM sleep and a decreased day:night difference in the amount of time spent sleeping (Stone *et al*., 1989). They also perform significantly worse on a passive-avoidance memory task and have decreased choline acetyltransferase activity in both the frontal cortex and the striatum (Stone *et al*., 1989). Nucleus basalis magnocellularis lesions of young rats also change sleep in similar ways. For instance, these lesions cause a decrease in the number and length of REM sleep bouts, which is also apparent in old intact rats (Stone *et al*., 1989). Similarly, young nucleus basalis magnocellularis-lesioned and old intact rats both show a decrease in the percentage of time spent sleeping during the light phase (Stone *et al*., 1989). They also show similar changes in passive-avoidance learning and cortical choline acetyltransferase activity, compared to young intact rats (Stone *et al*., 1989). These data suggest that some of the age-related changes in REM sleep and memory[5] are caused by the decreases in cholinergic function, perhaps at the level of the nucleus basalis magnocellularis or the frontal cortex.

There is good evidence for age-related declines in the function of cholinergic neurotransmission in general and in the basal forebrain in particular (Gallagher and Rapp, 1997). As adenosine levels in the basal forebrain have recently been implicated in the regulation of slow-wave sleep in cats (Porkka-Heiskanen *et al*., 1997), it is an obvious candidate for investigations into the mechanisms underlying age-related changes in slow-wave sleep. Future studies of old animals in species showing age-related changes in process S (e.g., hamsters, Fischer 344 rats) should examine adenosine levels in brain regions associated with sleep, especially the basal forebrain, as well as the efficacy of these cholinergic pathways in transmitting neural impulses to their targets.

There is also tentative evidence that the GABAergic system is involved in age-related changes in both REM and non-REM sleep regulation. Systemic administration of the $GABA_B$ antagonist CGP 35348 to old Wistar rats during the early portion of the night increased both REM and non-REM sleep duration and decreased the latency to REM onset (Puigcerver *et al*., 1996). However, due to limitations in the experimental design (no young control animals were studied), it is not possible to conclude if this effect is specific to aging animals (as is the effect of glucose on REM duration; see Stone *et al*., 1992) or is a general enhancer of REM (as is the effect of auditory stimulation, see below). Additionally, this study did not take into account the potential phase-shifting effects of this GABAergic compound; other GABAergic drugs [e.g., benzodiazepines (Turek and Losee-Olson, 1986)] can alter the phase of the circadian clock. GABA is a major neurotransmitter within the suprachiasmatic nucleus itself (Moore and Speh, 1993). Thus apparent changes in sleep may be the result of change in the phase of the circadian clock. Finally, no age-related changes in REM have been reported in Wistar rats, the strain used in this study. In fact, the authors cite data from other strains to support their claim that old rats show changes in REM sleep, when there seems to be an effect of genetic background on aging of sleep systems (Eleftheriou *et al*., 1975). Yet, the finding that a $GABA_B$ antagonist might increase REM suggests that this particular subclass of receptors is involved in sleep regulation and points to another possible mechanism underlying age-related changes in sleep.

2. Environmental Manipulations

Some data suggest that nonpharmacological environmental manipulations can influence age-related changes in sleep. For example, auditory stimulation (2 kHz, 75–80 dB, delivered for 100 msec every 20 sec) was delivered to both young and old rats. In young rats, stimuli were programmed to coincide with REM sleep bouts. In old rats, which show significantly shorter bouts of REM sleep, stimuli were presented for 10 min every 25 min (Arankowsky-Sandoval *et al*., 1992). On the day of stimulation both young and old rats showed increased time spent in REM sleep (Arankowsky-Sandoval *et al*., 1992); this effect is similar to that seen in both cats and humans (cf. Arankowsky-Sandoval *et al*., 1992). While the authors of this study suggest that auditory stimulation may increase REM sleep via the pons, they do not present data to show its involvement in this

[5]The relationship between REM sleep and long-term memory is beyond the scope of this review. Generally, preventing REM sleep inhibits consolidation and both sleep and memory disturbances are common in old age.

study (Arankowsky-Sandoval *et al*., 1992). Future studies are needed to determine if this relatively benign intervention can be used as a tool to explore the mechanisms of both age-related changes in REM bout duration, their functional significance, and ways to reverse them.

Interestingly, housing in an enriched environment also seems to offer the ability to counter some of the age-related changes in sleep. Van Gool and Mirmiran (1986) found that housing animals in an enriched environment for approximately 1 month following the baseline sleep-recording session led to an increase in both slow-wave (non-REM) and REM sleep during the light period in both young and old animals (van Gool and Mirmiran, 1986). This finding is particularly exciting because it suggests that the age-related changes in sleep quality are not necessarily permanent. Since the authors did not report delta power during non-REM sleep, it may be that the effect of the enriched environment is on circadian amplitude and not sleep *per se*. However, by increasing the circadian amplitude of the old rats, this intervention may be able to effect an output rhythm, i.e., the day:night difference in sleep duration.

3. Genetics

The severity of age-related changes in sleep parameters is at least partly under genetic control. As described above, males from two inbred strains (C57BL/6J and DBA/2J) of mice show a differential effect of aging on sleep parameters (Eleftheriou *et al*., 1975). In general, sleep in the DBA/2J mice ages faster, as measured by the age-related decrease in both REM and non-REM sleep. In fact, by the time they are 2 years old, DBA/2J mice show no REM sleep at all (Eleftheriou *et al*., 1975). Since the mice in a given strain are genetically identical, and all mice were maintained in the same environment, differences in the rate of aging are due to genetic differences between strains. Interestingly there is no difference between the two strains when they are young, suggesting that these strains have allelic differences in the way gene products underlying sleep change with age.

Little progress has been made in discovering genes that contribute to the aging of the sleep phenotype. However, two preliminary reports suggest the involvement of specific genes in the age-related changes of sleep. One report suggests that *Clock* plays an important role in regulating the set point of process S. *Clock* mutant mice were discovered in a screen for animals with altered circadian rhythms. Animals that are heterozygous for this mutation have long a circadian period (approximately 25 hr) and those that are homozygous for the mutation have a 28 hr period before losing circadian rhythmicity (Vitaterna *et al*., 1994). Compared to wild-type C57BL/6J mice, *Clock* mutants have decreased total sleep time without a change in power in the delta range (Naylor *et al*., 1998b). Most of the difference between the genotypes over a 24 hr period is due to decreased sleep time during the light phase. This suggests that the *Clock* mutation, which clearly alters process C (see Vitaterna *et al*., 1994; King *et al*., 1997; Antoch *et al*., 1997, and Jin *et al*., 1999, for a complete description of the effects and molecular basis of *Clock*), also has effects on process S. Further research will elucidate whether the difference in the homeostatic sleep mechanism is due to effects of the mutation on the circadian timing system or if there are other brain areas that are also affected by the mutation. It is not yet known if the CLOCK protein is expressed in brain regions that are thought to control sleep. A second report indicates that old rats have significantly less non-REM sleep during recovery from sleep deprivation, and that they also had less c-Fos and AP-1 production compared to young rats (Shiromani *et al*., 1998). Of course, *c-fos* and *Clock* are probably not the only genes involved in the age-related changes in sleep regulation. As new mutants and knockouts become available, they will serve as tools with which one can dissect the interactions between process C, process S, and aging.

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References

Akiyama, M., Kouzu, Y., Takahashi, S., Wakamatsu, H., Moriya, T., Maetani, M., Watanabe, S., Tei, H., Sakaki, Y., and Shibata, S. (1999). Inhibition of light- or glutamate-induced *mPer1* expression represses the phase shifts into the mouse circadian locomotor and suprachiasmatic firing rhythms. *J. Neurosci.* **19**, 1115–1121.

Arankowsky-Sandoval, G., Stone, W. S., and Gold, P. E. (1992). Enhancement of REM sleep with auditory stimulation in young and old rats. *Brain Res.* **589**, 353–357.

Arendt, J., and Deacon, S. (1997). Treatment of circadian rhythm disorders—Melatonin. *Chronobiol. Int.* **14**, 185–204.

Antoch, M. P., Song, E. J., Chang, A. M., Vitaterna, M. H., Zhao, Y., Wilsbacher, L. D., Sangoram, A. M., King, D. P., Pinto, L. H., and Takahashi, J. S. (1997). Functional identification of the mouse circadian *Clock* gene by transgenic BAC rescue. *Cell* **89**, 655–667.

Bell-Pedersen, D., Garceau, N., and Loros, J. J. (1996). Circadian rhythms in fungi. *J. Genet.* **75**, 387–401.

Benloucif, S., Masana, M. I., and Dubocovich, M. L. (1997a). Light-induced phase shifts of circadian activity rhythms and immediate early gene expression in the suprachiasmatic nucleus are attenuated in old C3H/HeN mice. *Brain Res.* **747**, 34–42.

Benloucif, S., Masana, M. I., and Dubocovich, M. L. (1997b). Responsiveness to melatonin and its receptor expression in the aging circadian clock of mice. *Am. J. Physiol.* **273**, R1855–R1860.

Borbély, A. A. (1982). A two process model of sleep regulation. *Hum. Neurobiol.* **1**, 195–204.

Borbély, A. A. (1998). Processes underlying sleep regulation. *Horm. Res.* **49**, 114–117.

Bundlie, S. R. (1998). Sleep in aging. *Geriatrics* **53**, S41–S43.

Buresová, M., Benesová, O., and Illnerová, H. (1990). Aging alters resynchronization of the circadian system in rats after a shift of the light-dark cycle. *Experientia* **46**, 75–77.

Cai, A., and Wise, P. M. (1996). Age-related changes in light-induced Jun-B and Jun-D expression: Effects of transplantation of fetal tissue containing the suprachiasmatic nucleus. *J. Biol. Rhythm* **11**, 284–290.

Cai, A. H., Lehman, M. N., Lloyd, J. M., and Wise, P. M. (1997a). Transplantation of fetal suprachiasmatic nuclei into middle-aged rats restores diurnal Fos expression in host—Special communication. *Am. J. Physiol.* **41**, R422–R428.

Cai, A. H., Scarbrough, K., Hinkle, D. A., and Wise, P. M. (1997b). Fetal grafts containing suprachiasmatic nuclei restore the diurnal rhythm of

CRH and POMC mRNA in aging rats. *Am. J. Physiol.* **42**, R1764–R1770.

Carrier, J., Monk, T. H., Buysse, D. J., and Kupfer, D. J. (1996). Amplitude reduction of the circadian temperature and sleep rhythms in the elderly. *Chronobiol. Int.* **13**, 373–386.

Copinschi, G., and Van Cauter, E. (1995). Effects of ageing on modulation of hormonal secretions by sleep and circadian rhythmicity. *Horm. Res.* **43**, 20–24.

Davis, F. C., and Viswanathan, N. (1998). Stability of circadian timing with age in Syrian hamsters. *Am. J. Physiol.* **275**, R960–R968.

DeCoursey, P. J., and Krulas, J. R. (1998). Behavior of SCN-lesioned chipmunks in natural habitat: A pilot study. *J. Biol. Rhythm* **13**, 229–244.

DeCoursey, P. J., Krulas, J. R., Mele, G., and Holley, D. C. (1997). Circadian performance of suprachiasmatic nuclei (SCN)-lesioned antelope ground squirrels in a desert enclosure. *Physiol. Behav.* **62**, 1099–1108.

Dijk, D.-J., Brunner, D. P., Bressma, D. G. M., and Borbély, A. A. (1990). Electroencephalogram power density and slow wave sleep as a function of prior waking and circadian phase. *Sleep* **13**, 430–440.

Dunlap, J. C. (1996). Genetic and molecular analysis of circadian rhythms. *Annu. Rev. Genet.* **30**, 579–601.

Dunlap, J. C. (1999). Molecular bases for circadian clocks. *Cell (Cambridge, Mass.)* **96**, 271–290.

Eleftheriou, B. E., Zolovick, A. J., and Elias, M. F. (1975). Electroencephalographic changes with age in male mice. *Gerontologia* **21**, 21–30.

Frank, S. A., Roland, D. C., Sturis, J., Byrne, M. M., Refetoff, S., Polonsky, K. S., and Van Cauter, E. (1995). Effects of aging on glucose regulation during wakefulness and sleep. *Am. J. Physiol.* **269**, E1006–E1016.

Franken, P., Tobler, I., and Borbély, A. A. (1991). Sleep homeostasis in the rat: stimulation of the time course of slow-wave EEG activity. *Neurosci. Lett.* **130**, 141–144.

Gallagher, M., and Rapp, P. R. (1997). The use of animal models to study the effects of aging on cognition. *Annu. Rev. Psychol.* **48**, 339–370.

Hillman, W. S. (1956). Injury of tomato plants by continuous light and unfavorable photperiodic cycles. *Am. J. Bot.* **43**, 89–96.

Huhman, K. L., and Albers, H. E. (1994). Neuropeptide Y microinjected into the suprachiasmatic nucleus region phase shifts circadian rhythms in constant darkness. *Peptides* **15**, 1475–1478.

Hurd, M. W., and Ralph, M. R. (1998). The significance of circadian organization for longevity in the golden hamster. *J. Biol. Rhythms* **13**, 430–436.

Inouye, S. T., Takahashi, J. S., Wollnik, F., and Turek, F. W. (1988). Inhibitor of protein synthesis phase shifts a circadian pacemaker in mammalian SCN. *Am. J. Physiol.* **255**, R1055–R1058.

Jin, X., Shearman, L. P., Weaver, D. R., Zylka, M. J., De Vries, G. J., Reppert, S. M. (1999). A molecular mechanism regulating the rhythmic output from the suprachiasmatic circadian clock. *Cell* **96**, 57–68.

Kawachi, I., Colditz, G. A., Stampfer, M. J., Willett, W. C., Manson, J. E., Speizer, F. E., and Hennekens, C. H. (1995). Prospective study of shift work and risk of coronary heart disease in women. *Circulation* **92**, 3178–3182.

Kawakami, F., Okamura, H., Tamada, Y., Maebayashi, Y., Fukui, K., and Ibata, Y. (1997). Loss of day-night differences in VIP mRNA levels in the suprachiasmatic nucleus of aged rats. *Neurosci. Lett.* **222**, 99–102.

Khalsa, S. B., Whitmore, D., and Block, G. D. (1992). Stopping the circadian pacemaker with inhibitors of protein synthesis. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 10862–10866.

King, D. P., Zhao, Y., Sangoram, A. M., Wilsbacher, L. D., Tanaka, M., Antoch, M. P., Steeves, T. D., Vitaterna, M. H., Kornhauser, J. M., Lowrey, P. L., Turek, F. W., Takahashi, J. S. (1997). Positional cloning of the mouse circadian *Clock* gene. *Cell* **89**, 641–653.

Klein, D. C., Moore, R. Y., and Reppert, S. M. (1991). "Suprachiasmatic Nucleus: The Mind's Clock." Oxford University Press, New York.

Kornhauser, J. M., Nelson, D. E., Mayo, K. E., and Takahashi, J. S. (1990). Photic and circadian regulation of *c-fos* gene expression in the hamster suprachiasmatic nucleus. *Neuron* **5**, 127–34.

Kort, W. J., and Weijma, J. M. (1982). Effect of chronic light-dark shift stress on the immune response of the rat. *Physiol. Behav.* **29**, 1083–1087.

Kort, W. J., Zondervan, P. E., Hulsman, L. O., Weijma, I. M., and Westbroek, D. L. (1986). Light-dark-shift stress, with special reference to spontaneous tumor incidence in female BN rats. *J. Natl. Cancer Inst.* **76**, 439–446.

Koster-van Hoffen, G. C., Mirmiran, M., Bos, N. P., Witting, W., Delagrange, P., and Guardiola-Lemaitre, B. (1993). Effects of a novel melatonin analog on circadian rhythms of body temperature and activity in young, middle-aged, and old rats. *Neurobiol. Aging* **14**, 565–569.

Krajnak, K., Kashon, M. L., Rosewell, K. L., and Wise, P. M. (1998). Aging alters the rhythmic expression of vasoactive intestinal polypeptide mRNA but not arginine vasopressin mRNA in the suprachiasmatic nuclei of female rats. *J. Neurosci.* **18**, 4767–4774.

Kumar, V., Goguen, D. M., Guido, M. E., and Rusak, B. (1997). Melatonin does not influence the expression of *c-fos* in the suprachiasmatic nucleus of rats and hamsters. *Mol. Brain Res.* **52**, 242–248.

Labyak, S. E., Turek, F. W., Wallen, E. P., and Zee, P. C. (1998). Effects of bright light on age-related changes in the locomotor activity of Syrian hamsters. *Am. J. Physiol.* **274**, R830–R839.

Li, H., and Satinoff, E. (1995). Changes in circadian rhythms of body temperature and sleep in old rats. *Am. J. Physiol.* **269**, R208–R214.

Liu, C., Weaver, D. R., Strogatz, S. H., and Reppert, S. M. (1997). Cellular construction of a circadian clock: Period determination in the suprachiasmatic nuclei. *Cell (Cambridge Mass.)* **91**, 855–860.

Madeira, M. D., Sousa, N., Santer, R. M., Paula-Barbosa, M. M., and Gundersen, H. J. (1995). Age and sex do not affect the volume, cell numbers, or cell size of the suprachiasmatic nucleus of the rat: An unbiased stereological study. *J. Comp. Neurol.* **361**, 585–601.

Markowska, A. L., Stone, W. S., Ingram, D. K., Reynolds, J., Gold, P. E., Conti, L. H., Pontecorvo, M. J., Wenk, G. L., and Olton, D. S. (1989). Individual differences in aging: Behavioral and neurobiological correlates. *Neurobiol. Aging* **10**, 31–43.

Mayeda, A. R., Hofstetter, J. R., and Possidente, B. (1997). Aging lengthens TauDD in C57BL/6J, DBA/2J, and outbred SWR male mice *(Mus musculus). Chronobiol. Int.* **14**, 19–23.

Mendelson, W. B., and Bergmann, B. M. (1999). Age-related changes in sleep in the rat. *Sleep* **22**, 145–150.

MeyerBernstein, E. L., Blanchard, J. H., and Morin, L. P. (1997). The serotonergic projection from the median raphe nucleus to the suprachiasmatic nucleus modulates activity phase onset, but not other circadian rhythm parameters. *Brain Res.* **755**, 112–120.

Moore, R. Y., and Speh, J. C. (1993). GABA is the principal neurotransmitter of the circadian system. *Neurosci. Lett.* **150**, 112–116.

Morin, L. P. (1988). Age-related changes in hamster circadian period, entrainment, and rhythm splitting. *J. Biol. Rhythms* **3**, 237–248.

Morin, L. P. (1993). Age, but not pineal status, modulates circadian periodicity of golden hamsters. *J. Biol. Rhythms* **8**, 189–197.

Morin, L. P., Blanchard, J., and Moore, R. Y. (1992). Intergeniculate leaflet and suprachiasmatic nucleus organization and connections in the golden hamster. *Visual Neurosci.* **8**, 219–230.

Mrosovsky, N. (1996). Locomotor activity and non-photic influences on circadian clocks. *Biol. Rev. Cambridge Philos. Soc.* **71**, 343–372.

Mrosovsky, N., and Biello, S. M. (1994). Nonphotic phase shifting in the old and the cold. *Chronobiol. Int.* **11**, 232–252.

Naylor, E., Buxton, O., Bergmann, B. M., Easton, A., Zee, P. C., and Turek, F. W. (1998a). Effects of aging on sleep in the golden hamster. *Sleep* **21**, 687–693.

Naylor, E., Vitaterna, M. H., Takahashi, J. S., and Turek, F. W. (1998b). Sleep in the *Clock* mutant mouse. *Sleep* **21** (Suppl.), 9.

Nelson, W., and Halberg, F. (1986). Schedule-shifts, circadian rhythms and lifespan of freely-feeding and meal-fed mice. *Physiol. Behav.* **38**, 781–788.

Pang, S. F., and Tang, P. L. (1983). Decreased serum and pineal concentrations of melatonin and *N*-acetylserotonin in aged male hamsters. *Horm. Res.* **17**, 228–234.

Penev, P. D., Turek, F. W., and Zee, P. C. (1993). Monoamine depletion alters the entrainment and the response to light of the circadian activity rhythm in hamsters. *Brain Res.* **612**, 156–164.

Penev, P. D., Zee, P. C., and Turek, F. W. (1994). Monoamine depletion blocks triazolam-induced phase advances of the circadian clock in hamsters. *Brain Res.* **637**, 255–261.

Penev, P. D., Zee, P. C., Wallen, E. P., and Turek, F. W. (1995). Aging alters the phase-resetting properties of a serotonin agonist on hamster circadian rhythmicity. *Am. J. Physiol.* **268**, R293–R298.

Penev, P. D., Turek, F. W., Wallen, E. P., and Zee, P. C. (1997). Aging alters the serotonergic modulation of light-induced phase advances in golden hamsters. *Am. J. Physiol.* **272**, R509–R513.

Penev, P. D., Zee, P. C., and Turek, F. W. (1998a). Quantitative analysis of age-related fragmentation of hamster 24-h activity rhythms. *Am. J. Physiol.* **273**, R2132–R2137.

Penev, P. D., Kolker, D. E., Zee, P. C., and Turek, F. W. (1998b). Chronic circadian desynchronization decreases the survival of animals with cardiomyopathic heart disease. *Am. J. Physiol.* **275**, H2334–H2337.

Pittendrigh, C. S., and Daan, S. (1974). Circadian oscillations in rodents: A systematic increase of their frequency with age. *Science* **186**, 548–551.

Pittendrigh, C. S., and Minis, D. H. (1972). Circadian systems: Longevity as a function of circadian resonance in Drosophila melanogaster. *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1537–1539.

Porkka-Heiskanen, T., Strecker, R. E., Thakkar, M., Bjørkum, A. A., Greene, R. W., and McCarley, R. W. (1997). Adenosine: A mediator of the sleep-inducing effects of prolonged wakefulness. *Science* **276**, 1265–1268.

Puigcerver, A., Van Luijtelaar, E. L. J. M., Drinkenburg, W. H. I. M., and Coenen, A. L. M. (1996). Effects of the $GABA_B$ antagonist CGP 35348 on sleep-wake states, behaviour, and spike-wave discharge in old rats. *Brain Res. Bull.* **40**, 157–162.

Rechtschaffen, A., and Kales, A., eds. (1968). A manual of standarized terminology, techniques and scoring system for sleep stages of human subjects. U.S. National Institute of Neurological Diseases and Blindness, Neurological Information Network, Bethesda, MD.

Reiter, R. J., Richardson, B. A., Johnson, L. Y., Ferguson, B. N., and Dinh, D. T. (1980). Pineal melatonin rhythm: reduction in aging Syrian hamsters. *Science* **210**, 1372–1373.

Roozendaal, B., van Gool, W. A., Swaab, D. F., Hoogendijk, J. E., and Mirmiran, M. (1987). Changes in vasopressin cells of the rat suprachiasmatic nucleus with aging. *Brain Res.* **409**, 259–264.

Rosenberg, R. S., Zepelin, H., and Rechtschaffen, A. (1979). Sleep in young and old rats. *J. Gerontol.* **34**, 525–532.

Rosenberg, R. S., Zee, P. C., and Turek, F. W. (1991). Phase response curves to light in young and old hamsters. *Am. J. Physiol.* **261**, R491–R495.

Saint Paul, U. V., and Aschoff, J. (1978). Longevity among blowflies *Phormia terraenovae* R.D. kept in non-24-hour light-dark cycles. *J. Comp. Physiol.* **127**, 191.

Sanchez-Barcelo, E. J., Megias, M., Verduga, R., and Crespo, D. (1997). Differences between the circadian system of two strains of senescence-accelerated mice (SAM). *Physiol. Behav.* **62**, 1225–1229.

Sangiah, S., and Caldwell, D. F. (1988). Reduction of rapid eye movement (REM) sleep by glucose alone or glucose and insulin in rats. *Life Sci.* **42**, 1425–1429.

Satinoff, E. (1998). Patterns of circadian body temperature rhythms in aged rats. *Clin. Exp. Pharmacol. Physiol.* **25**, 135–140.

Satinoff, E., Li, H., Tcheng, T. K., Liu, C., McArthur, A. J., Medanic, M., and Gillette, M. U. (1993). Do the suprachiasmatic nuclei oscillate in old rats as they do in young ones? *Am. J. Physiol.* **265**, R1216–R1222.

Sharma, V. K., and Chandrashekaran, M. K. (1998). Age-dependent modulation of circadian parameters in the field mouse *Mus booduga*. *J. Exp. Zool.* **280**, 321–326.

Shiromani, P. J., Basheer, R., Thakkar, J., Thakkar, M., and McCarley, R. W. (1998). Wakefulness-induced sleep and c-Fos/AP-1 in old and young rats. *Sleep* **21** (Suppl), 15.

Silver, R., LeSauter, J., Tresco, P. A., and Lehman, M. N. (1996). A diffusible coupling signal from the transplanted suprachiasmatic nucleus controlling circadian locomotor rhythms. *Nature (London)* **382**, 810–813.

Stone, W. S., Altman, H. J., Berman, R. F., Caldwell, D. F., and Kilbey, M. M. (1989). Association of sleep parameters and memory in intact old rats and young rats with lesions of the nucleus basalis magnocellularis. *Behav. Neurosci.* **103**, 755–764.

Stone, W. S., Wenk, G. L., Olton, D. S., and Gold, P. E. (1990). Poor blood glucose regulation predicts sleep and memory deficits in normal aged rats. *J. Gerontol.* **45**, B169–B173.

Stone, W. S., Wenk, G. L., Stone, S. M., and Gold, P. E. (1992). Glucose attenuation of paradoxical sleep deficits in old rats. *Behav. Neural Biol.* **57**, 79–86.

Stone, W. S., Rudd, R. J., Parsons, M. W., and Gold, P. E. (1997). Memory scores in middle-aged rats predict later deficits in memory, paradoxical sleep, and blood glucose regulation in old age. *Exp. Aging Res.* **23**, 287–300.

Sutin, E. L., Dement, W. C., Heller, H. C., and Kilduff, T. S. (1993). Light-induced gene expression in the suprachiasmatic nucleus of young and aging rats. *Neurobiol. Aging* **14**, 441–446.

Tani, Y., and Ishihara, T. (1988). Changes in EEG associated with sleep-awake behavior in young adult versus aged adult Fischer-344 rats. *Physiol. Behav.* **44**, 389–392.

Turek, F. W., and Losee-Olson, S. (1986). A benzodiazepine used in the treatment of insomnia phase-shifts the mammalian circadian clock. *Nature (London)* **321**, 167–168.

Turek, F. W., Penev, P., Zhang, Y., Van Reeth, O., Takahashi, J. S., and Zee, P. C. (1995a). Alterations in the circadian system in advanced age. *Ciba Found Symp.* **183**, 212–234.

Turek, F. W., Penev, P., Zhang, Y., Van Reeth, O., and Zee, P. (1995b). Effects of age on the circadian system. *Neurosci. Biobehav. Rev.* **19**, 53–58.

Valentinuzzi, V. S., Scarbrough, K., Takahashi, J. S., and Turek, F. W. (1997). Effects of aging on the circadian rhythm of wheel-running activity in C57BL/6 mice. *Am. J. Physiol.* **273**, R1957–R1964.

Van Cauter, E., Plat, L., Leproult, R., and Copinschi, G. (1998). Alterations of circadian rhythmicity and sleep in aging: endocrine consequences. *Horm. Res.* **49**, 147–152.

van den Pol, A. (1991). The suprachiasmatic nucleus: Morphological and cytochemical substrates for cellular interaction. *In* ''Suprachiasmatic Nucleus: The Mind's Clock'' (D. C. Klein, R. Y. Moore, and S. M. Reppert, eds.), pp. 17–50. Oxford University Press, New York.

van Gool, W. A., and Mirmiran, M. (1983). Age-related changes in the sleep pattern of male adult rats. *Brain Res.* **279**, 394–398.

van Gool, W. A., and Mirmiran, M. (1986). Effects of aging and housing in an enriched environment on sleep-wake patterns in rats. *Sleep* **9**, 335–347.

Van Reeth, O., and Turek, F. W. (1989). Stimulated activity mediates phase shifts in the hamster circadian clock induced by dark pulses or benzodiazepines. *Nature (London)* **339**, 49–51.

Van Reeth, O., Zhang, Y., Zee, P. C., and Turek, F. W. (1992). Aging alters feedback effects of the activity-rest cycle on the circadian clock. *Am. J. Physiol.* **263**, R981–R986.

Van Reeth, O., Zhang, Y., Reddy, A., Zee, P. C., and Turek, F. W. (1993). Aging alters the entraining effects of an activity-inducing stimulus on the circadian clock. *Brain Res.* **607**, 286–292.

Van Reeth, O., Zhang, Y., Zee, P. C., and Turek, F. W. (1994). Grafting fetal suprachiasmatic nuclei in the hypothalamus of old hamsters restores responsiveness of the circadian clock to a phase shifting stimulus. *Brain Res.* **643**, 338–342.

Van Reeth, O., Olivares, E., Mocaer, E., Zee, P. C., and Turek, F. W. (1997). A melatonin agonist increases the phase-shifting effects of dark pulses in old hamsters. 27th Annu. Meet., Soc. Neurosci., New Orleans, LA, Vol. 420.

Vitaterna, M. H., King, D. P., Chang, A.-M., Kornhauser, J. M., Lowrey, P. L., McDonald, J. D., Dove, W. F., Pinto, L. H., Turek, F. W., and Takahashi, J. S. (1994). Mutagenesis and mapping of a mouse gene, *Clock*, essential for circadian behavior. *Science* **264**, 719–725.

Watanabe, A., Shibata, S., and Watanabe, S. (1995). Circadian rhythm of spontaneous neuronal activity in the suprachiasmatic nucleus of old hamster in vitro. *Brain Res.* **695**, 237–239.

Wax, T. M. (1975). Runwheel activity patterns of mature-young and senscent mice: The efect of constant lighting conditions. *J. Gerontol.* **30**, 22–27.

Welsh, D. K., Richardson, G. S., and Dement, W. C. (1986). Effect of age on the circadian pattern of sleep and wakefulness in the mouse. *J. Gerontol.* **41**, 579–586.

Welsh, D. K., Logothetis, D. E., Meister, M., and Reppert, S. M. (1995). Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. *Neuron* **14**, 697–706.

Whealin, J. M., Burwell, R. D., and Gallagher, M. (1993). The effects of aging on diurnal water intake and melatonin binding in the suprachiasmatic nucleus. *Neurosci. Lett.* **154**, 149–152.

Wickland, C., and Turek, F. W. (1994). Lesions of the intergeniculate leaflet block activity-induced phase shifts in the circadian activity rhythm of the golden hamster. *Brain Res.* **660**, 293–300.

Wise, P. M., Cohen, I. R., Weiland, N. G., and London, E. D. (1988). Aging alters the circadian rhythm of glucose utilization in the suprachiasmatic nucleus. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5305–5309.

Wise, P. M., Kashon, M. L., Krajnak, K. M., Rosewell, K. L., Cai, A., Scarbrough, K., Harney, J. P., McShane, T., Lloyd, J. M., and Weiland, N. G. (1997). Aging of the female reproductive system: A window into brain aging. *Recent Prog. Horm. Res.* **52**, 279–303; discussion: pp. 303–305.

Witting, W., Mirmiran, M., Bos, N. P. A., and Swaab, D. F. (1993). Effect of light intensity on diurnal sleep-wake distribution in young and old rats. *Brain Res. Bull.* **30**, 157–162.

Witting, W., Mirmiran, M., Bos, N. P. A., and Swaab, D. F. (1994). The effect of old age on the free-running period of circadian rhythms in rat. *Chronobiol. Int.* **11**, 103–112.

Wollnik, F., Brysch, W., Uhlmann, E., Gillardon, F., Bravo, R., Zimmermann, M., Schlingensiepen, K. H., and Herdegen, T. (1995). Block of c-Fos and JunB expression by antisense oligonucleotides inhibits light-induced phase shifts of the mammalian circadian clock. *Eur. J. Neurosci.* **7**, 388–393.

Woods, W. H., Powell, E. W., Andrews, A., and Ford, C. W. (1993). Light and electron microscopic analysis of two divisions of the suprachiasmatic nucleus in the young and aged rat. *Anat. Rec.* **237**, 71–88.

Young, M. W. (1998). The molecular control of circadian behavioral rhythms and their entrainment in *Drosophila. Annu. Rev. Biochem.* **67**, 135–152.

Zee, P. C., and Turek, F. W. (1999). Introduction to sleep and circadian rhythms. *In* "Regulation of Sleep and Circadian Rhythms" (F. W. Turek and P. C. Zee, eds.), pp. 1–17. Dekker, New York.

Zee, P. C., Rosenberg, R. S., and Turek, F. W. (1992). Effects of aging on entrainment and rate of resychronization of circadian locomotor activity. *Am. J. Physiol.* **263**, R1099–R1103.

Zhang, Y., Kornhauser, J. M., Zee, P. E., Mayo, K. E., Takahashi, J. S., and Turek, F. W. (1996). Effects of aging on light-induced phase-shifting of circadian behavioral rhythms, Fos expression and CREB phosphorylation in the hamster suprachiasmatic nucleus. *Neuroscience* **70**, 951–961.

Zhang, Y., Brainard, G. C., Zee, P. C., Pinto, L. H., Takahashi, J. S., and Turek, F. W. (1998). Effects of aging on lens transmittance and retinal input to the suprachiasmatic nucleus on golden hamsters. *Neurosci. Lett.* **258**, 1–4.

Paul J. Lucassen and E. Ron De Kloet

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Glucocorticoids and the Aging Brain: Cause or Consequence?

In this review we discuss if changes in corticosteroid levels are the cause or consequence of the aging process and age-related brain pathology. Accumulating evidence suggests that corticosteroid levels are not consistently elevated in aging. However, if corticosteroid levels circulate in aberrant concentrations, they do increase the vulnerability to cognitive decline and disease, rather than aging *per se*. Corticosteroid hormone action is mediated through mineralocorticoid (MR) and glucocorticoid receptors (GR) that are colocalized in hippocampal neurons. The high-affinity MR operates in a proactive mode, limiting homeostatic disturbance and promoting neuronal stability. The low-affinity GR on the other hand, facilitates, in a reactive manner, the recovery of homeostasis following perturbation by stress. Together, steroid hormone-mediated MR and GR actions involve the expression of specific target genes that control brain processes like neuronal excitability, calcium homeostasis, energy metabolism, and cell division in discrete hippocampal regions. Given this capacity, MR and GR are critical for the set point regulation of many homeostatic processes during aging. Depending on additional environmental and genetic factors, an imbalance in their actions can therefore result in altered stress regulation, cognitive decline, and impaired behavioral adaptation, through dysregulation of the same genes that are otherwise essential for maintaining neuronal homeostasis and health. Corticosteroid receptors and their responsive genes are thus not only critical for successful aging, they form at the same time excellent drug targets, through which homeostasis can be reestablished and the restorative capacity still present within the diseased aging brain, promoted.

I. Introduction

Many changes associated with aging are experienced as deleterious, leading to slow, but progressive deterioration of peripheral organs, physical, and mental functions. In particular the senescent central nervous system (CNS) displays many neurophysiological, neurochemical, and neuroanatomical changes that are not only different from each other in nature, but also affect different parts of the brain to a different extent (Price *et al*., 1991; Mouton *et al*., 1994; Morrison and Hof, 1997; Braak *et al*., 1998).

Since many of our brain and bodily functions are governed by hormones, impaired endocrine control has been implicated in the etiology of several age-related dysfunctions and disease. A well-known definition of aging in this respect is a decreased ability to maintain homeostasis (Dilman *et al*., 1979; Everitt and Meites, 1989). Frolkis *et al*. (1972a,b), Landfield (1978), Finch (1979), and Sapolsky (1996, 1999) have studied this concept in more detail and implicated one class of steroid hormones, the glucocorticoid hormones, as major contributors to age-related pathology.

Glucocorticoids (GCs) are essential in mediating survival from stressors that threaten an organism's homeostasis. As such, they are involved in almost all aspects of peripheral physiology, such as energy metabolism, growth, immune function, but also in such diverse CNS processes as cognition, learning, memory, and behavioral adaptation. Small disruptions in their homeostatic control yield an altered neuroendocrine set point that, through aberrant hormone levels, induces slowly evolving functional changes, that can eventually accumulate in pathology and cognitive decline in senescence. Critical for neuroendocrine control of the GC set point are corticosteroid receptors localized in brain and anterior pituitary, through which the well-known classical action of these steroid hormones on gene transcription is mediated.

In this review we will focus on just that class of hormones and review changes in and consequences of GC action observed during mammalian brain aging. We further discuss to what extent structural and behavioral changes with aging are induced by GC alterations and at what point the central action of GCs shifts from a predominantly protective one to an endangering and damaging condition. This discussion is embedded in a

literature review of the role of GCs and the hypothalamic-pituitary-adrenal (HPA) axis in the adaptation to stress, which may be impaired with age.

II. Normal Physiology

A. The Hypothalamic-Pituitary-Adrenal (HPA) Axis and Hippocampus

Aging has been defined as a decreased ability to maintain homeostasis and a consequent failure to adapt to environmental changes and challenges. The view has evolved that the impact of age-related degeneration, in particular in higher organisms, is far more dramatic when the integrative and homeostatic communication and control systems are affected too (Everitt and Meites, 1989). Key elements in the communication of these adaptive changes within the organism are the autonomic nervous system with its sympathetic and parasympathetic components and the HPA axis, which uses amines, peptides, and steroids to integrate and exert complex homeostatic control functions.

The main function of the HPA axis is basically to mediate responses to environmental stressors and challenges, and to coordinate daily activities and sleep-related events. With a strong circadian rhythmicity, or after stimulation by stress, the release of the hypothalamic hormones corticotrophin-releasing hormone (CRH) and vasopressin (VP), is increased, leading in turn to the release of ACTH and endorphins from the pituitary. The latter peptides are end products of the proteolytic processing of the proopiomelanocortin (POMC) precursor. ACTH subsequently stimulates the adrenocortical release of the glucocorticoids, i.e., corticosterone in rodents and cortisol in primates. Peak levels of corticosterone, rising about 40-fold after stress, are usually reached within 15–30 min and return to prestress levels 60–90 min later. Through negative feedback action, glucocorticoids control their own release by downregulating the increases of CRH, vasopressin, and POMC peptides, whereas the secretion of mineralocorticoids is only slightly affected.

The primary sites of this negative feedback action are the pituitary corticotrophs and the parvocellular neurons of the paraventricular nucleus (PVN), where corticosteroids block stress-induced activation of the HPA axis. An extensive literature also points to the hippocampus as an additional site of control of HPA activity (Herman *et al*., 1989; Sapolsky *et al*., 1991b). Under basal conditions, however, the hippocampus exerts an overall inhibitory influence, whereas following stress, the hippocampus appears to be involved in the magnitude, onset, and termination phase of the HPA response. This hippocampal influence is mainly transsynaptic and occurs predominantly via the bed nucleus of the stria terminalis, from which an inhibitory GABAergic input innervates the PVN (see Fig. 62.2). This input is then integrated with input from several other areas, that, together, determine the composition of the secretagogue cocktail released to the pituitary (Herman *et al*., 1994, 1995, 1996; Herman and Cullinan, 1997). Glucocorticoid control of this neuroendocrine stress system is mediated by nuclear receptors that will be discussed below.

B. Brain Corticosteroid Receptors

Two types of corticosteroid receptors are expressed in brain: the mineralocorticoid receptors (MR), which bind corticosterone and cortisol with high affinity, and the glucocorticoid receptors (GR), with an approximately 10-fold lower affinity (Reul and De Kloet, 1985). Neurons in the hippocampus contain both receptor types in high densities, whereas cells in most other brain regions, including glia, mainly express GRs, and the anterior pituitary contains them as well (Van Eekelen *et al*., 1991; Drouin *et al*., 1993). Since synthetic steroids like dexamethasone, poorly penetrate the blood–brain barrier (De Kloet *et al*., 1975; De Kloet, 1997; Meijer *et al*., 1998; De Kloet *et al*., 1998), the pituitary GRs are the main site where these steroids exert their feedback action. The naturally occuring GCs on the other hand, are highly lipophylic and readily pass the blood–brain barrier, where their principal sites of action are the CRH neurons of the paraventricular nucleus where GRs are also found in high densities.

Due to the high affinity of MR, these hippocampal receptors are already largely occupied when corticosteroid levels are low, for example during rest. Under these conditions, GRs are, however, only partially occupied and do not become fully activated until corticosteroid levels rise considerably, such as after stress. Although, classically, homodimers of MR or GR are known to bind to glucocorticoid responsive elements on the DNA, and from there affect gene transcription, many additional genomic steroid actions require protein–protein interactions between corticosteroid receptor monomers and other transcription factors: see Fig. 62.1, and also coactivators have been implicated (Meijer *et al.*, 2000). The effects of steroids on brain cells and their function therefore critically depend also on the nature and timing of other inputs that acti-

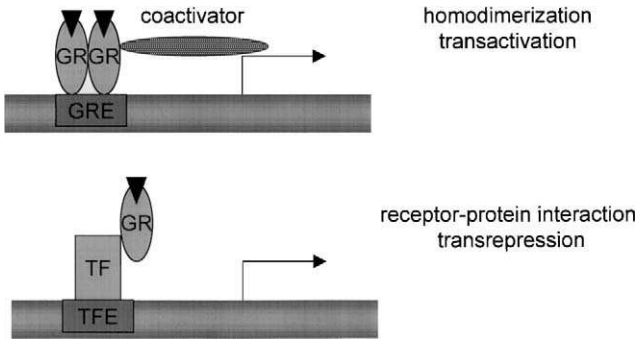

FIG. 62.1. Corticosteroid receptor-mediated actions on gene transcription. The interaction of the steroid receptors with other proteins in the cell constitutes a molecular basis for the conditional or context-dependent effects of corticosteroid hormones and consists of two modes of action. (Top) Transactivation: Upon ligand binding, corticosteroid steroid receptor can form homodimers, that bind to glucocorticoid response elements (GRE) in the DNA and recruit RNA polymerase II that contains a pre-initiation complex of multiple proteins and can subsequently stimulate the synthesis of mRNA. This mode of steroid action requires interaction of the receptor with additional coactivator proteins. (Bottom) Transrepression: In this mode of action, GRE-dependent target genes in the nucleus are left unstimulated, but transcription factors (TF) can bind specific response elements (TFE) and stimulate transcription. The receptor interferes in its monomeric form with transcription, that is activated by interactions with other TFs (transrepression).

vate transcription factors, like cyclic AMP response element binding protein (CREB) or other immediate–early genes (Vreugdenhil and DeKloet, 1998; Reichardt *et al*., 1999).

A wide variety of cellular and molecular actions by corticosteroids has been described, particularly for cells of the hippocampus. In general, situations where mostly MRs, but few GRs, are activated, are associated with small calcium currents, and thus a reduced spike frequency accommodation, stable responses to repeated stimulation of glutamatergic pathways, and relatively small responses to biogenic amines (Joëls and De Kloet, 1990, 1992; Joëls *et al*., 1991; Karten *et al*., 1999). MR activation thus seems to guarantee a stable background of neuronal firing which has been described as a proactive mode with respect to its role in maintaining neuronal homeostasis (De Kloet *et al*., 1998, 1999).

Activation of GR, in addition to MR, as occurs after exposure to a stressor or during the circadian peak, results in enhanced calcium influx, stronger spike frequency accommodation, changes in ion channel conductances, and marked responses to biogenic amines like serotonin (Joëls and De Kloet, 1989; Nair *et al*., 1998; Joëls *et al*., 1991; Karten *et al*., 1999). GR activation thus reduces cellular activity after acutely stressful situations, which is in accordance with their proposed reactive mode of action by which corticosteroids facilitate the recovery from disturbances in homeostasis (De Kloet *et al*., 1998).

Interestingly, in the absence of corticosteroids, many cellular properties and synaptic plasticity related events, resemble the situation as seen after high corticosteroid levels with simultaneous MR and GR activation. The effects of stress exposure on synaptic plasticity thus seems to follow essentially the same pattern in the two conditions (Diamond *et al*., 1992), revealing an (inverted) U-shaped, dose dependency for the action of these steroids (Joëls and De Kloet, 1990, 1992; De Kloet, 1991). The properties of the entire neuronal network may either change in parallel with or result from the steroid effects on individual cell characteristics, such as calcium influx and responsiveness to glutamatergic input.

C. Behavior

Steroid actions on hippocampal cells and the circuits they form also modulate learning and memory processes. For example, it has become clear that predominant MR activation results in completely different actions on these processes than concomittant MR and GR activation. Recent studies have shown that MR plays a role in behavioral reactivity during novel situations, whereas GR is involved in the consolidation of learned information (Fig. 62.2). This is partly based on studies using the Morris water maze. Intracerebroventricular administration of selective GR antagonists before or immediately after the first training session in a water maze resulted in impaired retention of the task 24 hr later (Oitzl and De Kloet, 1992; Oitzl *et al*., 1994, 1997b). Since treatment before the retrieval test was ineffective, GR blockade apparently interferes with the consolidation rather than the retrieval of acquired spatial information. MR blockage did not influence the latencies to find the platform, irrespective of the timing of the treatment. However, MR blockade significantly changed the search strategy in the free swim trial. The rats still headed directly to the former platform location (hence retention is undisturbed), but subsequently explored other areas of the pool as well, rather than remaining in the platform quadrant, which the controls did (Fig. 62.2 and Oitzl *et al*., 1994, 1995, 1997b, 1998a,b).

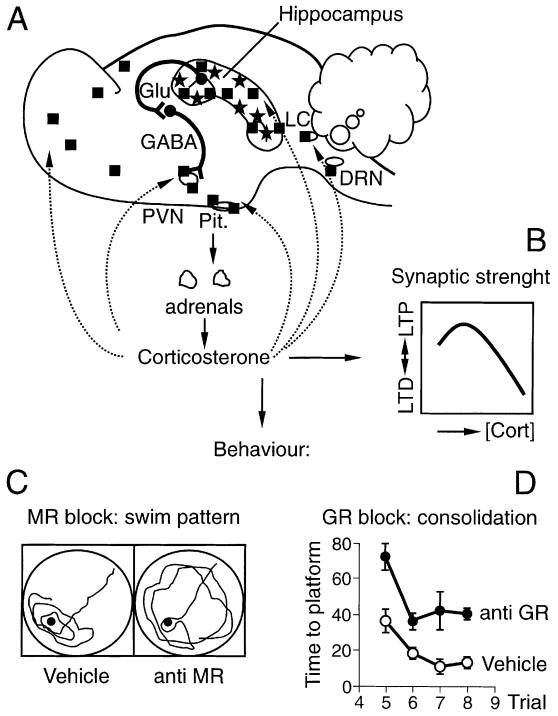

FIG. 62.2. MR and GR-mediated corticosteroid regulation of HPA-activity, of synaptic strength in hippocampus, and spatial learning behaviour. (A) HPA-axis: Schematic view of the HPA-axis comprising the hippocampus with mineralocorticoid receptors (MR, stars) and glucocorticoid receptors (GR, squares), the CRH neurons in the paraventricular nucleus (PVN), the corticotrophs in the anterior pituitary gland and the adrenal cortex. A major inhibitory GABAergic input to the PVN is formed by excitatory glutamatergic input originating from the hippocampus. Stress-induced rises of corticosteroids exert a negative feedback following binding to GRs in the pituitary corticotrophs and the CRH neurons of the PVN. The MR is involved in potentiation of the hippocampal output and thus enhances neural inhibition of the HPA-axis, whereas GRs in the hippocampus mediate opposite actions of corticosteroids, and dampen hippocampal output. GRs also mediate corticosteroid activation of ascending aminergic inputs to the PVN coming from the brain stem (LC, locus coeruleus; DRN, dorsal raphe nucleus) (De Kloet *et al.*, 1998). (B) Synaptic strength: LTP is induced in an optimal manner when corticosteroid levels are mildy elevated, that is, when MR and some GR are activated. High levels of corticosteroids in contrast, occupying most GRs, not only inhibit long term potentiation (LTP), but also induce long term depression (LTD). These data point to a bell-shaped dose-dependency for the formation of LTP in the hippocampus (De Kloet *et al.*, 1990). (C and D) Spatial learning behavior: (C) Following testing in the Morris water maze, the application of an MR antagonist i.c.v., 30 min prior to the retrieval test 24 hr later, clearly influenced the swim pattern and interfered with the selection of a behavioral response (left panel, MR block: swim pattern). Although treated rats (anti MR) approach the former platform position directly they subsequently spend less time searching in the vicinity of the platform quadrant, and rather search for an escape route elsewhere in the pool. (D) Inhibition of GR (anti GR) immediately during the consolidation process on day 1, resulted in an impaired performance when tested 24 hr later, indicating the role of GR in memory consolidation. These MR- and GR-mediated effects on information processing facilitate behavioral adaptation (Oitzl and de Kloet, 1992).

Specific MR- and GR-mediated effects can thus be disentangled by administration of selective agonists and antagonists for one of the receptor types, at specific stages of information processing. This should be done with care, in view of the possibility of changed blood–brain barrier kinetics for synthetic steroid receptor agonists, like dexamethasone (Meijer *et al*., 1998). The resulting suppression of HPA activity after dexamethasone takes place at the level of the pituitary, but leaves hormone receptors within the brain unoccupied. This explains why systemic administration of corticosterone to adrenalectomized rats, which activates both MR and GR, can reinstate contextual fear conditioning, whereas dexamethasone failed to do so (Bohus and De Kloet, 1981). These behavioral studies underscore the notion that MR activation appears essential for the correct interpretation of environmental stimuli and selection of a proper behavioral response. The MR- and GR-mediated effects are different, but interact and proceed in a coordinative manner, linked in time to the particular stage in information processing (De Kloet *et al*., 1998, 1999).

D. Neuroendocrine Regulation

Observations on the selective effects of glucocorticoids on HPA regulation have often been made without considering their additional effects on higher brain functions involved in arousal and information processing. GR-mediated effects on brain areas projecting to the PVN, for example, can have profound and long-lasting consequences for glucocorticoid feedback regulation of the HPA axis. First, the ascending aminergic inputs excite the PVN, an action that is potentiated by glucocorticoids and stress. Inputs from other nuclei, such as the suprachiasmatic nucleus and an intrahypothalamic GABAergic network, are also under control of glucocorticoids (Herman *et al*., 1996; Herman and Cullinan, 1997; De Kloet *et al*., 1998).

Limbic inputs into the intrahypothalamic GABAergic network, coming from the hippocampus, can be either excitatory, and thus enhance the inhibitory GABAergic tone, or inhibitory, when they originate from the amygdala, in which case they reduce the inhibitory tone (Fig. 62.2). This implies that with an enhanced exitatory input from the hippocampus, the HPA axis becomes relatively more suppressed. MR-mediated actions in the hippocampus maintain a high excitatory tone in the hippocampal circuit (Deuschle *et al*., 1998b), which, via the subsequent activation of the GABAergic input to the paraventricular nucleus, are expected to suppress HPA activity. Consistent with this hypothesized mechanism, central administration of an MR antagonist indeed disinhibited the HPA axis, and elevated basal levels of corticosterone were observed (Ratka *et al*., 1989; Oitzl *et al*., 1995; Spencer *et al*., 1998).

GR-mediated effects generally oppose those mediated by MRs in the hippocampus (Joëls and De Kloet, 1992). Accordingly, during a rise in GC levels after stress, GR activation suppresses hippocampal excitatory output, which results in disinhibition of the GABAergic input to the paraventricular nucleus. This disinhibitory influence persists as long as adaptation to the stressor fails, and both HPA activity and GC levels remain elevated. Behavioral adaptation eventually eliminates the HPA hyperdrive and is facilitated by GC action in the hippocampus. Accordingly, GC action in higher brain regions like the hippocampus, appears to be primarily concerned with behavioral adaptation and only secondarily with the neuroendocrine consequences of information processing (De Kloet *et al*., 1998).

III. Aging

A. Glucocorticoid Changes in Aging Mammals

A vast and already somewhat older literature has generally demonstrated elevated basal GC and ACTH levels in aged rodents, frequently accompanied by a flattened circadian GC rhythm. Following stress, HPA reactivity displays a profoundly different pattern in young and old animals. Peak levels of ACTH and/or corticosterone are elevated, but there are large variations between individuals and between strains. It has been reported that aged animals furthermore generally display a reduced ability to terminate the stress response (Sapolsky, 1992, 1996) and hence are exposed to increased cumulative GC levels as compared to young animals (Landfield *et al*., 1978, 1981; Angelucci *et al*., 1987; Brodish and Odio, 1989; Dellwo and Beauchene, 1990; Erisman *et al*., 1990; Sabatino *et al*., 1991; Sapolsky, 1992, 1996; Hauger *et al*., 1994). Exceptions exist as well, as several studies failed to find elevations in basal GC levels, reported on decreases in GC or ACTH levels, or, such as found an enhanced ACTH rather than GC response to an emotional stressor, in old as compared to young animals (Fig. 62.3 and Sonntag *et al*., 1987; Issa *et al*., 1990; Lorens *et al*., 1990; Scaccianoce *et al*., 1990, 1995; Van Eekelen *et al*., 1991, 1992; Dhabhar *et al*., 1993; Morano *et al*., 1994; Cizza *et al*., 1995; Seckl and Olsson, 1995).

Following exposure to a prolonged or repeated stressor, GC-mediated feedback of the HPA axis is reduced. This may in part be due to decreases in the number or affinity of the GR, that were found in at least some rat and mouse strains and may reflect functional alterations as well (Nicholson *et al*., 1987, 1988; Eldridge *et al*., 1989a,b; Patacchioli *et al*., 1989, 1990; Zoli *et al*., 1991; Meaney *et al*., 1992; Sapolsky, 1992, 1996, 1999; Talmi *et al*., 1993). However, many of these binding studies did not discriminate between MR and GR. Moreover, they considered the hippocampus as a sole and primary neuroendocrine feedback site through which GR-mediated effects suppress HPA activity, and failed to include GR expression in the hypothalamic paraventricular nucleus or the pituitary corticotrophs.

In old Brown Norway rats, differential changes in MR and GR number in hippocampus and hypothalamus have been reported (De Kloet *et al*., 1991; De Kloet, 1992; Van Eekelen *et al*., 1991, 1992). One consistent finding is that regardless of the strain of rats examined, a reduction in MR binding capacity at senescence is found (Lorens *et al*., 1990; De Kloet, 1992). In addition, both MR mRNA and GR mRNA levels in the hippocampus as well as GR mRNA in the PVN and frontal cortex were found to be reduced in aged rats, which suggests a decreased transcriptional activity in these areas (Plotsky *et al*., 1993; Morano *et al*., 1994; Makino *et al*., 1995; McEwen, 1996; Workel *et al*., 2000). These observations are further supported by reductions in GR immunoreactivity in the CA1 and CA2 fields of the hippocampus (Van Eekelen *et al*., 1991, 1992; Zoli *et al*., 1991). However, with immuno-

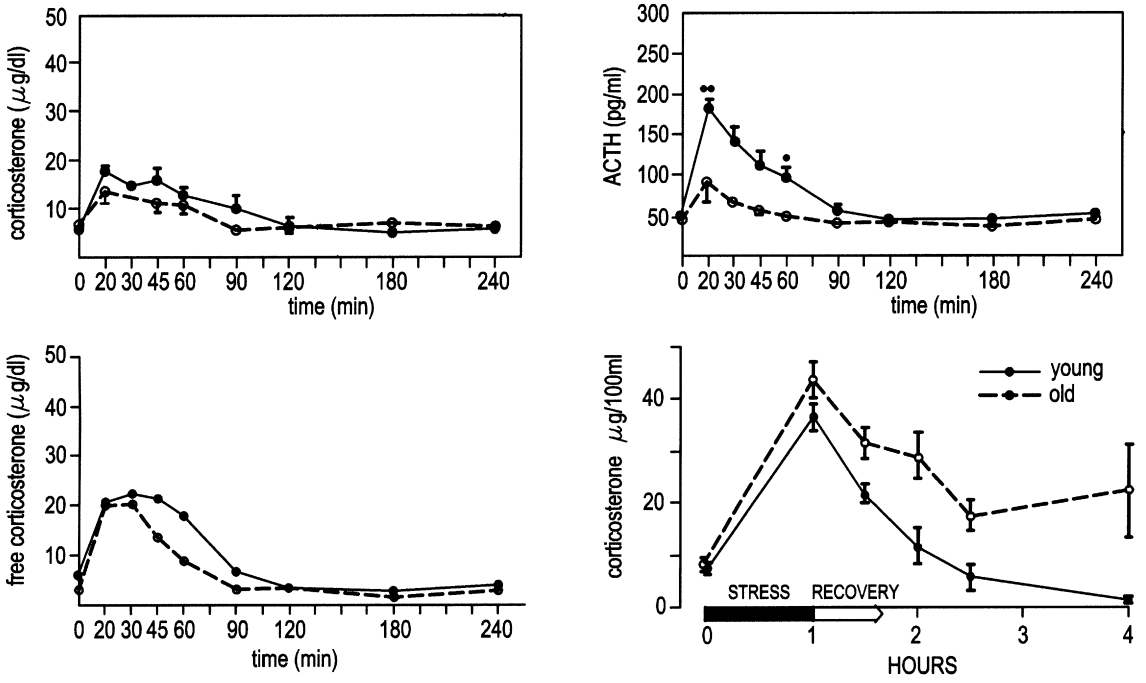

FIG. 62.3. Effect of stress on the plasma ACTH and corticosterone levels of the senescent rat. (Upper right and left) Effects of a conditional emitional response on corticosterone and ACTH levels in young (open circles, n=8, dashed line) and aged (closed circles, n=6, continous line) male Brown Norway rats. Note that corticosterone levels are not significantly different, whereas the ACTH response to stress is significantly enhanced (from Van Eekelen *et al.*, 1991, with permission). (Lower left) Effect of a conditional emotional response on free corticosterone levels in plasma of the male Brown Norway animals shown above (open circles, young; and closed circles, old animals). Plasma corticosteroid binding is suppressed during aging and the small differences in free corticosterone levels between the groups indicate a delayed recovery from stress (from Van Eekelen *et al.*, 1991, with permission). (Lower right) Corticosterone levels in young and aged Fisher rats during 60 min of immobilization stress during the light period, monitored for 3 subsequent hr of recovery. In contrast to the Brown Norway rat, corticosterone levels of aged subjects of the Fisher strain did not recover to basal values after this stressor (from Sapolsky *et al.*, 1984, with permission).

cytochemistry for the MR in these old animals, no consistent changes could be found, either in number of MR immunopositive cells or in MR immunoreactivity per cell (Bhatnagar *et al.*, 1997). MR mRNA was altered in the hippocampus, but only in discrete areas, like the dentate gyrus (Workel *et al.*, 2000). This discrepancy concerning the age-related downregulation of MR binding vs MR mRNA levels and immunoreactivity may be due to translation, receptor processing, or posttranslational events.

Another mammal that is relatively well studied in relation to the effects of stress and GC exposure is the day-active tree shrew, which is highly sensitive to social hierarchy differences (Fuchs and Flügge, 1998). When two males are confronted with each other in a dominant–subordinate setting, profound changes in several behavioral and physiological parameters of HPA axis activity are induced. The resulting psychosocial, rather than physical, stress induces many changes like elevated GC levels in the subordinate animal, dendritic atrophy of CA3, downregulation of GR mRNA in the hippocampus as well as suppression of neurogenesis (Johren *et al.*, 1994; Fuchs *et al.*, 1995; Magarinos *et al.*, 1996; Jamieson *et al.*, 1997; Fuchs and Flügge, 1998). In a recent aging study in this species, gradual increases were reported until adulthood, after which no further rise was found in basal urinary free cortisol concentrations, or after reaching senescence (7–8 years) (Van Kampen and Fuchs, 1998). Interestingly, no indications could be found for amyloid pathology in the brain of the old tree shrew, which contrasts with the situation in all other aged non-human primates studied to date and may possibly relate to the absence of increased GC levels in aging (Pawlik *et al.*, 1999).

B. Aging Studies in Rodents: General Considerations

As is clear from the above, HPA axis activation in aging rats has been reported in many, but not all studies (Sonntag *et al.*, 1987; Issa *et al.*, 1990; Lorens *et al.*, 1990; Van Eekelen *et al.*, 1991, 1992; Dhabhar *et al.*, 1993; Morano *et al.*, 1994; Cizza *et al.*, 1995; Sapolsky, 1996). These differences largely depend on differences in strain and sex or experimental design (see Sapolsky, 1992, 1996). Spraque–Dawley, Long–Evans, Fisher, and Wistar rats, furthermore, generally show increased basal and prolonged stress-induced GC levels during aging. However, in aged Brown–Norway rats, neither basal plasma GC levels nor GC rises induced by a novel environment or a conditioned emotional response were altered (Fig. 62.3 and Van Eekelen *et al.*, 1991, 1992; Gomez *et al.*, 1998). In the latter strain, the capacity of GC-binding globulin was strongly reduced, which yields an increased free fraction of nonbound GC. Together with the increased peak values, but not the duration of the ACTH response, and without clear changes in adre-

nocortical GC secretion, this suggests a reduced adrenal sensitivity to ACTH or a reduced bioactivity of ACTH in old animals of this particular strain.

When comparing aging studies in rodents, clear differences in the 50% survival age are apparent between rat strains (Masoro, 1980a,b). Criteria formulated for an animal model for aging include an almost rectangular survival curve and thus a high estimated 50% survival age, which should be accompanied by the occurrence of age-related, multiple pathologies, rather than inbred strain specific diseases like the high prevalence of testis or pituitary tumors in F344 or Wistar rats (Masoro, 1980a,b). The animals used in many of the previous aging studies in fact either fail to meet these criteria for a good model for aging or should be considered middle-aged rather than old (i.e., less than the lowest estimate of the 50% survival age for the strain) which can have important consequences when studying age-related changes (Coleman *et al*., 1990). The Brown Norway strain does meet these criteria and is as such generally accepted as a good rodent model to study aging.

Another important consideration in this type of studies is the individual variation within a genetically homogeneous cohort of animals, which becomes much more pronounced at old age. With respect to many behavioral and neurobiological parameters such as memory performance, individual animals can often be classified in an impaired, an intermediate, and a nonimpaired subgroup (Gage *et al*., 1984b; Rots *et al*., 1996b,c; Oitzl *et al*., 2000a,b; Workel *et al*., 2000). In a large group of aged rats, approximately 30% appeared to be unaffected in their memory performance, whereas the rest had severe impairments, notably in relation to their GC levels (Issa *et al*., 1990).

Interestingly, individual differences in cognitive decline that appear at old age, are subject to manipulation by early life events. Procedures like maternal deprivation or postnatal handling during the first 3 weeks of the rat's life, for example, can alter the animal's stress responsiveness permanently. Animals handled postnataly showed lower basal GC levels at old age than non-handled, aged animals, as well as reduced rises in plasma GC levels following stress, less age-related hippocampal cell loss and less cognitive impairment (Levine *et al*., 1991; Meaney *et al*., 1991, 1992; Van Oers *et al*., 1998, 1999; Workel *et al*., 2000).

Another example of the long-lasting effects of early life events is maternal deprivation, which influences several aspects of HPA axis reactivity during development, adulthood, and aging. The outcome of maternal deprivation, however, depends on the frequency and age at which separation occurs, while also strain and sex are important variables (Levine *et al*., 1991; Meaney *et al*., 1991; Rots *et al*., 1996a; Workel *et al*., 1997, 2000). Deprivation for 24 hr at day 3 of the rat pup's life was recently shown to cause a striking pattern of endocrine and behavioral changes during life. The stress-induced corticosterone output of the deprived animals showed a pronounced increase at midlife, but senescent animals displayed attenuated corticosterone responses as compared to their control, mother-reared littermates. At midlife they showed a transient increase in expression of MR mRNA in the hippocampal dentate gyrus prior to downregulation at senescence and age-related changes in GR expression as well (Workel *et al*., 2000).

The most striking effect of maternal deprivation was on cognitive performance at senescence. Based on current ideas, it was expected that an early traumatic life event would cause cognitive decline in *every* individual. This was, however, not observed and maternal deprivation was found to amplify the individual differences present at old age and gave rise to a complete shift in the percentages of animals classified as good, intermediate, and bad learners, on the basis of their performance in the Morris water maze. The large group of average performers in the mother-reared rats, fell apart in two extremes of cognitive performance in the maternally deprived group. The senescent, mother-reared animals were mostly partially impaired, whereas deprived littermates were either very poor, or very good performers in terms of cognition. Notably, senescent animals that were maternally deprived in the first days after birth were either good or impaired learners at old age, with only a few animals showing intermediate performance. Their frequency distribution, consequently, resembled a ''U-shaped'' curve. In contrast, the learning ability of nonhandled, control animals at old age showed a bell, or ''inverted U-shaped'' distribution, with the majority of the animals showing intermediate performance. This dichotomy of cognitive performance in the deprived animals developed in association with attenuated corticosterone responses to stress, but was preceded by a midlife surge in stress reponsiveness and hypercorticism. Whether such a ''midlife'' crises in the rat programs the subsequent aging trajectory remains to be established (Oitzl *et al*., 2000a,b; Workel *et al*., 2000).

Regarding handling of newborn animals, this procedure in female animals resulted in consistent increases in GR binding (Meaney *et al*., 1991, 1992) and probably also involves changes in serotonin metabolism and in sensitivity to antidepressants. Both restraint stress induced atrophy of the dendritic tree in CA3 as well as the parallel memory impairment could be prevented by serotonin uptake enhancers in nonhandled adult rats (Magarinos *et al*., 1998, 1999). The antidepressant amitriptyline also significantly improved spatial memory in young rats and increased hippocampal MR, but not GR mRNA expression, whereas in aged rats, this antidepressant had no effect on spatial memory or hippocampal corticosteroid receptor gene expression, either in cognitively unimpaired or cognitively impaired animals (Yau *et al*., 1995). Amitriptyline did not influence basal morning plasma corticosterone levels in either young or aged rats, but significantly decreased evening corticosterone levels in aged rats, consistent with a selective activation of hippocampal MR. In addition, age-related decreases in various hippocampal 5HT transporter sites could be upregulated by 10 weeks of antidepressant treatment, which may contribute to the general lower effectiveness of tricyclic antidepressants with aging (Yau *et al*., 1995, 1999). In another study, administration of antidepressants reversed the age-related changes in corticosterone and feedback function in old animals, but not in GR levels or cognitive decline (Rowe *et al*., 1997), whereas *in vitro* treatment of cultured hippocampal neurons increased both GR binding as well as GR gene expression (Okugawa *et al*., 1999).

Together, this suggests that early life events can permanently alter the responsiveness of the serotonergic and the HPA systems throughout life. These changes may have important consequences for the development of differential sensitivity to adult and senescent cognitive decline, but are very difficult

to influence or correct once steady-state conditions have been reached in adulthood.

In conclusion, on the basis of animal studies, several scenarios have been put forward that link rises in GC levels to aging. First, stress and hypercorticism were proposed to promote cognitive aging. Second, aging may cause initial hypercorticism, which then, in a feedforward cascade of increasing feedback insensitivity, would further promote the aging process. Third, stress together with hypercorticism would amplify the individual variation in cognitive aging. The evidence for these three scenarios will be discussed below in different species.

C. Glucocorticoid-Related Changes in Aging Primates and Humans

Similar to rodents, increased HPA activity is not a universal feature of human aging either, although these species also differ in several respects. Aging does not seem to lead to a change in basal HPA activity as measured by morning cortisol levels that were not different between young or old subjects, nor did cortisol-binding globulin levels change with age, and no changes in the sensitivity of the feedback system to dexamethasone were observed with human age (Huizenga *et al*., 1998). The circadian release pattern of cortisol also appears intact in terms of rhythmicity and pulsatile hormone release, but a reduced amplitude and a phase-advance in the cortisol peak have been reported as well (Colucci *et al*., 1975; Touitou *et al*., 1983; Huizenga *et al*., 1988; Van Cauter *et al*., 1996; Deuschle *et al*., 1997, 1998a). Following a (prolonged) challenge, furthermore, the stress response seems to remain intact, as does adrenal sensitivity to ACTH and feedback sensitivity to dexamethasone with age (Cartlidge *et al*., 1970; Riegle and Hess, 1972; Zimmerman and Coryell, 1987; Roberts *et al*., 1990; Gotthardt *et al*., 1995). A few studies reported an increased ACTH and corticosterone response in aged individuals, that was sex dependent in some but not all studies. A sex difference was also found in the cortisol response in aged females that was larger compared to males, which may represent an enhanced age-related decline in feedback inhibition of the HPA axis of females (Heuser *et al*., 1994; Wilkinson *et al*., 1997; Deuschle *et al*., 1998a).

In addition, in studies on the postmortem human hypothalamus, increased numbers of CRH expressing neurons, and increased CRH-vasopressin colocalization in the paraventricular nucleus were found with aging in males indicative of an increased chronic CRH activation during human aging (Raadsheer *et al*., 1994, 1995). Related activational changes, like activation of vasopressin neurons, increases in cortisol concentrations in the cerebrospinal fluid (Swaab *et al*., 1994), were also observed during aging, in Alzheimer's disease, and in depression (Raadsheer *et al*., 1993, 1994, 1995; Swaab, 1995; Lucassen *et al*., 1994, 1997a; Swaab *et al*., 1999).

As most of the human studies are done retrospectively and many different paradigms and criteria for the definition of "aged" have been used, it is difficult to draw final conclusions on the effects of aging on human HPA parameters or vice versa. Two studies, however, followed a prospective approach in which cortisol was related to cognitive function. First, Lupien observed that individuals with increased cortisol levels over a period of 4 years, showed impaired performance in explicit memory and selective attention tasks. However, when these individuals were classified into three subgroups (low, medium, and high cortisol levels), only individuals with rising corticosterone levels over the 4-year period, showed impairment in cognition, indicating a relation between basal corticosterone and cognitive deficits at old age, that seems to be predicted by early changes in GC levels (Lupien *et al*., 1997; Lupien and McEwen, 1997). Furthermore, stress responsiveness was involved, since a (stressful) public speech task revealed that the subgroup with an early (anticipatory) increase in cortisol in response to the stimulus, was impaired in declarative memory, whereas elderly without this early rise in cortisol showed no memory deficits (Lupien *et al*., 1997, 1998) (Fig. 62.4).

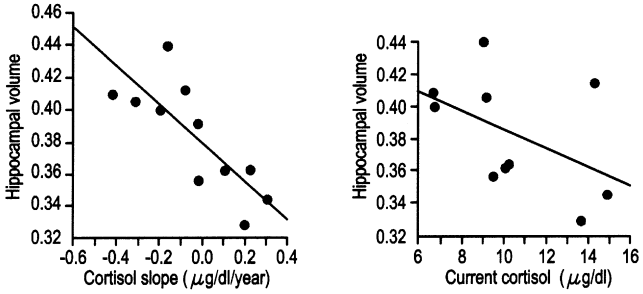

FIG. 62.4. Cortisol and hippocampal volume in aged human subjects. (Left) Correlations between cortisol slope (i.e., the change in average amount of circulating cortisol per year) and (Right) current cortisol levels with the volume of the hippocampus, as measured by MRI, in 11 elderly subjects (from Lupien *et al.*, 1998, with permission).

Second, in another prospective follow-up study in Rotterdam, the ratio of free cortisol over dehydroepiandrosterone (DHEA) was significantly related to cognitive impairment, which confirms the notion that changes in basal free cortisol influence cognitive decline (Kalmijn *et al*., 1998). Regarding the possible inverse relationship between another important adrenal steroid, dehydroepiandrosterone sulfate (DHEA-S) and cognitive changes in aging, no final conclusions can yet be drawn (Kalmijn *et al*., 1998; Wolf *et al*., 1998, 1999; Wolf and Kirschbaum, 1999).

These data suggest that some of the results from rat studies, especially those showing that long-term GC overexposure in later life can accelerate aspects of brain aging and may initiate memory disturbance and cognitive decline, can be partly extrapolated to the human situation. However, it should be realized that the design and extent of the stressors compared have been quite different.

1. Memory and Cognitive Changes in Aging Humans

Studies in humans and primates suggest a similar sensitivity of brain and hippocampal functioning to aberrant GC exposure, as occurs in aging rodents (Issa *et al*., 1990; Geinisman *et al*., 1995; McEwen and Sapolsky, 1995; Van Cauter *et al*., 1996; Wilkinson *et al*., 1997; Newcomer *et al*., 1999b; Ward *et al*., 1999). First, declines in cognitive function frequently accompany normal aging in human and nonhuman primates and are often paralleled by gradual increases in GC levels (Luine, 1997; Lupien *et al*., 1997, 1998). In addition, exogen-

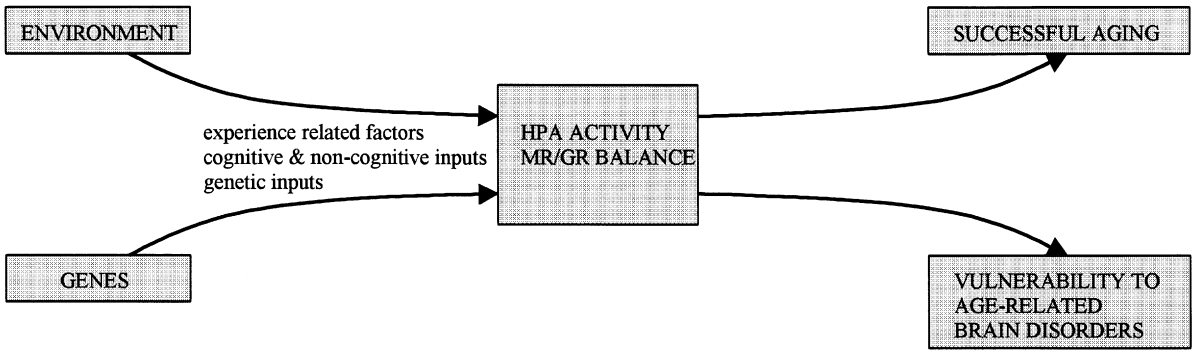

FIG. 62.5. Stress and aging. Scheme depicting genetic and environmental factors in relation to the set point of HPA activity and the balance of MR- and GR-mediated effects in hippocampal cells. Dysregulation of HPA activity and MR/GR imbalance are thought to be critical factors determining the individual vulnerability to age- and stress-related disorders in senescence (see also de Kloet *et al.*, 1998).

ous application of high doses of GCs to humans, for example, by oral administration, causes acute cognitive impairment of declarative memory and disruption of memory formation (Newcomer *et al*., 1994; Luine, 1997; Lupien *et al*., 1997; McEwen *et al*., 1999). Furthermore, women exhibiting increases in cortisol excretion over a 2.5-year follow-up period, preferentially showed declines in memory performance, that were, however, reversible upon normalization of their cortisol levels (Seeman *et al*., 1997). Moreover, in human conditions associated with prolonged cortisol elevation or activation, such as Cushing's disease, following (synthetic) steroid treatment, aging, Alzheimer's disease or in major depression, memory disturbances are frequently observed as well (Starkman *et al*., 1992; Newcomer *et al*., 1994, 1999b; Kirschbaum *et al*., 1996; Lupien *et al*., 1997; Lupien and McEwen, 1997; Wolf *et al*., 1998; McEwen, 1999; McEwen *et al*., 1999).

A word of caution is required regarding the interpretation of GC application in human studies. First, often synthetic glucocorticoids are given, that, at least in rat, can have rather the opposite effect, as they deplete the brain MRs of their endogenous ligands, and thus create a condition resembling that of chemical adrenalectomy, which is expected to affect neuronal viability (De Kloet, 1997; De Kloet *et al*., 1998; Meijer *et al*., 1998; Sousa *et al*., 1999). Furthermore, steroids given to humans are often administered out of context with respect to the particular stage of information processing as outlined in the behavioral studies above (Oitzl *et al*., 1994, 1995, 1998a,b). This can exert differential, and even opposite effects regarding the selective MR- or GR-mediated modes of action (De Kloet *et al*., 1999; see above). Accordingly, altered cognitive performance and behavior is redirected under such conditions to a more opportune response that may be quite unrelated to the original test situation (Fig. 62.5).

2. Hippocampal Volume Changes

Interestingly, changes in hippocampal function are often associated with reductions in hippocampal volume as measured by, for example, MRI in live persons. These are already apparent in elderly individuals who display still only mild cognitive or memory impairment. Such changes have also been observed in many of the GC-associated conditions mentioned above (Convit *et al*., 1995; De Leon *et al*., 1995, 1997a,b; Double *et al*., 1996; Sheline *et al*., 1996, 1999; Bobinski *et al*., 1998; Kumar *et al*., 1998; McEwen, 1999; McEwen *et al*., 1999; Bremner *et al.*, 2000). Although direct correlations with learning deficits in the same animals were not described in this species, studies on tree shrews subjected to a 4 week treatment with cortisol, also showed a significant reduction in hippocampal volume in relation to memory performance (Ohl and Fuchs, 1998, 1999; Ohl *et al*., 1999).

Furthermore, in a prospective study in man, increased basal cortisol levels correlated with reductions in explicit memory and attention tasks (Lupien and McEwen, 1997) that were paralleled by a 14% decrease in hippocampal volume (Lupien *et al*., 1998) (Fig. 62.4). Significant correlations have furthermore been found between hippocampal formation size and delayed, but not primary or immediate memory performance, that was independent of sex, generalized cerebral atrophy, or age. The extent of these early atrophic changes of the hippocampus that was found to be a risk factor for accelerated memory dysfunction in normal aging (Golomb *et al*., 1993, 1994, 1996; Lupien *et al*., 1998; Reiman *et al*., 1998), appeared to correlate with the neurofibrillary pathology in Alzheimer's disease (Bobinski *et al*., 1996), and was even proposed to predict the decline in memory performance (Golomb *et al*., 1996).

Notably, in aged but cognitively normal individuals, changes in hippocampal volume were preceded by changes in glucose metabolism. Both changes depended on the genotype of the patients. For example, both hippocampal volume and hippocampal metabolism were strongly reduced in carriers of the apolipoprotein E (ApoE) epsilon 4 allele, which is a well-known risk factor for age-related cognitive deficits and Alzheimer's disease (Reiman *et al*., 1996, 1998; Haan *et al*., 1999). Furthermore, clear changes in hippocampal or even entire cerebral volume were found in conditions associated with altered HPA functioning, such as aging (Golomb *et al*., 1994; Double *et al*., 1996), depression (Sheline *et al*., 1996, 1999; Kumar *et al*., 1998; Lupien *et al*., 1998; Bremner *et al.*, 2000), or Cushing's disease (Starkman *et al*., 1992). In some reports on the effect of high-dose GC exposure, the consequences appeared reversible once the steroid exposure had decreased or stopped, suggesting that the atrophy observed is in fact transient and may relate to atrophy or changes in water content rather than to actual cell loss (Starkman *et al*., 1992; Sapolsky, 1996; Seeman *et al*., 1997).

D. Age-Related Glucocorticoid Changes in Mice

The current generation of many transgenic mice models has allowed the detailed analysis of several genes or proteins in isolation that are relevant to neurodegenerative or cognitive changes during aging. The actual phenotype of these mice in terms of cognitive or learning capacities, is, however, heavily influenced by the genetic background, that in many of them result in non-specific cognitive deficits that are not related to the manipulated gene (Ingram and Jucker, 1999). One explanation is that behaviors like learning and cognition are so complex that their genetic regulation probably involves many interrelated genes and gene products. Changes in any one of these single genes may consequently lead to a perturbation of the behavioral aspect under study (Keverne, 1997; Nelson and Young, 1998; Picciotto, 1999).

In spite of these considerations, unbiased analysis of the related anatomical substrates or studies on the relation between cognition and glucocorticoid levels in aging (transgenic) mice have been limited so far (Bernstein *et al*., 1985; Fordyce and Wehner, 1993a,b; Talmi *et al*., 1993; Ammassari-Teule *et al*., 1994; Barkats *et al*., 1996). Calhoun *et al*. (1998) failed to find age-related deficits in learning performance, number of synaptophysin-positive boutons, or total neuron number of the DG or CA1 area of aged C57BL/6J mice, although a significant relationship between DG synaptophysin-positive bouton number and water maze performance was found. Other studies in a substrain of aged B6 mice reported some impairment in water maze performance, but this finding has not been related to cell numbers (Fordyce and Wehner, 1993a,b). Similarly, radial arm maze performance tested in young and old mice in two studies was found not to differ either (Bernstein *et al*., 1985; Ammassari-Tuele *et al*., 1994). Furthermore, in old β-amyloid precursor protein knockout mice, learning deficits were clearly apparent, but could not be related to hippocampal neuron numbers (Phinney *et al*., 1999), whereas in aging mice lacking neurotrophin receptor p75 or high-affinity nicotine receptors, significant central neuronal loss was found, associated with (Peterson *et al*., 1999) as well as without behavioral impairment (Zoli *et al*., 1999).

Another major risk factor for age-related cognitive deficits, hippocampal volume reductions as well as the development of Alzheimer's disease, is apolipoprotein E (ApoE), a lipoprotein that plays an important role in peripheral fat metabolism, which is found in three variants, ApoE ϵ 2, 3, and 4 (Soininen and Riekkinen, 1996; Roses, 1997). In the brain, where a separate ApoE population appears to be present, this lipoprotein is thought to play a role in repair and lipid redistribution in response to damage or insults. Some cues as to the function of ApoE in the brain are becoming clear from studies in ApoE knockout mice. For example, adult ApoE knockout mice display a severe learning deficit when tested in a Morris water maze and also show electrophysiological changes in hippocampal function (Gordon *et al*., 1995, 1996a; Krugers *et al*., 1997; Oitzl *et al*., 1997a; Anderson *et al*., 1998; Veinbergs *et al*., 1998). Furthermore, some indications for neurodegenerative alterations in these mice have been observed at old age (Masliah *et al*., 1995; Montine *et al*., 1999) that may relate to changes in ApoE expression during life (Masliah *et al*., 1996; Robertson *et al*., 1998) and appear isoform specific (Raber *et al*., 1998; Buttini *et al*., 1999; Teter *et al*., 1999). Also neurotrophic alterations were found (Veinbergs *et al*., 1999). In adult ApoE knockout animals, tau alterations also have been described (Genis *et al*., 1995) while reintroduction of ApoE in these knockout mice was shown to prevent the cognitive deficit as well as the neurodegenerative changes from occurring (Masliah *et al*., 1997).

Interestingly, ApoE knockout mice appear increasingly sensitive to subsequent damage or insults, like ischemia or kainic acid injections. These treatments induce ApoE expression in neurons, the same cell type in which ApoE is found in the Alzheimer brain (Masliah *et al*., 1996; Boschert *et al*., 1999; Horsburgh *et al*., 1999; Grootendorst *et al*., 2000a). Furthermore, in addition to the learning impairment, these mice show aberrant basal GC levels and alterations in stress responsiveness which suggests a reduced adrenocortical synthetic capacity (Gordon *et al*., 1996b; Zhou *et al*., 1998; Grootendorst *et al*., 2000b), and thus decreased dynamics in the corticosterone response. Furthermore, the observed learning deficit in ApoE knockout mice could be reversed by either GC suppletion or the application of chronic stress, whereas in wild-type littermates, this induced learning impairment (Grootendorst *et al.*, 2000b) indicating that the ApoE genotype is apparently crucial for the direction of the effects of aberrant GC levels in mice.

IV. Corticosteroid Exposure and Hippocampal Damage

A. Rat Studies

The hippocampus has frequently been implicated in the negative feedback regulation of corticosterone and is thought to exert a tonic inhibitory control on HPA axis activity (see above, and Ratka *et al*., 1989; Herman *et al*., 1989; Sapolsky *et al*., 1991b; Jacobson and Sapolsky, 1991). More recent studies have revealed that this inhibition is exerted through several, often indirect neural pathways to which also frontal cortical regions and the ventral subiculum contribute (Herman *et al*., 1994, 1995, 1996; Herman and Cullinan, 1997). On the other hand, GC feedback of the HPA axis, takes place primarily at the level of the paraventricular nucleus in the hypothalamus (De Kloet and Joëls, 1998; Kretz *et al*., 1999). Also this structure may thus be involved in a gradual insensitivity to feedback signals, slowly causing a loss of control over vital homeostatic processes as proposed before (Frolkis *et al.* 1972b; Dilmean *et al.*, 1979). Nevertheless, the hippocampus remains an important brain region involved in modulating aspects of learning and memory processing, mood, affect, and adaptation, each with their own associated effects on neuroendocrine regulation.

In spite of the indirect connections between hippocampus and PVN, glucocorticoid or stress-induced damage to either area can impair HPA axis inhibition, as was shown before after mechanical or pharmacological intervention studies (negative feedback Herman *et al*., 1989, 1996; Jacobson and Sapolsky, 1991; Herman and Cullinan, 1997). It has thus been proposed that this glucocorticoid neuronal damage can give rise to an altered negative feedback set point and a feedforward cascade of cumulative GC overexposure, from which the entire brain and body suffer. With the rapid effects on memory and cogni-

tion in mind, establishing the impact of (acute or chronic) GC overexposure on function and structure of an important neuroendocrine control center like the hippocampus, has become a highly relevant subject of study in recent years.

Regarding GC-related damage, it is important to note that a clearly differential vulnerability is apparent between the main hippocampal subareas with respect to different GC concentrations. First, GCs are essential for neuronal viability in the dentate gyrus, since GC absence, induced after adrenalectomy, induces widespread apoptotic cell death throughout the granule cell layer of the dentate gyrus (Sloviter *et al*., 1989, 1993a,b; Sapolsky *et al*., 1991a; Adem *et al*., 1994; Sousa *et al*., 1997; MacLennan *et al*., 1998) to which also androgens contribute (Frye and McCormick, 2000). The cell loss appears specific for the dentate gyrus since pyramidal cells, calbindin or somatostatin containing hippocampal interneurons, and areas outside the dentate gyrus remain intact (Sapolsky *et al*., 1991a; Sloviter *et al*., 1993a,b; Adem *et al*., 1994; MacLennan *et al*., 1998).

As part of the trisynaptic circuit in the hippocampus, the dentate gyrus is important for information processing to CA1 and subsequent cortical regions. Indeed, adrenelectomy-induced dentate gyrus apoptosis is accompanied by abnormal electrophysiological responses to perforant path stimulation, calcium currents, and learning performance (Armstrong *et al*., 1993; Karst *et al*., 1997; Stienstra *et al*., 1998; Phan *et al*., 1999). These effects are thought to be mediated through the MR, since the effects of adrenelectomy-induced apoptosis can be prevented by low doses of corticosterone that occupy only MR and not GR. Furthermore, treatment with the MR agonist, but not with GR agonists like dexamethasone, prevents the occurrence of apoptosis. Treatment with the latter even seems to increase hippocampal damage in primates (Uno *et al*., 1994), whereas in rat, shorter periods are already effective and reveal changes within anatomical subregions (Hassan *et al*., 1996; Hornsby *et al*., 1996; Sousa *et al*., 1997, 1999). As outlined earlier, this dexamethasone effect is likely to be indirect, depleting MR of its endogeneous ligand. Aging rats in particular, were reported to have an increased sensitivity to dexamethasone (Hassan *et al*., 1996, 1999). In addition to hypercorticism, hypocorticism can thus be deleterious for rat hippocampal viability as well. For many human somatic and CNS functions, reduced glucorticoid concentrations and hypocorticism are indeed considered endangering (Heim *et al*., 1999).

Elevated GC levels, on the other hand, primarily affect pyramidal neurons in the CA layers and are thought to induce hippocampal dysfunction predominantly through GR action (Sapolsky *et al*., 1985, 1990; Uno *et al*., 1989; Virgin *et al*., 1991; Diamond *et al*., 1992; Dachir *et al*., 1993; Arbel *et al*., 1994; Levy *et al*., 1994; Stein-Behrens *et al*., 1994; Bodnoff *et al*., 1995; Fuchs *et al*., 1995; Hassan *et al*., 1996; De Kloet *et al*., 1998, 1999; Sousa *et al*., 1998a, 1998b, 1999). Consequences of hypercorticism are dependent on the duration and concentration applied, and range from early functional deficits and selective, but still transient, changes in neuronal morphology, via an increased vulnerability to subsequent insults, to eventually neuropathology and cell death under extreme conditions. More specifically, atrophy of the apical dendrites of neurons in the CA3c subarea is observed first, in association with reversible memory impairment (Magarinos *et al*., 1996, 1997; Luine, 1997; Lupien and McEwen, 1997), which is influenced by excitatory amino acid transmission (Armanini *et al*., 1990; McEwen, 1996, 1999). Even short exposures to aberrant GC levels or stress can endanger hippocampal neurons in their response to subsequent insults and effects of, for example, ischemia or kainic acid, which are much more severe when applied following stress or high GC exposure than under low corticosterone conditions, both *in vivo* and *in vitro* (Sapolsky and Pulsinelli, 1985; Koide *et al*., 1986; Morse and Davis, 1990; Tombaugh *et al*., 1992; Elliott *et al*., 1993; White-Gbadebo and Hamm, 1993; Lawrence and Sapolsky, 1994; Stein-Behrens *et al*., 1994; Bodnoff *et al*., 1995; Krugers *et al*., 1998; Porter and Landfield, 1998).

When stress becomes severe and prolonged or hypercorticism has been chronic, the atrophy of CA3 neurons has been proposed to eventually lead to cell death, which may, through subsequent anterograde degeneration, partly affect CA1 as well (Sapolsky *et al*., 1985, 1990; Uno *et al*., 1989; Kerr *et al*., 1991; Stein-Behrens *et al*., 1994; Sapolsky, 1996, 1999). The extent of the deleterious effects of high glucocorticoid exposure during the course of aging (Sapolsky and Altmann, 1991; Sapolsky, 1992), for example, the hippocampal volume changes in rat (Rapp *et al*., 1999), also seems to depend on additional factors like neuronal energy metabolism (see below) and sex steroids (McEwen, 1996, 1999; Luine, 1997; Rivier, 1999). For example, castration significantly worsened the extent of the cell loss in CA3 and CA4 after severe restraint stress (Mizoguchi *et al*., 1992; Phan *et al*., 1999).

On the basis of observations on adrenocortical activity and GC levels, that positively correlated with hippocampal pathology, the relationship between increased GC levels and hippocampal function has been causally implicated in aging. This was judged from increased numbers of reactive astrocytes, activation of microglia, and changes in membrane conductances of hippocampal neurons (Landfield *et al*., 1978, 1981; Kerr *et al*., 1991; Nichols *et al*., 1993; Seckl and Olsson, 1995; Sugaya *et al*., 1996; Rozovsky *et al*., 1998). In later studies in Long–Evans rats, also other markers like superoxide dismutase, β-amyloid precursor protein, and nitric oxide synthase were found to be increased in the aged hippocampus, which paralleled the extent of the age-related learning impairment (Sugaya *et al*., 1996).

Importantly, adrenalectomy of rats at middle age, attenuated hippocampal degeneration and cognitive decline later in life, when compared to aged, intact animals (Landfield *et al*., 1981). Although the latter study seems to confirm that elevated basal GC levels contribute to the development of age-related cognitive impairment and pathology, it should be noted that these animals were supplemented from midlife onward with low levels of corticosterone. This indeed excludes GR effects, but, in view of the above-mentioned differential receptor affinities for cortisol (De Kloet, 1991; De Kloet *et al*., 1998), still allows for a predominant MR occupation, a condition known to promote stability, which may explain the lack of deleterious effects on neuronal viability (De Kloet *et al*., 1998, 1999).

1. Functional Changes

Hypercorticism in young and middle-aged rats frequently results in senescent-like neurophysiological patterns that are

characterized by a general failure to regulate intracellular calcium levels properly. Spatial learning deficits are further found in aged rats, suffering from hypercorticism which often parallels the extent of pathology. Such deficits can also be induced following corticosterone injections, which cause altered electrophysiological function (Gage *et al*., 1984b; Issa *et al*., 1990; Gallagher and Holland, 1992; Dachir *et al*., 1993; Gallagher and Nicolle, 1993; Geinisman *et al*., 1995; Sapolsky, 1996, 1999; Conrad *et al*., 1999; Hebda-Bauer *et al*., 1999). Interestingly, in one study, elevated plasma GC levels were found only in aged rats with spatial memory deficits but not in animals without such deficits, suggesting that not age itself, but rather the extent of the associated hypercorticism, is crucial for alterations in hippocampal function (Issa *et al*., 1990). As was pointed out above, this notion is being disputed, since in aged individuals of the Brown Norway rat strain cognitive decline occurs in the absence of hypercorticism (Van Eekelen *et al*., 1991), while in Fisher 344 × Brown Norway rats, spatial learning impairment was not found even in the very old animals group (32 months), except when changes were made in the environment. Furthermore, after injections with corticosterone, very old Fisher 344 × Brown Norway rats even demonstrated enhanced spatial learning (Hebda-Bauer *et al*., 1999).

Previous studies have already revealed that GCs act via a GR-mediated mechanism to increase the voltage-dependent calcium influx into hippocampal neurons. Hypercorticism induces prolonged and increased calcium dependent after hyperpolarizations, reduced thresholds for eliciting excitatory postsynaptic potentials (EPSP), reduced EPSP amplitudes, and overall increased calcium currents, all conditions know to hamper proper synaptic transmission. The importance of altered calcium regulation in aging is also supported by changes observed in aged animals and associated with neuronal degeneration, in factors involved in intracellular calcium buffering, such as calbindin, calretinin, calmodulin, etc. Furthermore, spatial learning was found to be related to changes in calcium-dependent protein kinases (Fordyce and Wehner, 1993a,b; Colombo *et al*., 1997; Krugers *et al*., 1997b). Pharmacological manipulation of intracellular calcium was further shown to be effective in influencing aspects of cognition in the aged brain (Landfield *et al*., 1978, 1981; Joëls and de Kloet, 1989; Kerr *et al*., 1991; Nair *et al*., 1998; Vekhratsky and Toescu, 1998).

Glucocorticoids also influence long-term potentiation (LTP), an electrophysiological model for memory formation. Interestingly, this also seems to follow a U-shaped dose–response relationship, since in hippocampal slices the long-term potentiation response is impaired only when GC concentrations are either too high or too low (Joëls and De Kloet, 1992; De Kloet *et al*., 1999). The inability to generate LTP in the hippocampus of aged rats, has been attributed to aberrant, high GC exposure in these animals (Kerr *et al*., 1991). A similar dose–response relationship of GCs exists for voltage-dependent ion conductances such as calcium and serotonin 1a-receptor-mediated hyperpolarization (Joëls and De Kloet, 1992; Joëls, 1997; De Kloet *et al*., 1998).

2. Structural Changes

Persistently elevated GC exposure, either resulting from stress or from exogenous application, has been reported to lead to reactive glial cell proliferation, a reduction of the dendritic branching in the CA3 area, reductions in volume, and reduced cell numbers in CA1 and CA3 (Landfield *et al*., 1978, 1981; Sapolsky *et al*., 1985, 1990; Sapolsky, 1996, 1999). Chronic exposure for 6 months to foot shock stress for 4 hr per day resulted in endogenous hypercorticism and induced CA1 pyramidal neuronal loss, but only in senescent rats (Kerr *et al*., 1991). Also, increases in glial fibrillary acidic protein and astrocytic activation have been reported in aged rats quite consistently and in parallel to the extent of the learning deficit (Nichols *et al*., 1993; Sugaya *et al*., 1996). Most studies on high GC exposure, stress, hippocampal viability, and aging, however, were done in rats, applying either rather extreme, often physical, stressors or pharmacologically high GC concentrations (Sapolsky *et al*., 1985; Sapolsky, 1996, 1999; see also Seckl and Olsson, 1995). Studies performed under less extreme, more physiologically relevant conditions, or, for example, in nonrodent species, have so far provided conflicting data (see below).

In most older studies, shrinkage-sensitive density measures were used to assess structural changes, which may cause bias when counting numbers of cells. In order to study the true extent and localization of structural alterations in, for example, the aged hippocampus and neocortex, modern stereology has been a powerful tool, that has modified some of the earlier views considerably (Gundersen *et al*., 1988; Korbo *et al*., 1990; Morrison and Hof, 1997; West, 1999; Long *et al*., 1999). When assessed by means of these tools, rat hippocampal neuron numbers or volume remain relatively stable with age, nor do they seem to be related to cognitive deficits, or be influenced by stress or GC treatment (Rapp and Gallagher, 1996; Rasmussen *et al*., 1996; Sousa *et al*., 1998b, 1999). Also, in both young and aged rats subjected to stress as well as to several steroid treatments, no changes could be found in neuron number after high GC exposure, although some volume reductions were measured in specific (neuron-sparse) subareas of the hippocampus and some loss in DG and CA3 after dexamethasone (Sousa *et al*., 1998a,b, 1999; Rapp *et al*., 1999). Volumetric analysis of the main circuits in the aged rat hippocampus revealed regionally selective and circuit-specific effects of aging, that, for example, were shown to spare the temporal hippocampus (Rapp *et al*., 1999).

Whether the age-related GC changes in conditions of GC overexposure in rodents (Sapolsky, 1996), have indeed structural or neuropathological consequences for the human and primate hippocampus, has been addressed only to a limited extent, and a structural basis for the observed age-related declines in volume and cognition, remains, therefore, so far unclear (Gallagher and Holland, 1992; Convit *et al*., 1995; Gallagher *et al*., 1996; Hyman and Gómez-Isla, 1996; Landfield *et al*., 1996; Reagan and McEwen, 1997; Lupien *et al*., 1998). In humans, the reduction in neuron numbers in the brain during normal aging and in Alzheimer's disease, as assessed with stereological methods, is relatively small and appears to occur only in specific subregions of the cortex, hippocampus, and locus coeruleus (West, 1993; Mouton *et al*., 1994; Hoogendijk *et al*., 1995; Gómez-Isla *et al*., 1996; Morrison and Hof, 1997; Simic *et al*., 1997). The number of neurons in the CA1–3 hippocampal regions, is also relatively preserved during human aging, in spite of the changes in hippocampal

volume as measured by magnetic resonance imaging (West, 1993; Simic *et al*., 1997). Interestingly, the latter changes are in fact related to reductions in memory, which in turn, are preceded by reductions in glucose metabolism in this area (West, 1993; Convit *et al*., 1995; De Santi *et al*., 1995; De Leon *et al*., 1997b; McEwen *et al*., 1999), while also genetic factors like ApoE contribute to this effect (Reiman *et al*., 1998). Furthermore, recent studies using disector methodology to count neuron numbers in the hippocampal of chronical cortisol treated and control, aged rhesus monkeys, failed to find any difference between the groups (Leverenz *et al*., 1999), consistent with similar studies on the tree shrew and rat hippocampus (Vollmann-Honsdorf *et al*., 1997; Sousa *et al*., 1998a,b, 1999).

In major depression, a condition generally associated with an activated HPA axis, GC feedback resistance, hypercorticism as well as a decreased volume of the hippocampus (Raadsheer *et al*., 1994, 1995; Holsboer and Barden, 1996; Sheline *et al*., 1996; Rowe *et al*., 1997; Deuschle *et al*., 1998a; Bremner *et al*., 2000) and reductions in prefrontal lobe and whole brain volume (Kumar *et al*., 1998), detrimental effects on hippocampal viability may be expected as well (Chan *et al*., 1996; Landfield *et al*., 1996; Sapolsky, 1996; Reagan and McEwen, 1997). However, in a well characterized cohort of depressed and steroid-treated patients, no indications could be found for gross anatomical changes nor any obvious neuronal loss or damage was observed using various histological markers, although only very few apoptotic cells were present suggesting that in spite of the enhanced HPA activity, at least large structural changes were not induced in areas at risk for SL over-exposure (Lucassen *et al*., 1997b, 2000; Muller *et al*., 1998, 2000).

These and other studies seem to suggest that hippocampal cell loss does not account for the behavioral or cognitive impairments observed following prolonged stress or hypercorticism, but that these changes are more likely to be reflected by changes in the neuronal network function, its dendrites, or its synapses, rather than in structural changes, like neuronal number (Rapp and Gallagher, 1996; Rasmussen *et al*., 1996; Sousa *et al*., 2000). This conclusion is also consistent with the transient nature of GC-induced brain or neuronal atrophy that appears to allow for recovery or reversal, once the treatment is stopped (Starkman *et al*., 1992; Sapolsky, 1996; Seeman *et al*., 1997). Should massive neuronal loss have been induced, this would be difficult to explain. Stereological studies addressing the extent of synaptic loss, at least in man, are just beginning to appear and also fail to suggest neuronal loss (DeKosky *et al*., 1996; Sze *et al*., 1997; West, 1999). An alternative possibility is that structural degenerative hippocampal changes are induced indirectly, in subcortical neuronal populations, that project to and regulate the function of the hippocampal and cortical circuitry (Arbel *et al*., 1994; Aubert *et al*., 1995; Bodnoff *et al*., 1995; Geinisman *et al*., 1995; Calhoun *et al*., 1998; Dawson *et al*., 1999; Nicolle *et al*., 1999; Phinney *et al*., 1999; West, 1999).

B. Possible Mechanisms Underlying Glucocorticoid-Related Damage

In the previous sections, MR- and GR-mediated GC effects on membrane permeability for ions such as calcium were described. While changes in ion regulation have important consequences for cell function and viability in aging, the underlying mechanisms are not known. Glucocorticoids may induce genes encoding specific membrane proteins, receptors, and subunits of ion channels, but these genomic effects rarely explain the electrophysiological changes in a direct way. Glucocorticoid-induced genes may instead be, for example, kinases involved in activation of ion channel function, but presently there is no evidence for this assumption. In the course of the research on GCs and aging a number of factors and processes have been proposed that could be implicated in the cascade of degenerative changes that accompany the aging process.

1. Metabolic Factors

In terms of possible underlying mechanisms, acute and chronic increases in cortisol suppress hippocampal glucose metabolism, which is known to reflect learning and memory capacity (Gage *et al*., 1984a; Newcomer *et al*., 1994, 1999b; De Leon *et al*., 1997a; Ouchi *et al*., 1998). In rat, GC overexposure accelerated ATP loss (Lawrence and Sapolsky, 1994), inhibited glucose transport in neurons and glia *in vitro*, as well as glutamate uptake in hippocampal astrocytes (Horner *et al*., 1990; Virgin *et al*., 1991). Consistent with these observations, memory impairment was found to be associated with decreased glucose metabolism, appearing most pronounced in the hippocampus compared to the proisocortex and neocortex (Stein *et al*., 1998). Positron emission tomography measurements of cerebral glucose metabolism showed that elderly subjects, as well as individuals at risk for Alzheimer's disease, were characterized by a generalized hypometabolism in the cortical and hippocampal areas, that preceeded subsequent hippocampal volume reductions and reduced performance on a long-term memory test (Convit *et al*., 1995, 1996; De Santi *et al*., 1995; De Leon *et al*., 1997a; Ouchi *et al*., 1998). Furthermore, unlike healthy elderly, no effect of cortisol on the inhibition of glucose metabolism could be demonstrated in patients with Alzheimer's disease (De Leon *et al*., 1997a).

Furthermore, restoration of energy levels, by means of glucose supplementation, could protect the brain and hippocampus from various insults, and improved memory when the brain was exposed to aberrant GC conditions as occurs during aging (Gage *et al*., 1984a; Fordyce and Wehner, 1993a; Sapolsky, 1996; Winocur and Gagnon, 1998; Newcomer *et al*., 1999a). Also, genetic overexpression of the glucose transporter (Lawrence *et al*., 1995, 1996) was shown to protect against stroke or seizure induced cell loss. Similarly, growth-promoting factors, like insulin-like growth factor I expression, which can have beneficial effects on neuronal viability, are suppressed in socially stressed monkeys, attenuated after deafferentiation in aged rats, and modulated by GCs as well (Sapolsky and Spencer, 1997; Islam *et al*., 1998; Woods *et al*., 1998; Sonntag *et al*., 1999). *In vitro* data on cortical neurons further demonstrate similar beneficial effects of high glucose protection against *N*-methyl-D-aspartate, free radicals, and oxygen deprivation (Seo *et al*., 1999). The beneficial effects not only of glucose, but also of insulin supplementation on aspects of rat and human memory and cognition, are interesting to mention here, although this effect is not unequivocal (Winocur and Gagnon, 1998; Craft *et al*., 1999; Newcomer *et al*., 1999a). Together, these observations suggest that inhibition of energy utilization is a critical feature of GC action, and may be a possible mechanism mediating the deleterious consequences of high glucocorticoid exposure.

2. Neurogenesis

Another possibility relates to a unique feature of the dentate gyrus, in which ongoing neurogenesis persists in a large number of rodent species as well as in adult tree shrew, marmosets, rhesus monkey, and human brain (Eriksson *et al*., 1998; Gould *et al*., 1999; Kornack and Rakic, 1999). In contrast to other brain areas, neurogenesis persists from development into old age only in this part of the hippocampus and in the lateral ventricle well. The former is thought to allow the dentate gyrus to continuously rejuvenate its neuronal population in order to adapt to changing situations or demands. Interestingly, not only does stress or enhanced GC levels suppress the frequency of neuronal birth in young, adult (Cameron and Gould, 1994; Gould *et al*., 1998; Gould and Tanapat, 1999), and aged rats, but a number of steroid- and non-steroid-mediated conditions modulate neuronal birth in the adult brain as well. These include enriched environmental housing, increased physical activity through voluntary running, estrogen treatment, amygdala kindling, or training in a hippocampal dependent learning task (Kempermann *et al*., 1998; Parent *et al*., 1998; Tanapat *et al*., 1999; Van Praag *et al*., 1999). Newborn cells were further shown to become functional, as increases in synaptic plasticity, learning performance, long-term potentiation and have been demonstrated (Patel *et al*., 1997; Lee *et al*., 1998; Nilsson *et al*., 1999; Palmer *et al*., 1999).

In aged rats and mice, neuronal birth persists, but at a considerably slower pace. As aging is associated with increased GC levels in some strains, reduction of GC levels in old rats by adrenalectomy was found to restore the rate of cell proliferation to that observed in young animals and resulted in increased numbers of new granule neurons (Cameron and McKay, 1999). These data require scrutiny, however, since these studies were actually performed in middle-aged animals, no information was given on the circulating corticosterone levels, and the data analysis was based on a small number of animals showing substantial individual variation.

Recently, serotonin metabolism, which is regulated by GCs as well (Meijer and De Kloet, 1998; Brezun and Daszuta, 1999), has also been implicated in controlling hippocampal neurogenesis that consequently, may also influence changes in hippocampal volume. This evidence suggests that increased serotonin levels enhance the production of new neurons, that may be mediated via activation of the serotonin 1a receptor (Gould, 1999). This makes it possible to speculate that the inhibitory effects of stress on granule cell production would be preventable by serotonin 1a agonists (Gould, 1999) which is consistent with the idea that volume reductions in parts of the hippocampus after high GC exposure, could be prevented by serotonin reuptake inhibitors and indeed with a study showing that depletion of serotonin decreased neurogenesis in the DG (Brezun and Daszuta, 1999). Whether inhibition of neurogenesis influences synaptic plasticity, or is a main factor in the many GC effects in aging, remains to be further established.

3. Growth Factors

Many investigators have speculated on the role of growth factors like brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and their receptors, in the aging process. Most of this interest was based on the early and age-related cholinergic neuronal soma atrophy, which forms an important source of the cholinergic innervation of the hippocampus and neocortex (Sugaya *et al*., 1998). Perturbation of these systems with age has been related to deficits in attention and memory. Interestingly, these behavioral deficits could be prevented or reversed by the administration of some but not all growth factors (Fischer *et al*., 1994), whereas the tyrosine kinase B receptor has also been strongly implicated in hippocampal mediated learning (Minichiello *et al*., 1999). Also, administration of recombinant human NGF was able to prevent the retrograde degeneration of axotomized basal forebrain cholinergic neurons in the rat (Koliatsos *et al*., 1991). In addition, glucocorticoids are known to downregulate the expression and protein levels of growth factors, like BDNF and its receptor, tyrosine kinase B (Smith and Cizza, 1996; Chao *et al*., 1998; Schaaf *et al*., 1998). As growth factors are highly expressed in the hippocampus and involved in many aspects of neuronal viability, they are potentially important for the age-related changes observed in this brain area. Age-related rises in GC levels, for example, could potentially disrupt the mobilization of neurotrophins, whereas abnormal neurotrophic function has been implicated also in human aging and Alzheimer's disease (Salehi *et al*., 1996; Murer *et al*., 1999).

The literature on effects of aging on the expression of hippocampal BDNF mRNA is rather confusing, which may partly relate to the same differences in animal strain, criteria for aging, and methodology as indicated above for studies on GC levels in rodents (see above). BDNF mRNA levels, for example, were found to be decreased in CA3 and CA1 of 18- to 24-month-old Fisher rats (Smith and Cizza, 1996), increased in 28-month-old Long–Evans rats (Sugaya *et al*., 1998) and in hippocampal homogenates of 18-month-old animals of the same strain (Narisawa-Saito and Nawa, 1996), but remained unchanged in hippocampal fields of Spraque–Dawley and Fisher rats up to 24 months of age (Lapchak *et al*., 1993). Furthermore, the low and high affinity receptors for NGF, were found to be reduced in the basal forebrain and septal area of aged rats, in parallel with spatial learning deficits. While NGF concentrations in the hippocampus were reduced in aged and spatially impaired rats, other reports show no change or even increased levels. Chronic environmental enrichment was shown to increase NGF concentrations in the adult rat hippocampus and also to induce GR, but not MR gene expression in specific hippocampal subfields. In addition, it proved to be protective against focal brain ischemia (Dahlqvist *et al*., 1999; Phan *et al*., 1999; Smith *et al*., 1999). However, in brains from patients with Alzheimer's disease, significant, differential reductions were found in the expression of the tyrosine kinase receptors trkA, trkB, and trkC in the nucleus basalis of Meynert, which implies a decreased neurotrophin responsiveness of the cholinergic system in this condition (Salehi *et al*., 1996).

Taken together, these data suggest that altered hippocampal synaptic plasticity as it may occur in the aged brain, is not consistently reflected by changes in the expression of growth factors, or their receptors. However, the age-associated spatial learning impairments in rats could be reversed by administration of some but not all growth factors (Fischer *et al*., 1994), that proved beneficial also after lesions (Mandel *et al*., 1999). Recently, in rhesus monkeys, learning impairments as well as the associated atrophy of subcortical cholinergic neurons could even be reversed using neurotrophin gene therapy (Smith *et al*.,

1999). Together with its protective role in ischemia, this suggests that growth factors still hold some potential for treating age- or stress-related changes, and possibly in the hippocampus as well.

V. Concluding Remarks

In this chapter we have discussed whether corticosteroids are the cause or consequence of brain aging and age-related brain pathology. Although the lability of the HPA axis is increased during aging, recent data challenge the view that elevated corticosteroid levels are generally, and consistently, associated with aging. However, if corticosteroid levels circulate in chronically aberrant concentrations, either too high, or too low, they increase the vulnerability to cognitive decline and disease, rather than the aging process *per se*. The mechanisms underlying one of the most obvious characteristics of the aging process, the great individual variation within an aged group, is poorly understood, but early life events appear to be important for the quality of cognitive performance at old age.

Glucocorticoids mediate their action through mineralocorticoid and glucocorticoid receptors, which are colocalized in hippocampal neurons, but have different affinities and opposing actions. The MR operates in a proactive mode, whereas GR facilitates in a reactive manner, the recovery of homeostasis following perturbation by stress. Together, these steroid receptors effect changes and adaptive processes in the brain and its behavior, by controlling expression of specific target genes involved in many fundamental brain processes like neuronal excitability, calcium homeostasis, energy metabolism, and cell division. Therefore, both MR and GR are critical in the maintenance of homeostasis during neuronal aging. Depending on additional environmental and genetic factors, an imbalance in their actions can result in altered stress regulation, cognitive decline and impaired behavioral adaptation, which could further result in dysregulation through the same genes that are otherwise essential for maintaining homeostasis and health.

The corticosteroid receptors and genes under their control, are therefore not only critical for successful aging, they form at the same time excellent molecular targets for drugs, based on restoring a disturbed neuronal homeostasis through correction of the MR/GR imbalance, and thus to promote the restorative capacity that is still present within the aging brain.

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References

Adem, A., Islam, A., Bogdanovic, N., Carlstrom, K., and Winblad, B. (1994). Loss of neurones after long-term adrenalectomy in the adult rat hippocampal formation. *NeuroReport* **21**, 2285–2288.

Ammassari-Teule, M., Fagioli, S., and Rossi-Arnaud, C. (1994). Radial maze performance and open-field behaviours in aged C57BL/6 mice: Further evidence for preserved cognitive abilities during senescence. *Physiol. Behav.* **55**, 341–345.

Anderson, R., Barnes, J. C., Bliss, T. V., Cain, D. P., Cambon, K., Davies, H. A., Errington, M. L., Fellows, L. A., Gray, R. A., Hoh, T., Stewart, M., Large, C. H., and Higgins, G. A. (1998). Behavioural, physiological and morphological analysis of a line of apolipoprotein E knockout mouse. *Neuroscience* **85**, 93–110.

Angelucci, L., Patacchioli, F. R., Scaccianoce, S., Di Sciullo, A., Catalani, A., Taglialatela, G., and Ramacci, M. T. (1987). Hypothalamo-pituitary-adrenocortical function and process of brain aging. *Adv. Biochem. Psychopharmacol*. **43**, 293–304.

Arbel, I., Kadar, T., Silbermann, M., and Levy, A. (1994). The effects of long-term corticosterone administration on hippocampal morphology and cognitive performance of middle-aged rats. *Brain Res*. **657**, 227–235.

Armanini, M. P., Hutchins, C., Stein, B. A., and Sapolsky, R. M. (1990). Glucocorticoid endangerment of hippocampal neurons is NMDA-receptor dependent. *Brain Res*. **5**, 532, 7–12.

Armstrong, J. N., McIntyre, D. C., Neubort, S., and Sloviter, R. S. (1993). Learning and memory after adrenalectomy-induced hippocampal dentate granule cell degeneration in the rat. *Hippocampus* **3**, 359–371.

Aubert, I., Rowe, W., Meaney, M. J., Gauthier, S., and Quirion, R. (1995). Cholinergic markers in aged cognitively impaired Long-Evans rats. *Neuroscience* **67**, 277–292.

Barkats, M., Bertholet, J. Y., and Cohen-Salmon, C. (1996). Age-related morphological changes in the hippocampus in two mouse strains. *Mech. Ageing Dev.* **87**, 155–164.

Bernstein, D., Olton, D. S., Ingram, D. K., Waller, S. B., Reynolds, M. A., and London, E. D. (1985). Radial maze performance in young and aged mice: Neurochemical correlates. *Pharmacol. Biochem. Behav.* **22**, 301–307.

Bhatnagar, M., Cintra, A., Chadi, G., Lindberg, J., Oitzl, M., De Kloet, E. R., Moller, A., Agnati, L. F., and Fuxe, K. (1997). Neurochemical changes in the hippocampus of the Brown Norway rat during aging. *Neurobiol. Aging* **18**, 319–327.

Bobinski, M., Wegiel, J., Wisniewski, H. M., Tarnawski, M., Bobinski, M., Reisberg, B., De Leon, M. J., and Miller, D. C. (1996). Neurofibrillary pathology—correlation with hippocampal formation atrophy in Alzheimer's disease. *Neurobiol. Aging* **17**, 909–919.

Bobinski, M., De Leon, M. J., Tarnawski, M., Wegiel, J., Reisberg, B., Miller, D. C., and Wisniewski, H. M. (1998). Neuronal and volume loss in CA1 of the hippocampal formation uniquely predicts duration and severity of Alzheimer disease. *Brain Res*. **805**, 267–269.

Bodnoff, S. R., Humphreys, A. G., Lehman, J. C., Diamond, D. M., Rose, G. M., and Meaney, M. J. (1995). Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats. *J. Neurosci*. **15**, 61–69.

Bohus, B., and De Kloet, E. R. (1981). Adrenal steroids and extinction be-havior: Antagonism by progesterone, deoxycorticosterone and dexamethasone of a specific effect of corticosterone. *Life Sci*. **28**, 433–440.

Boschert, U., Merlo-Pich, E., Higgins, G., Roses, A. D., and Catsicas, S. (1999). Apolipoprotein E expression by neurons surviving excitotoxic stress. *Neurobiol. Dis*. **6**, 508–514.

Braak, H., de Vos, R. A., Jansen, E. N., Bratzke, H., and Braak, E. (1998). Neuropathological hallmarks of Alzheimer's and Parkinson's diseases. *Prog. Brain Res*. **117**, 267–285.

Bremmer, J. D., Narayan, M., Anderson, E. R., Staib, L. H., Miller, H. L., and Charney, D. S. (2000). Hippocampal volume reduction in major depression. *Am. J. Psychiatry.* **157**, 115–118.

Brezun, J. M., and Daszuta, A. (1999). Depletion in serotonin decreases neurogenesis in the dentate gyrus and the subventricular zone of adult rats. *Neuroscience* **89**, 999–1002.

Brodish, A., and Ohio, M. (1989). Age-dependent effects of chronic stress on ACTH and corticosterone responses to an acute novel stress. *Neuroendocrinology* **49**, 496–501.

Buttini, M., Orth, M., Bellosta, S., Akeefe, H., Pitas, R. E., Wyss-Coray, T., Mucke, L., and Mahley, R. W. (1999). Expression of human apolipoprotein E3 and E4 in the brains of ApoE-/-mice: Isoform-specific effects on neurodegeneration. *J. Neurosci.* **19**, 4867–4880.

Calhoun, M. E., Kurth, D., Phinney, A. L., Long, J. M., Hengemihle, J., Mouton, P. R., Ingram, D. K., and Jucker, M. (1998). Hippocampal neuron and synaptophysin-positive bouton number in aging C57BL/6 mice. *Neurobiol. Aging* **19**, 599–606.

Cameron, H. A., and Gould, E. (1994). Adult neurogenesis is regulated by adrenal steroids in the dentate gyrus. *Neuroscience* **61**, 203–209.

Cameron, H. A., and McKay, R. D. (1999). Restoring production of hippocampal neurons in old age. *Nat. Neurosci.* **2**, 894–897.

Cartlidge, N. E., Black, M. M., Hall, M. R., Hall, R. *et al*. (1970). Pituitary function in the elderly. *Gerontol. Clin.* **12**, 65–70.

Chan, R. S., Huey, E. D., Maecker, H. L., Cortopassi, K. M., Howard, S. A., Iyer, A. M., McIntosh, L. J., Ajilore, O. A., Brooke, S. M., and Sapolsky, R. M. (1996). Endocrine modulators of necrotic neuron death. *Brain Pathol.* **6**, 481–491.

Chao, H. M., Sakai, R. R., Ma, L. Y., and McEwen, B. S. (1998). Adrenal steroid regulation of neurotrophic factor expression in the rat hippocampus. *Endocrinology (Baltimore)* **139**, 3112–3118.

Cizza, G., Pacak, K., Kvetnansky, R., Palkovits, M., Goldstein, D. S., Brady, L. S., Fukuhara, K., Bergamini, E., Kopin, I. J., and Blackman, M. R. (1995). Decreased stress responsivity of central and peripheral catecholaminergic systems in aged 344/N Fischer rats. *J. Clin. Invest.* **95**, 1217–1224.

Coleman, P., Finch, C., and Joseph, J. (1990). The need for multiple time points in aging studies. *Neurobiol. Aging* **11**, 1–2.

Colombo, P. J., Wetsel, W. C., and Gallagher, M. (1997). Spatial memory is related to hippocampal subcellular concentrations of calcium-dependent protein kinase C isoforms in young and aged rats. *Proc. Natl. Acad. Sci. U. S. A.* **94**, 14195–91419.

Colucci, C. F., D'Alessandro, B., Bellastella, A., and Montalbetti, N. (1975). Circadian rhythm of plasma cortisol in the aged (Cosinor method). *Gerontol. Clin.* **17**, 89–95.

Conrad, C. D., Lupien, S. J., and McEwen, B. S. (1999). Support for a bimodal role for type II adrenal steroid receptors in spatial memory. *Neurobiol. Learn. Mem.* **72**, 39–46.

Convit, A., De Leon, M. J., Tarshish, C., De Santi, S., Kluger, A., Rusinek, H., and George, A. E. (1995). Hippocampal volume losses in minimally impaired elderly. *Lancet* **345**, 266.

Craft, S., Asthana, S., Newcomer, J. W., Wilkinson, C. W., Matos, I. T., Baker, L. D., Cherrier, M., Lofgreen, C., Latendresse, S., Petrova, A., Plymate, S., Raskind, M., Grimwood, K., and Veith, R. C. (1999). Enhancement of memory in Alzheimer disease with insulin and somatostatin, but not glucose. *Arch. Gen. Psychiatry* **56**, 1135–1140.

Dachir, S., Kadar, T., Robinzon, B., and Levy, A. (1993). Cognitive deficits induced in young rats by long-term corticosterone administration. *Behav. Neural Biol.* **60**, 103–109.

Dahlqvist, P., Zhao, L., Johansson, I. M., Mattsson, B., Johansson, B. B., Seckl, J. R., and Olsson, T. (1999). Environmental enrichment alters nerve growth factor-induced gene A and glucocorticoid receptor messenger RNA expression after middle cerebral artery occlusion in rats. *Neuroscience* **93**, 527–535.

Dawson, G. R., Seabrook, G. R., Zheng, H., Smith, D. W., Graham, S., O'Dowd, G., Bowery, B. J., Boyce, S., Trumbauer, M. E., Chen, H. Y., Van der Ploeg, L. H., and Sirinathsinghji, D. J. (1999). Age-related cognitive deficits, impaired long-term potentiation and reduction in synaptic marker density in mice lacking the beta-amyloid precursor protein. *Neuroscience* **90**, 1–13.

De Kloet, R., Wallach, G., and McEwen, B. S. (1975). Differences in corticosterone and dexamethasone binding to rat brain and pituitary. *Endocrinology (Baltimore)* **96**, 598–609.

De Kloet, E. R. (1991). Brain corticosteroid receptor balance and homeostatic control. *Front. Neuroendocrinol.* **12**, 95–164.

De Kloet, E. R. (1992). Corticosteroids, stress, and aging. *Ann. N. Y. Acad. Sci.* **663**, 357–371.

De Kloet, E. R. (1997). Why dexamethasone poorly penetrates in brain. *Stress* **2**, 13–20.

De Kloet, E. R., Sutanto, W., Rots, N., van Haarst, A., van den Berg, D., Oitzl, M., Van Eekelen, A., and Voorhuis, D. (1991). Plasticity and function of brain corticosteroid receptors during aging. *Acta Endocrinol. (Copenhagen)* **124** (Suppl. 1), 65–67.

De Kloet, E. R., Vreugdenhil, E., Oitzl, M. S., and Joëls, M. (1998). Brain corticosteroid receptor balance in health and disease. *Endocr. Rev.* **19**, 269–301.

De Kloet, E. R., Oitzl, M. S., and Joëls, M. (1999). Stress and cognition: Are corticosteroids good or bad guys? *Trends Neurosci.* **22**, 422–426.

DeKosky, S. T., Scheff, S. W., and Styren, S. D. (1996). Structural correlates of cognition in dementia: Quantification and assessment of synapse change. *Neurodegeneration* **5**, 417–421.

De Leon, M. J., Convit, A., De Santi, S., Golomb, J., Tarshish, C., Rusinek, H., Bobinski, M., Ince, C., Miller, D. C., and Wisniewski, H. M. (1995). The hippocampus in aging and Alzheimer's disease. *Neuroimaging Clin. North Am.* **5**, 1–17.

De Leon, M. J., McRae, T., Rusinek, H., Convit, A., De Santi, S., Tarshish, C., Golomb, J., Volkow, N., Daisley, K., Orentreich, N., and McEwen, B. (1997a). Cortisol reduces hippocampal glucose metabolism in normal elderly, but not in Alzheimer's disease. *J. Clin. Endocrinol. Metab.* **82**, 3251–3259.

De Leon, M. J., George, A. E., Golomb, J., Tarshish, C., Convit, A., Kluger, A., De Santi, S., McRae, T., Ferris, S. H., Reisberg, B., and Ince, C. (1997b). Frequency of hippocampal formation atrophy in normal aging and Alzheimer's disease. *Neurobiol. Aging* **18**, 1–11.

Dellwo, M., and Beauchene, R. E. (1990). The effect of exercise, diet restriction, and aging on the pituitary—adrenal axis in the rat. *Exp. Gerontol.* **25**, 553–562.

De Santi, S., De Leon, M. J., Convit, A., Tarshish, C., Rusinek, H., Tsui, W. H., Sinaiko, E., Wang, G. J., Bartlet, E., and Volkow, N. (1995). Age-related changes in brain: II. Positron emission tomography of frontal and temporal lobe glucose metabolism in normal subjects. *Psychiatr. Q.* **66**, 357–370.

Deuschle, M., Gotthardt, U., Schweiger, U., Weber, B., Korner, A., Schmider, J., Standhardt, H., Lammers, C. H., and Heuser, I. (1997). With aging in humans the activity of the hypothalamus-pituitary-adrenal system increases and its diurnal amplitude flattens. *Life Sci.* **61**, 2239–2246.

Deuschle, M., Weber, B., Colla, M., Depner, M., and Heuser, I. (1998a). Effects of major depression, aging and gender upon calculated diurnal free plasma cortisol concentrations: A reevaluation study. *Stress* **2**, 281–287.

Deuschle, M., Weber, B., Colla, M., Muller, M., Kniest, A., and Heuser, I. (1998b). Mineralocorticoid receptor also modulates basal activity of hypothalamus-pituitary-adrenocortical system in humans. *Neuroendocrinology* **68**, 355–360.

Dhabhar, F. S., McEwen, B. S., and Spencer, R. L. (1993). Stress response, adrenal steroid receptor levels and corticosteroid-binding globulin levels—a comparison between Sprague-Dawley, Fischer 344 and Lewis rats. *Brain Res.* **616**, 89–98.

Diamond, D. M., Bennet, M. C., Fleshner, M., and Rose, G. M. (1992). Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal primed burst potentiation. *Hippocampus* **2**, 421–340.

Dilman, V. M., Ostroumova, M. N., and Tsyrlina, E. V. (1979). Hypothalamic mechanisms of ageing and of specific age pathology. II. On the sensitivity threshold of hypothalamo-pituitary complex to homeostatic stimuli in adaptive homestasis. *Exp. Gerontol.* **14**, 175–181.

Double, K. L., Halliday, G. M., Kril, J. J., Harasty, J. A., Cullen, K., Brooks, W. S., Creasey, H., and Broe, G. A. (1996). Topography of brain atrophy during normal aging and Alzheimer's Disease. *Neurobiol. Aging* **17**, 513–521.

Drouin, J., Sun, Y. L., Chamberland, M., Gauthier, Y., De Lean, A., Nemer, M., and Schmidt, T. J. (1993). Novel glucocorticoid receptor complex with DNA element of the hormone-repressed POMC gene. *EMBO J*. **12**, 145–156.

Eldridge, J. C., Brodish, A., Kute, T. E., and Landfield, P. W. (1989a). Apparent age-related resistance of type II hippocampal corticosteroid receptors to down-regulation during chronic escape training. *J. Neurosci*. **9**, 3237–3242.

Eldridge, J. C., Fleenor, D. G., Kerr, D. S., and Landfield, P. W. (1989b). Impaired upregulation of type II corticosteroid receptors in hippocampus of aged rats. *Brain Res*. **478**, 248–256.

Elliott, E. M., Mattson, M. P., VenderKlish, P., Lynch, G., Chang, I., and Sapolsky, R. M. (1993). Corticosterone exacerbates kainate-induced alterations in hippocampal tau immunoreactivity and spectrin proteolysis in vivo. *J. Neurochem*. **61**, 57–67.

Eriksson, P. S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A., and Gage, F. H. (1989). Neurogenesis in the adult human hippocampus. *Nat. Med*. **4**, 1313–1317.

Erisman, S., Carnes, M., Takahashi, L. K., and Lent, S. J. (1990). The effects of stress on plasma ACTH and corticosterone in young and aging pregnant rats and their fetuses. *Life Sci*. **47**, 1527–1533.

Erkut, Z. A., Pool, C., and Swaab, D. F. (1998). Glucocorticoids suppress corticotropin-releasing hormone and vasopressin expression in human hypothalamic neurons. *J. Clin. Endocrinol. Metab.* **83**, 2066–2073.

Everitt, A., and Meites, J. (1989). Aging and antiaging effects of hormones. *J. Gerontol.* **44**, B139–B147.

Finch, C. E. (1979). Neuroendocrine mechanisms and aging. *Fed. Proc. Fed. Am. Soc. Exp. Biol*. **38**, 178–183.

Fischer, W, Sirevaag, A., Wiegand, S. J., Lindsay, R. M., and Bjorklund, A. (1994). Reversal of spatial memory impairments in aged rats by nerve growth factor and neurotrophins 3 and 4/5 but not by brain-derived neurotrophic factor. *Proc. Natl. Acad. Sci. U. S. A*. **91**, 8607–8611.

Fordyce, D. E., and Wehner, J. M. (1993a). Physical activity enhances spatial learning performance with an associated alteration in hippocampal protein kinase C activity in C57BL/6 and DBA/2 mice. *Brain Res*. **619**, 111–119.

Fordyce, D. E., and Wehner, J. M. (1993b). Effects of aging on spatial learning and hippocampal protein kinase C in mice. *Neurobiol. Aging* **14**, 309–317.

Frolkis, V. V., Bezrukov, V. V., Duplenko, Y. K., and Genis, E. D. (1972a). The hypothalamus in aging. *Exp. Gerontol*. **7**, 169–184.

Frolkis, V. V., Bezrukov, V. V., and Sinitsky, V. N. (1972b). Sensitivity of central nervous structures to humoral factors in aging. *Exp. Gerontol*. **7**, 185–194.

Frye, C. A., and McCormick, C. M. (2000). Androgens are neuroprotective in the dentate gyrus of adrenalectomized female rats. *Stress* **3**, 185–194.

Fuchs, E., and Flügge, G. (1998). Stress, glucocorticoids and structural plasticity of the hippocampus. *Neurosci. Biobehav. Rev.* **23**, 295–300.

Fuchs, E., Uno, H., and Flügge, G. (1995). Chronic psychosocial stress induces morphological alterations in hippocampal pyramidal neurons of the tree shrew. *Brain Res*. **673**, 275–282.

Fuchs, E., Flugge, G., Ohl, F., Lucassen, P. J., Vollmann-Honsdorf, G. K., and Michaelis, T. (2000). Psychosocial stress, glucocorticoids and structural alteration in the tree shrew hippocampus. *Physiol. Behav.*, in press.

Gage, F. H., Kelly, P. A., and Björklund, A. (1984a). Regional changes in brain glucose metabolism reflect cognitive impairments in aged rats. *J. Neurosci*. **4**, 2856–2865.

Gage, F. H., Dunnett, S. B., and Björklund, A. (1984b). Spatial learning and motor deficits in aged rats. *Neurobiol. Aging* **5**, 43–48.

Gallagher, M., and Holland, P. C. (1992). Preserved configural learning and spatial learning impairment in rats with hippocampal damage. *Hippocampus* **2**, 81–88.

Gallagher, M., and Nicolle, M. M. (1993). Animal models of normal aging: Relationship between cognitive decline and markers in hippocampal circuitry. *Behav. Brain Res*. **57**, 155–162.

Gallagher, M., Landfield, P. W., McEwen, B., Meaney, M. J., Rapp, P. R., Sapolsky, R., and West. M. J. (1996). Hippocampal neurodegeneration in aging. *Science* **274**, 484–485.

Geinisman, Y., DeToledo-Morrell, L., Morrell, F., and Heller, R. E. (1995). Hippocampal markers of age-related memory dysfunction: Behavioral, electrophysiological and morphological perspectives. *Prog. Neurobiol*. **45**, 223–252.

Genis, I., Gordon, I., Sehayek, E., and Michaelson, D. M. (1995). Phosphorylation of tau in apolipoprotein E-deficient mice. *Neurosci. Lett*. **199**, 5–8.

Golomb, J., De Leon, M. J., Kluger, A., Geroge, A. E., Tarshish, C., and Ferris, S. H. (1993). Hippocampal atrophy in normal aging. An association with recent memory impairment. *Arch. Neurol. (Chicago)* **50**, 967–973.

Golomb, J., Kluger, A., De Leon, M. J., Ferris, S. H., Convit, A., Mittelman, M. S., Cohen, J., Rusinek, H., De Santi, S., and George, A. E. (1994). Hippocampal formation size in normal human aging: A correlate of delayed secondary memory performance. *Learn. Mem*. **1**, 45–54.

Golomb, J., Kluger, A., De Leon, M. J., Ferris, S. H. Mittelman, M., Cohen, J., and George, A. E. (1996). Hippocampal formation size predicts declining memory performance in normal aging. *Neurology* **47**, 810–813.

Gomez, F., De Kloet, E. R., and Armario, A. (1998). Glucocorticoid negative feedback on the HPA axis in five inbred rat strain. *Am. J. Physiol*. **274**, R420–R427.

Gómez-Isla, T., Price, J. L., McKeel, D. W., Jr., Morris, J. C., Growdon, J. H., and Hyman, B. T. (1996). Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *J. Neurosci*. **16**, 4491–4500.

Gordon, I., Grauer, E., Genis, I., Sehayek, E., and Michaelson, D. M. (1995). Memory deficits and cholinergic impairments in apolipoprotein E-deficient mice. *Neurosci. Lett*. **199**, 1–4.

Gordon, I., Genis, I., Grauer, E., Sehayek, E., and Michaelson, D. M. (1996a). Biochemical and cognitive studies of apolipoprotein-E-deficient mice. *Chem. Neuropathol*. **28**, 97–103.

Gordon, I., Ben-Eliyahu, S., Rosenne, E., Sehayek, E., and Michaelson, D. M. (1996b). Derangement in stress response of apolipoprotein E-deficient mice. *Neurosci. Lett*. **206**, 212–214.

Gotthardt, U., Schweiger, U., Fahrenberg, J., Lauer, C. J., Holsboer, F., and Heuser, I. (1995). Cortisol, ACTH, and cardiovascular response to a cognitive challenge paradigm in aging and depression. *Am. J. Physiol*. **268**, R865–R873.

Gould, E. (1999). Serotonin and Hippocampal neurogenesis. *Neuropsychopharmacology* **21** (2, Suppl.), 46S–51S.

Gould E., and Tanapat, P. (1999). Stress and hippocampal neurogenesis. *Biol. Psychiatry* **46**, 1472–1479.

Gould, E., Tanapat, P., McEwen, B. S., Flügge, G., and Fuchs, E. (1998). Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. *Proc. Natl. Acad. Sci. U. S. A*. **95**, 3168–3171.

Gould, E., Reeves, A. J., Fallah, M., Tanapat, P., Gross, C. G., and Fuchs, E. (1999). Hippocampal neurogenesis in adult Old World primates. *Proc. Natl. Acad. Sci. U. S. A*. **96**, 5263–5267.

Grootendorst, J., Mulder, M., Haasdijk, E., de Kloet, E. R., and Jaarsma, D. (2000a). Presence of apolipoprotein E immunoreactivity in degenerating neurones of mice is dependent on the severity of kainic acid-induced lesion. *Brain Res*. **868**, 165–175.

Grootendorst, J., de Kloet, E. R., Lucassen, P. J., Kempes, M. M., Dalm, S., and Oitzl, M. S. (2000b). Stress and corticosterone restore the behavioral deficit of apolipoproteinE 0/0 mice. Submited.

Gundersen, H. J., Bagger, P., Bendtsen, T. F., Evans. S. M., Korbo, L., Marcussen, N., Moller, A., Nielsen, K., Nyengaard, J. R., and Pakkenberg, B. (1988). The new stereological tools: Disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. *Acta Pathol. Microbiol. Immunol. Scand.* **96**, 857–881.

Haan, M. N., and Shemanski, L., Gagust, W. J., Manolio, T. A., and Kuller, L. (1999). The role of ApoE e4 in modulating effects of other risk factors for cognitive decline in eldely persons. *JAMA, J. Am. Med. Assoc*. **282**, 40–46.

Hassan, A. H., von Rosenstiel, P., Patchev, V. K., Holsboer, F., and Almeida, O. F. (1996). Exacerbation of apoptosis in the dentate gyrus of the aged rat by dexamethasone and the protective role of corticosterone. *Exp. Neurol*. **140**, 43–52.

Hassan, A. H., Patchev, V. K., von Rosenstiel, P., Holsboer, F., and Almeida, O. F. (1999). Plasticity of hippocampal corticosteroid receptors during aging in the rat. *FASEB J*. **13**, 115–122.

Hauger, R. L., Thrivikraman, K. V., and Plotsky, P. M. (1994). Age-related alterations of hypothalamic-pituitary-adrenal axis function in male Fischer 344 rats. *Endocrinology (Baltimore)* **134**, 1528–1536.

Hebda-Bauer, E. K., Morano, M. I., and Therrien, B. (1999). Aging and corticosterone injections affect spatial learning in Fisher 344 × Brown Norway rats. *Brain Res*. **827**, 93–103.

Heim, C., Ehlert, U., and Hellhammer, D. H. (1999). The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. *Psychoneuroendocrinology* **25**, 1–35.

Herman, J. P., and Cullinan, W. E. (1997). Neurocircuitry of stress: Central control of the hypothalamo-pituitary-adrenocortical axis. *Trends Neurosci*. **20**, 78–84.

Herman, J. P., Schafer, M. K., Young, E. A., and Thompson, R., Douglass, J., Akil, H., and Watson, S. J. (1989). Evidence for hippocampal regulation of neuroendocrine neurons of the hypothalamo-pituitary-adrenocortical axis. *J. Neurosci*. **9**, 3072–3082.

Herman, J. P., Cullinan, W. E., and Watson, S. J. (1994). Involvement of the bed nucleus of the stria terminalis in tonic regulation of paraventricular hypothalamic CRH and AVP mRNA expression. *J. Neuroendocrinol*. **6**, 433–442.

Herman, J. P., Cullinan, W. E., Morano, M. I., Akil, H., and Watson, S. J. (1995). Contribution of the ventral subiculum to inhibitory regulation of the hypothalamo-pituitary-adrenocortical axis. *J. Neuroendocrinol*. **7**, 475–482.

Herman, J. P., Prewitt, C. M., and Cullinan, W. E. (1996). Neuronal circuit regulation of the hypothalamo-pituitary-adrenocortical stress axis. *Crit Rev. Neurobiol*. **10**, 371–394.

Heuser, I. J., Gotthardt, U., Schweiger, U., Schmider, J., Lammers, C. H., Dettling, M., and Holsboer, F. (1994). Age-associated changes of pituitary-adrenocortical hormone regulation in humans: Importance of gender. *Neurobiol. Aging* **15**, 227–231.

Holsboer, F., and Barden, N. (1996). Antidepressants and hypothalamic-pituitary-adrenocortical regulation. *Endocr. Rev.* **17**, 187–205.

Hoogendijk, W. J., Pool, C. W., Troost, D., van Zwieten, E., and Swaab, D. F. (1995). Image analyser-assisted morphometry of the locus coeruleus in Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. *Brain* **118**, 131–143.

Horner, H. C., Packan, D. R., and Sapolsky, R. M. (1990). Glucocorticoids inhibit glucose transport in cultured hippocampal neurons and glia. *Neuroendocrinology* **52**, 57–64.

Hornsby, C. D., Grootendorst, J., and De Kloet, E. R. (1996). Dexamethasone does not prevent seven-day ADX-induced apoptosis in the dentate gyrus of the rat hippocampus. *Stress* **1**, 51–64.

Horsburgh, K., Kelly, S., McCulloch, J., Higgins, G. A., Roses, A. D., and Nicoll, J. A. (1999). Increased neuronal damage in apolipoprotein E-deficient mice following global ischaemia. *NeuroReport* **10**, 837–841.

Huizenga, N. A., Koper, J. W., de Lange, P., Pols, H. A., Stolk, R. P., Grobbee, D. E., de Jong, F. H., and Lamberts, S. W. (1998). Interperson variability but intraperson stability of baseline plasma cortisol concentrations, and its relation to feedback sensitivity of the hypothalamo-pituitary-adrenal axis to a low dose of dexamethasone in elderly individuals. *J. Clin. Endocrinol. Metab*. **83**, 47–54.

Hyman, B. T., and Gomez-Isla, T. (1996). Neuropathological markers of impaired cognition in the entorhinal cortex. *Neurobiol. Aging* **17**, 940–941.

Ingram, D. K., and Jucker, M. (1999). Developing mouse models of aging: A consideration of strain differences in age-related behavioral and neural parameters *Neurobiol. Aging* **20**, 137–145.

Islam, A., Ayer-LeLievre, C., Heigenskold, C., Bogdanovic, N., Winblad, B., and Adem, A. (1998). Changes in IGF-1 receptors in the hippocampus of adult rats after long-term adrenalectomy: Receptor autoradiography and in situ hybridization histochemistry. *Brain Res*. **797**, 342–346.

Issa, A. M., Rowe, W., Gauthier, S., and Meaney, M. J. (1990). Hypothalamic-pituitary-adrenal activity in aged, cognitively impaired and cognitively unimpaired rats. *J. Neurosci*. **10**, 3247–3254.

Jacobson, L., and Sapolsky, R. (1991). The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. *Endocr. Rev.* **12**, 118–134.

Jamieson, P. M., Fuchs, E., Flügge, G., and Seckl, J. R. (1997). Attenuation of hippocampal 11beta-hydroxysteroid dehydrogenase type 1 by chronic psychosocial stress in the tree shrew. *Stress* **2**, 123–132.

Joëls, M. (1997). Steroid hormones and excitability in the mammalian brain. *Front. Neuroendocrinol*. **18**, 2–48.

Joëls, M., and De Kloet, E. R. (1989). Effects of glucocorticoids and norepinephrine on the excitability in the hippocampus. *Science* **245**, 1502–1505.

Joëls, M., and De Kloet, E. R. (1990). Mineralocorticoid receptor mediated changes in membrane properties of rat CA1 pyramidal neurons in vitro. *Proc. Natl. Acad. Sci. U. S. A*. **87**, 4495–4498.

Joëls, M., and De Kloet, E. R. (1992). Control of neuronal excitability by corticosteroid hormones. *Trends Neurosci*. **15**, 25–30.

Joëls, M., Hesen, W., and De Kloet, E. R. (1991). Mineralocorticoid hormones suppress serotonin-induced hyperpolarization of rat hippocampal CA1 neurons. *J. Neurosci*. **11**, 2288–2294.

Johansson, I. M., Bjartmar, L., Marcusson, J., Ross, S. B., Seckl, J. R., and Olsson, T. (1998). Chronic amitriptyline treatment induces hippocampal NGFI-A, glucocorticoid receptor and mineralocorticoid receptor mRNA expression in rats. *Mol. Brain Res*. **62**, 92–95.

Johren, O., Flügge, G., and Fuchs, E. (1994). Hippocampal glucocorticoid receptor expression in the tree shrew: Regulation by psychosocial conflict. *Cell. Mol. Neurobiol*. **14**, 281–296.

Kalmijn, S., Launer, L. J., Stolk, R. P., de Jong, F. H., Pols, H. A., Hofman, A., Breteler, M. M., and Lamberts, S. W. (1998). A prospective study on cortisol, dehydroepiandrosterone sulfate, and cognitive function in the elderly. *J. Clin. Endocrinol. Metab*. **83**, 3487–3492.

Karst, H., Werkman, T. R., Struik, M., Bosma, A., and Joëls, M. (1997). Effects of adrenalectomy on Ca^{2+} Currents and Ca^{2+} channel subunit mRNA expression in hippocampal CA1 neurons of young rats. *Synapse* **26**, 155–165.

Karten, Y. J., Nair, S. M., van Essen, L., Sibug, R., and Joëls, M. (1999). Long-term exposure to high corticosterone levels attenuates serotonin responses in rat hippocampal CA1 neurons. *Proc. Natl. Acad. Sci. U. S. A*. **96**, 13456–13461.

Kempermann, G., Kuhn, H. G., and Gage, F. H. (1998). Experience-induced neurogenesis in the senescent dentate gyrus. *J. Neurosci*. **18**, 3206–3212.

Kerr, D. S., Campbell, L. W., Applegate, M. D., Brodish, A., and Landfield, P. W. (1991). Chronic stress-induced acceleration of electrophysiologic and morphometric biomarkers of hippocampal aging. *J. Neurosci*. **11**, 1316–1324.

Keverne, E. B. (1997). An evaluation of what the mouse knockout experiments are telling us about mammalian behaviour. *BioEssays* **19**, 1091–1098.

Kirschbaum, C., Wolf, O. T., May, M., Wippich, W., and Hellhammer, D. H. (1996). Stress- and treatment-induced elevations of cortisol levels associated with impaired declarative memory in healthy adults. *Life Sci*. **58**, 1475–1483.

Koide, T., Wieloch, T. W., and Siesjö, B. K. (1986). Chronic dexamethasone pretreatment aggravates ischemic neuronal necrosis. *J. Cereb Blood Flow Metab*. **6**, 395–404.

Koliatsos, V. E., Applegate, M. D., Knusel, B., Junard, E. O., Burton, L. E., Mobley, W. C., Hefti, F. F., and Price, D. L. (1991). Recombinant human nerve growth factor prevents retrograde degeneration of axotomized basal forebrain cholinergic neurons in the rat. *Exp. Neurol*. **112**, 161–173.

Korbo, L., Pakkenberg, B., Ladefoged, O., Gundersen, H. J., Arlien-Soborg, P., and Pakkenberg, H. (1990). An efficient method for estimating the total number of neurons in rat brain cortex. *J. Neurosci. Methods* **31**, 93–100.

Kornack, D. R., and Rakic, P. (1999). Continuation of neurogenesis in the hippocampus of the adult macaque monkey. *Proc. Natl. Acad. Sci. U. S. A*. **96**, 5768–5773.

Kretz, O., Reichardt, H. M., Schutz, G., and Bock, R. (1999). Corticotropin-releasing hormone expression is the major target for glucocorticoid feedback-control at the hypothalamic level. *Brain Res*. **818**, 488–491.

Krugers, H. J., Mulder, M., Korf, J., Havekes, L., De Kloet, E. R., and Joëls, M. (1997a). Altered synaptic plasticity in hippocampal CA1 area of apolipoprotein E-deficient mice. *NeuroReport* **8**, 2505–2510.

Krugers, H. J., Douma, B. R., Andringa, G., Bohus, B., Korf, J., and Luiten, P. G. (1997b). Exposure top chronic psychosocial stress and corticosterone in the rat: Effects on spatial discrimination learning and hippocampal protein kinase C gamma immunoreactivity. *Hippocampus* **7**, 427–436.

Krugers, H. J., Kemper, R. H., Korf, J., Ter Horst, G. J., and Knollema, S. (1998). Metyrapone reduces rat brain damage and seizures after hypoxia-ischemia: An effect independent of modulation of plasma corticosterone levels? *J. Cereb. Blood Flow Metab.* **18**, 386–390.

Kumar, A., Jin, Z., Bilker, W., Udupa, J., and Gottlieb, G. (1998). Late-onset minor and major depression: Early evidence for common neuroanatomical substrates detected by using MRI. *Proc. Natl. Acad. Sci. U. S. A*. **95**, 7654–7658.

Landfield, P. W. (1978). An endocrine hypothesis of brain aging and studies on brain endocrine correlations and monosynaptic neurophysiology during aging. *Adv. Exp. Med. Biol*. **113**, 179–199.

Landfield, P. W., Waymire, J. C., and Lynch, G. (1978). Hippocampal aging and adrenocorticoids: Quantitative correlations. *Science* **202**, 1098–1102.

Landfield, P. W., Baskin, R. K., and Pitler, T. A. (1981). Brain aging correlates: Retardation by hormonal-pharmacological treatments. *Science* **214**, 581–584.

Landfield, P. W., McEwen, B. S., Sapolsky, R. M., and Meaney, M. J. (1996). Hippocampal cell death. *Science* **272**, 1249–1251.

Lapchak, P. A., Araujo, D. M., Beck, K. D., Finch, C. E., Johnson, S. A., and Hefti, F. (1993). BDNF and trkB mRNA expression in the hippocampal formation of aging rats. *Neurobiol. Aging* **14**, 121–126.

Lawrence, M. S., and Sapolsky, R. M. (1994). Glucocorticoids accelerate ATP loss following metabolic insults in cultured hippocampal neurons. *Brain Res*. **646**, 303–306.

Lawrence, M. S., Ho, D. Y., Dash, R., and Sapolsky, R. M. (1995). Herpes simplex virus vectors overexpressing the glucose transporter gene protect against seizure-induced neuron loss. *Proc. Natl. Acad. Sci. U. S. A*. **92**, 7247–7251.

Lawrence, M. S., Sun, G. H., Kunis, D. M., Saydam, T. C., Dash, R., Ho, D. Y., Sapolsky, R. M., and Steinberg, G. K. (1996). Overexpression of the glucose transporter gene with a herpes simplex viral vector protects striatal neurons against stroke. *J. Cereb. Blood Flow Metab.* **16**, 181–185.

Lee, D. W., Miyasato, L. E., and Clayton, N. S. (1998). Neurobiological bases of spatial learning in the natural environment: Neurogenesis and growth in the avian and mammalian hippocampus. *NeuroReport* **9**, R15–R27.

Leverenz, J. B., Wilkinson, C. W., Wamble, M., Corbin, S., Grabber, J. E., Raskind, M. A., and Peskind, E. R. (1999). Effect of chronic high-dose exogenous cortisol on hippocampal neuronal number in aged nonhuman primates. *J. Neurosci*. **19**, 2356–2361.

Levine, S., Huchton, D. M., Wiener, S. G., and Rosenfeld, P. (1991). Time course of the effect of maternal deprivation on the hypothalamic-pituitary-adrenal axis in the infant rat. *Dev. Psychobiol*. **24**, 547–558.

Levy, A., Dachir, S., Arbel, I., and Kadar, T. (1994). Aging, stress, and cognitive function. *Ann. N. Y. Acad. Sci*. **717**, 79–88.

Long, J. M., Mouton, P. R., Jucker, M., and Ingram, D. K. (1999). What counts in brain aging? Design-based stereological analysis of cell number. *J. Gerontol.* **54**, B407–B417.

Lorens, S. A., Hata, N., Hanada, R. J., VandeKar, L. D., Guschwan, M., Goral, J., Lee, J. M., and Hamiton, M. E., Bethea, C. L., Clancey, J. (1990). Neurochemical, endocrine and immunological responses to stress in young and old Fischer 344 male rats. *Neurobiol. Aging* **11**, 139–150.

Lucassen, P. J., Salehi, A., Pool, C. W., Gonatas, N. K., and Swaab, D. F. (1994). Activation of vasopressin neurons in aging and in Alzheimer's Disease. *J. Neuroendocrinol*. **6**, 673–679.

Lucassen, P. J., Tilders, F. J. H., Salehi, A., and Swaab, D. F. (1997a). Neuropeptides — AVP, CRH and OXT — in the human hypothalamus; activity changes in aging, senile dementia and depression. *Aging Clin. Exp. Res*. **9** (Suppl.), 48–50.

Lucassen, P. J., De Kloet, E. R., Wouda, J., Holtrop, A., van Oers, H., and Swaab, D. F. (1997b). No increase in DNA damage in the hippocampus of depressed patients. *Soc. Neurosci. Abstr.* **698.2**.

Lucassen, P. J., Müller, M. B., Holsboer, F., Bauer, J., Holtrop, A., Wouda, J., Hoogendijk, W. J. G., De Kloet, E. R., and Swaab, D. F. (2000). Hippocampal apoptosis in major depression is a minor event and absent from subareas at risk for glucocorticoid overexposure. *Am. J. Pathol.*, in press.

Luine, V. N. (1997). Steroid hormone modulation of hippocampal dependent spatial memory. *Stress* **2**, 21–36.

Lupien, S. J., and McEwen, B. S. (1997). The acute effects of corticosteroids on cognition: Integration of animal and human model studies. *Brain Res. Rev.* **24**, 1–27.

Lupien, S. J., Gaudreau, S., Tchiteya, B. M., Maheu, F., Sharma, S., Nair, N. P., Hauger, R. L., McEwen, B. S., and Meaney, M. J. (1997). Stress-induced declarative memory impairment in healthy elderly subjects: Relationship to cortisol reactivity. *J. Clin. Endocrinol. Metab.* **82**, 2070–2075.

Lupien, S. J., de Leon, M. J., De Santi, S., Convit, A., Tarshish, C., Nair, N. P., Thakur, M., McEwen, B. S., Hauger, R. L., and Meaney, M. J. (1998). Cortisol levels during human aging predict hippocampal atrophy and memory deficits. *Nat. Neurosci*. **1**, 69–73.

MacLennan, K. M., Smith, P. F., and Darlington, C. L. (1998). Adernalectomy-induced neuronal degeneration. *Prog. Neurobiol*. **54**, 481–498.

Magarinos, A. M., McEwen, B. S., Flügge, G., and Fuchs, E. (1996). Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. *J. Neurosci*. **16**, 3534–3540.

Magarinos, A. M., Verdugo, J. M., and McEwen, B. S. (1997). Chronic stress alters synaptic terminal structure in hippocampus. *Proc. Natl. Acad. Sci. U. S. A*. **94**, 14002–14008.

Magarinos, A. M., Orchinik, M., and McEwen, B. S. (1998). Morphological changes in the hippocampal CA3 region induced by non-invasive glucocorticoid administration: A paradox. *Brain Res*. **809**, 314–318.

Magarinos, A. M., Deslandes, and A., McEwen, B. S. (1999). Effects of antidepressants and benzodiazepine treatments on the dendritic structure of CA3 pyramidal neurons after chronic stress. *Eur. J. Pharmacol*. **371**, 113–122.

Makino, S., Schulkin, J., Smith, M. A., Pacak, K., Palkovits, M., and Gold, P. W. (1995). Regulation of corticotropin-releasing hormone receptor messenger ribonucleic acid in the rat brain and pituitary by glucocorticoids and stress. *Endocrinology (Baltimore)* **136**, 4517–4525.

Mandel, R. J., Gage, F. H., Clevenger, D. G., Spratt, S. K., Snyder, R. O., and Leff, S. E. (1990). Nerve growth factor expressed in the medial septum following in vivo gene delivery using a recombinant adeno-

associated viral vector protects cholinergic neurons from fimbriafornix lesion-induced degeneration. *Exp. Neurol*. **155**, 59–64.

Masliah, E., Mallory, M., Ge, N., Alford, M., Veinbergs, I., and Roses, A. D. (1995). Neurodegeneration in the central nervous system of apoE-deficient mice. *Exp. Neurol*. **136**, 107–122.

Masliah, E., Mallory, M., Veinbergs, I., Miller, A., and Samuel, W. (1996). Alterations in apolipoprotein E expression during aging and neurodegeneration. *Prog. Neurobiol*. **50**, 493–503.

Masliah, E., Samuel, W., Veinbergs, I., Mallory, M., Mante, M., and Saitoh, T. (1997). Neurodegeneration and cognitive impairment in apoE-deficient mice is ameliorated by infusion or recombinant apoE. *Brain Res*. **751**, 307–314.

Masoro, E. J. (1980a). Biology of aging. Introduction. *Fed. Proc*. **39**, 3162.

Masoro, E. J. (1980b). Mortality and growth characteristics of rat strains commonly used in aging research. *Exp. Aging Res* **6**, 219–233.

McEwen, B. S. (1996). Gonadal and adrenal steroids regulate neurochemical and structural plasticity of the hippocampus via cellular mechanisms involving NMDA receptors. *Cell. Mol. Neurobiol*. **16**, 103–116.

McEwen, B. S. (1999). Stress and the aging hippocampus. *Front. Neuroendocrinol*. **20**, 49–70.

McEwen, B. S., and Sapolsky, R. M. (1995). Stress and cognitive function. *Curr. Opin. Neurobiol*. **5**, 205–216.

McEwen, B. S., De Leon, M., Lupien, S., and Meaney, M. J. (1999). Corticosteroids, the aging brain and cognitive. *Trends Endocrinol. Metab*. **10**, 92–96.

Meaney, M. J., Aitken, D. H., Bhatnagar, S., and Sapolsky, R. M. (1991). Postnatal handling attenuates certain neuroendocrine, anatomical, and cognitive dysfunctions associated with aging in female rats. *Neurobiol. Aging* **12**, 31–38.

Meaney, M. J., Aitken, D. H., Sharma, S., and Viau, V. (1992). Basal ACTH, corticosterone and corticosterone-binding globulin levels over the diurnal cycle, and age-related changes in hippocampal type I and type II corticosteroid receptor binding capacity in young and aged, handled and nonhandled rats. *Neuroendocrinology* **55**, 204–213.

Meijer, O. C., and De Kloet, E. R. (1998). Corticosterone and serotonergic neurotransmission in the hippocampus: Functional implications of central corticosteroid receptor diversity. *Crit. Rev. Neurobiol*. **12**, 1–20.

Meijer, O. C., de Lange, E. C., Breimer, D. D., de Boer, A. G., Workel, J. O., and De Kloet, E. R. (1998). Penetration of dexamethasone into brain glucocorticoid targets is enhanced in mdr1A P-glycoprotein knockout mice. *Endocrinology (Baltimore)* **139**, 1789–1793.

Meijer, O. C., Steenbergen, P. J., and de Kloet, E. R. (2000). Differential expression and regional distribution of steroid receptor coactivators SRC-1 and SRC-2 in brain and pituitary. *Endocrinology (Baltimore)*, in press.

Minichiello, L., Korte, M., Wolfer, D., Kuhn, R., Unsicker, K., Cestari, V., Rossi-Arnaud, C., Lipp, H. P., Bonhoeffer, T., and Klein, R. (1999). Essential role for TrkB receptors in hippocampus-mediated learning. *Neuron* **24**, 401–414.

Mizoguchi, K., Kunishita, T., Chui, D. H., and Tabira, T. (1992). Stress induces neuronal death in the hippocampus of castrated rats. *Neurosci. Lett*. **138**, 157–160.

Montine, T. J., Montine, K. S., Olson, S. J., Graham, D. G., Roberts, L. J., 2nd, Morrow, J. D., Linton, M. F., Fazio, S., and Swift, L. L. (1999). Increased cerebral cortical lipid peroxidation and abnormal phospholipids in aged homozygous apoE-deficient C57BL/6J mice. *Exp. Neurol*. **158**, 234–241.

Morano, M. I., Vazquez, D. M., and Akil, H. (1994). The role of the hippocampal mineralocorticoid and glucocorticoid receptors in the hypothalamo-pituitary-adrenal axis of the aged Fisher rat. *Mol. Cell. Neurosci*. **5**, 400–412.

Morrison, J. H., and Hof, P. R. (1997). Life and death of neurons in the aging brain. *Science* **278**, 412–419.

Morse, J. K., and Davis, J. N. (1990). Regulation of ischemic hippocampal damage in the gerbil: Adrenalectomy alters the rate of CA1 cell disappearance. *Exp. Neurol*. **110**, 86–92.

Mouton, P. R., Pakkenberg, B., Gundersen, H. J., and Price, D. L. (1994). Absolute number and size of pigmented locus coeruleus neurons in young and aged individuals. *J. Chem. Neuroanat*. **7**, 185–190.

Müller, M. B., Lucassen, P. J., Yassouridis, A., Hoogendijk, W. J. G., Holsboer F., and Swaab, D. F. (1998). The human hippocampus in major depression or following administration of glucocorticoids: No evidence for major structural alterations. *Soc. Neurosci. Abstr*. **386.14**.

Müller, M. B., Lucassen, P. J., Yassouridis, A., Hoogendijk, W. J. G., Holsboer, F., and Swaab, D. F. (2000). Major depression and glucocorticoid treatment do not affect the structural integrity of the human hippocampus. Submitted.

Murer, M. G., Boissiere, F., Yan, Q., Hunot, S., Villares, J., Faucheux, B., Agid, Y., Hirsch, E., and Raisman-Vozari, R. (1999). An immunohistochemical study of the distribution of brain-derived neurotrophic factor in the adult human brain, with particular reference to Alzheimer's disease. *Neuroscience* **88**, 1015–1032.

Nair, S. M., Werkman, T. R., Graig, J., Finnell, R., Joëls, M., and Eberwine, J. H. (1998). Corticosteroid regulation of ion channel conductances and mRNA levels in individual hippocampal CA1 neurons. *J. Neurosci*. **18**, 2685–2696.

Nelson, R. J., and Young, K. A. (1998). Behavior in mice with targeted disruption of single genes. *Neurosci. Biobehav. Rev*. **22**, 453–462.

Newcomer, J. W., Craft, S., Hershey, T., Askins, K., and Bardgett, M. E. (1994). Glucocorticoid-induced impairment in declarative memory performance in adult humans. *J. Neurosci*. **14**, 2047–2053.

Newcomer, J. W., Craft, S., Fucetola, R., Moldin, S. O., Selke, G., Paras, L., and Miller, R. (1999a). Glucose-induced increase in memory performance in patients with schizophrenia. *Schizophr. Bull*. **25**, 321–335.

Newcomer, J. W., Selke, G., Melson, A. K., Hershey, T., Craft, S., Richards, K., and Alderson, A. L. (1999b). Decreased memory performance in healthy humans induced by stress-level cortisol treatment. *Arch. Gen. Psychiatry* **56**, 527–533.

Nichols, N. R., Day, J. R., Laping, N. J., Johnson, and S. A., and Finch, C. E. (1993). GFAP mRNA increases with age in rat and human brain. *Neurobiol. Aging* **14**, 421–429.

Nicholson, S., Campbell, E., Torrellas, A., Beckford, U., Altaher, R., Sandford, R., Scraggs, R., Gillham, B., and Jones, M. (1984). Recovery of the hypothalamo-pituitary-adrenocortical axis in the rat after long-term dexamethasone treatment. *Neuroendocrinology* **39**, 343–349.

Nicholson, S. A., Campbell, E. A., Gillham, B., and Jones, M. T. (1987). Recovery of the components of the hypothalamo-pituitary-adrenocortical axis in the rat after chronic treatment with prednisolone. *J. Endocrinol*. **113**, 239–247.

Nicholson, S. A., Gillham, B., and Jones, M. T. (1988). Influence of prolonged glucocorticoid treatment on intracellular mechanisms involved in ACTH secretion in the rat. *J. Mol. Endocrinol*. **1**, 203–212.

Nicolle, M. M., Gallagher, M., and McKinney, M. (1999). No loss of synaptic proteins in the hippocampus of aged, behaviorally impaired rats. *Neurobiol. Aging* **20**, 343–348.

Nilsson, M., Perfilieva, E., Johansson, U., Orwar, O., and Eriksson, P. S. (1999). Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. *J. Neurobiol*. **39**, 569–578.

Ohl, F., and Fuchs, E. (1998). Memory performance in tree shrews: Effects of stressful experiences. *Neurosci. Biobehav. Rev*. **23**, 319–323.

Ohl, F., and Fuchs, E. (1999). Differential effects of chronic stress on memory processes in the tree shrew. *Cogn. Brain Res*. **7**, 379–387.

Ohl, F., Michaelis, T., Fujimori, H., Frahm, J., Rensing, S., and Fuchs, E. (1999). Volumetric MRI measurements of the tree shrew hippocampus. *J. Neurosci. Methods* **88**, 189–193.

Oitzl, M. S., and De Kloet, E. R. (1992). Selective corticosteroid antagonists modulate specific aspects of spatial orientation learning. *Behav. Neurosci*. **106**, 62–71.

Oitzl, M. S., Fluttert, M., and De Kloet, E. R. (1994). The effect of corticosterone on reactivity to spatial novelty is mediated by central mineralo-corticosteroid receptors. *Eur. J. Neurosci*. **6**, 1072–1079.

Oitzl, M. S., van Haarst, A. D., Sutanto, W., and De Kloet, E. R. (1995). Corticosterone, brain mineralocorticoid receptors (MRs) and the activity of the hypothalamic-pituitary-adrenal (HPA) axis: The Lewis rat as an example of increased central MR capacity and a hyporesponsive HPA axis. *Psychoneuroendocrinology* **20**, 655–675.

Oitzl, M. S., Mulder, M., Lucassen, P. J., Havekes, L. M., Grootendorst, J., and De Kloet, E. R. (1997a). Severe learning deficits in apolipoprotein E-knockout mice in a water maze task. *Brain Res*. **752**, 189–196.

Oitzl, M. S., van Haarst, A. D., and De Kloet, E. R. (1997b). Behavioral and neuroendocrine responses controlled by the concerted action of central mineralocorticoid (MRS) and glucocorticoid receptors (GRS). *Psychoneuroendocrinology* **22**(Suppl. 1), S87–S93.

Oitzl, M. S., Fluttert, M., Sutanto, W., and De Kloet, E. R. (1998a). Continuous blockade of brain glucocorticoid receptors facilitates spatial learning and memory in rats. *Eur. J. Neurosci*. **10**, 3759–3766.

Oitzl, M. S., Fluttert, M., and De Kloet, E. R. (1998b). Acute blockade of hippocampal glucocorticoid receptors facilitates spatial learning in rats. *Brain Res*. **797**, 159–162.

Oitzl, M. S., Workel, J., and De Kloet, E. R. (2000a). Maternal deprivation affects behaviour from youth to senescence. *Eur. J. Neurosci.*, in press.

Oitzl, M. S., Workel, J., and De Kloet, E. R. (2000b). Maternal deprivation selects for extremes of cognitive aging. *Proc. Natl. Acad. Sci. U.S.A.*, submitted (revision).

Okugawa, G., Omori, K., Suzukawa, J., Fujiseki, Y., Kinoshita, T., and Inagaki, C. (1999). Long-term treatment with antidepressants increases glucocorticoid receptor binding and gene expression in cultured rat hippocampal neurones. *Neuroendocrinology* **11**, 887–895.

Ouchi, Y. Nobezawa, S., Okada, H., Yoshikawa, E., Futatsubashi, M., and Kaneko, M. (1998). Altered glucose metabolism in the hippocampal head in memory impairment. *Neurology* **51**, 136–142.

Palmer, T. D., Markakis, E. A., Willhoite, A. R., Safar, and F., Gage, F. H. (1999). Fibroblast growth factor-2 activates a latent neurogenic program in neural stem cells from diverse regions of the adult CNS. *J. Neurosci*. **19**, 8487–8497.

Parent, J. M., Janumpalli, S., McNamara, J. O., and Lowenstein, D. H. (1998). Increased dentate granule cell neurogenesis following amygdala kindling in the adult rat. *Neurosci. Lett*. **247**, 9–12.

Patacchioli, F. R., Taglialatela, G., Angelucci, L., Cerbone, A., and Sadile, A. G. (1989). Adrenocorticoid receptor binding in the rat hippocampus: Strain-dependent covariations with arousal and habituation to novelty. *Behav. Brain Res*. **33**, 287–300.

Patacchioli, F. R., Casolini, P., Puglisi-Allegra, S., Sadile, A. G., and Angelucci, L. (1990). Hippocampal glucocorticoid receptor and behavior: A correlative study in rats and mice. *J. Steroid Biochem. Mol. Biol*. **37**, 405–409.

Patel, S. N., Clayton, N. S., and Krebs, J. R. (1997). Spatial learning induces neurogenesis in the avian brain *Behav. Brain Res*. **89**, 115–128.

Pawlik, M., Fuchs, E., Walker, L. C., and Levy, E. (1999). Primate-like amyloid-beta sequence but no cerebral amyloidosis in aged tree shrews. *Neurobiol. Aging* **20**, 47–51.

Peterson, D. A., Dickinson-Anson, H. A., Leppert, J. T., Lee, K. F., and Gage, F. H. (1999). Central neuronal loss and behavioral impairment in mice lacking neurotrophin receptor p75. *J. Comp. Neurol*. **404**, 1–20.

Phan, V. L., Su, T. P., Privat, A., and Maurice, T. (1999). Modulation of steroidal levels by adrenalectomy/castration and inhibition of neurosteroid synthesis enzymes affect sigmal receptor-mediated behaviour in mice. *Eur. J. Neurosci*. **11**, 2385–2396.

Phinney, A. L., Calhoun, M. E., Wolfer, D. P., Lipp, H. P., Zheng, H., and Jucker, M. (1999). No hippocampal neuron or synaptic bouton loss in learning-impaired aged beta-amyloid precursor protein-null mice. *Neuroscience* **90**, 1207–1216.

Picciotto, M. R. (1999). Knock-out mouse models used to study neurobiological systems. *Crit. Rev. Neurobiol*. **13**, 103–149.

Plotsky, P. M., Thrivikraman, K. V., and Meaney, M. J. (1993). Central and feedback regulation of hypothalamic corticotropin-releasing factor secretion. *Ciba Found. Symp*. **172**, 59–75; discussion: pp. 75–84.

Porter, N. M., and Landfield, P. W. (1998). Stress hormones, and brain aging: Adding injury to insult? *Nat. Neurosci.* **1**, 69–73.

Price, J. L., Davis, P. B., Morris, J. C., and White, D. L. (1991). The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. *Neurobiol. Aging* **12**, 295–312.

Raadsheer, F. C., Sluiter, A. A., Ravid, R., Tilders, F. J., and Swaab, D. F. (1993). Localization of corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus of the human hypothalamus; age-dependent colocalization with vasopressin. *Brain Res*. **615**, 50–62.

Raadsheer, F. C., Tilders, F. J., and Swaab, D. F. (1994). Similar age related increase of vasopressin colocalization in paraventricular corticotropin-releasing hormone neurons in controls and Alzheimer patients. *J. Neuroendocrinol*. **6**, 131–133.

Raadsheer, F. C., van Heerikhuize, J. J., Lucassen, P. J., Hoogendijk, W. J., Tilders, F. J., and Swaab, D. F. (1995). Corticotropin-releasing hormone mRNA levels in the paraventricular nucleus of patients with Alzheimer's disease and depression. *Am. J. Psychiatry* **152**, 1372–1376.

Raber, J., Wong, D., Buttini, M, Orth, M., Bellosta, S., Pitas, R. E., Mahley, R. W., and Mucke, L. (1998). Isoform-specific effects of human apolipoprotein E on brain function revealed in ApoE knockout mice: Increased susceptibility of females. *Proc. Natl. Acad. Sci. U. S. A*. **95**, 10914–10919.

Rapp, P. R., and Gallagher, M. (1996). Preserved neuron number in the hippocampus of aged rats with spatial learning deficits. *Proc. Natl. Acad. Sci. U. S. A*. **93**, 9926–9930.

Rapp, P. R., Stack, E. C., and Gallagher, M. (1999). Morphometric studies of the aged hippocampus: I. Volumetric analysis in behaviorally characterized rats. *J. Comp. Neurol*. **403**, 459–470.

Rasmussen, T., Schliemann, T., Sorensen, J. C., Zimmer, J., and West, M. J. (1996). Memory impaired aged rats: No loss of principal hippocampal and subicular neurons. *Neurobiol. Aging* **17**, 143–147.

Ratka, A., Sutanto, W., Bloemers, M., and De Kloet. E. R. (1989). On the role of brain mineralocorticoid (type I) and glucocorticoid (type II) receptors in neuroendocrine regulation. *Neuroendocrinology* **50**, 117–123.

Reagan, L. P., and McEwen, B. S. (1997). Controversies surrounding glucocorticoid-mediated cell death in the hippocampus. *J. Chem. Neuroanat*. **13**, 149–167.

Reichardt, H. M., Tronche, F., Berger, S., Kellendonk, C., and Schutz, G. (1999). New insights into glucocorticoid and mineralocorticoid signaling: Lessons from gene targeting. *Adv. Pharmacol*. **47**, 1–21.

Reiman, E. M., Caselli, R. J., Yun, L. S., Chen, K., Bandy, D., Minoshima, S., Thibodeau, S. N., and Osborne, D. (1996). Preclinical evidence of Alzheimer's disease in persons homozygous for the epsilon 4 allele for apolipoprotein E. *N. Engl. J. Med*. **334**, 752–758.

Reiman, E. M., Uecker, A., Caselli, R. J., Lewis, S., Bandy, D., De Leon, M. J., De Santi, S., Convit, A., Osborne, D., Weaver, A., and Thibodeau, S. N. (1998). Hippocampal volumes in cognitively normal persons at genetic risk for Alzheimer's disease. *Ann. Neurol*. **44**, 288–291.

Reul, J. M. H. M., and De Kloet, E. R. (1985). Two receptor systems for corticosterone in rat brain; Microdistribution and differential occupation. *Endocrinology (Baltimore)* **117**, 2505–2511.

Riegle, G. D., and Hess, G. D. (1972). Chronic and acute dexamethasone suppression of stress activation of the adrenal cortex in young and aged rats. *Neuroendocrinology* **9**, 175–187.

Rivier, C. (1999). Gender, sex steroids, corticotropin-releasing factor, nitric oxide, and the HPA response to stress. *Pharmacol. Biochem. Behav*. **64**, 739–751.

Roberts, N. A., Barton, R. N., and Horan, M. A. (1990). Ageing and the sensitivity of the adrenal gland to physiological doses of ACTH in man. *J. Endocrinol*. **126**, 507–513.

Robertson, T. A., Dutton, N. S., Martins, R. N., Roses, A. D., Kakulas, B. A., and Papadimitriou, J. M. (1998). Age-related congophilic inclusions in the brains of apolipoprotein E-deficient mice. *Neuroscience* **82**, 171–180.

Roses, A. D. (1997). Apolipoprotein E, a gene with complex biological interactions in the aging brain. *Neurobiol. Dis*. **4**, 170–185.

Rots, N. Y., de Jong, J., Workel, J. O., Levine, S., Cools, A. R., and De Kloet, E. R. (1996a). Neonatal maternally deprived rats have as adults elevated basal pituitary-adrenal activity and enhanced susceptibility to apomorphine. *J. Neuroendocrinol*. **8**, 501–506.

Rots, N. Y., Cools, A. R., Oitzl, M. S., de Jong, J., Sutanto, W., and De Kloet, E. R. (1996b). Divergent prolactin and pituitary-adrenal activity in rats selectively bred for different dopamine responsiveness. *Endocrinology (Baltimore)* **137**, 1678–1686.

Rots, N. Y., Workel, J., Oitzl, M. S., Berod, A., Rostene, W., Cools, A. R., and De Kloet, E. R. (1996c). Development of divergence in dopamine responsiveness in genetically selected rat lines is preceded by changes in pituitary-adrenal activity *Dev. Brain Res*. **92**, 164–171.

Rowe, W., Steverman, A., Walker, M., Sharma, S., Barden, N., Seckl, J. R., and Meaney, M. J. (1997). Antidepressants restore hypothalamic-pituitary-adrenal feedback function in aged, cognitively-impaired rats. *Neurobiol. Aging* **18**, 527–533.

Rozovsky, I., Finch, C. E., and Morgan, T. E. (1998). Age-related activation of microglia and astrocytes; in vitro studies show persistent phenotypes of aging, increase proliferation, and resistance to down regulation. *Neurobiol. Aging* **19**, 97–103.

Sabatino, F., Masoro, E. J., McMahan, C. A., and Kuhn, R. W. (1991). Assessment of the role of the glucocorticoid system in aging processes and in the action of food restriction. *J. Gerontol*. **46**, B171–B179.

Salehi, A., Verhaagen, J., Dijkhuizen, P. A., and Swaab, D. F. (1996). Co-localization of high-affinity neurotrophin receptors in nucleus basalis of Meynert neurons and their differential reduction in Alzheimer's disease. *Neuroscience* **75**, 373–387.

Sapolsky, R. M. (1992). Do glucocorticoid concentrations rise with age in the rat? *Neurobiol. Aging* **13**, 171–174.

Sapolsky, R. M. (1996). Stress, glucocorticoids, and damage to the nervous system: The current state of confusion. *Stress* **1**, 1–19.

Sapolsky, R. M. (1999). Glucocorticoids, stress, and their adverse neurological effects: Relevance to aging. *Exp. Gerontol*. **34**, 721–732.

Sapolsky, R. M., and Altmann, J. (1991). Incidence of hypercortisolism and dexamethasone resistance increase with age among wild baboons. *Biol. Psychiatry* **30**, 1008–1016.

Sapolsky, R. M., and Pulsinelli, W. A. (1985). Glucocorticoids potentiate ischemic injury to neurons: Therapeutic implications. *Science* **229**, 1397–1400.

Sapolsky, R. M., and Spencer, E. M. (1997). Insulin-like growth factor I is suppressed in socially subordinate male baboons. *Am. J. Physiol*. **273**, R1346–R1351.

Sapolsky, R. M., Krey, L. C., and McEwen, B. S. (1985). Prolonged glucocorticoid exposure reduces hippocampal neuron number: Implications for aging. *J. Neurosci*. **5**, 1221–1227.

Sapolsky, R. M., Uno, H., Rebert, C. S., and Finch, C. E. (1990). Hippocampal damage associated with prolonged glucocorticoid exposure in primates. *J. Neurosci*. **10**, 2897–2902.

Sapolsky, R. M., Stein-Behrens, B. A., and Armanini, M. P. (1991a). Long-term adrenalectomy causes loss of dentate gyrus and pyramidal neurons in the adult hippocampus. *Exp. Neurol*. **114**, 246–249.

Sapolsky, R. M., Zola-Morgan, S., and Squire, L. R. (1991b). Inhibition of glucocorticoid secretion by the hippocampal formation in the primate. *J. Neurosci*. **11**, 3695–3704.

Scacianoce, S., Di. Sciullo, A., and Angelucci, L. (1990). Age-related changes in hypothalamo-pituitary-adrenocortical axis activity in the rat. In vitro studies. *Neuroendocrinology* **52**, 150–155.

Scaccianoce, S., Nicolai, R., Cigliana, G., and Angelucci, L. (1995). Reduced glucocorticoid response to corticotropin secretagogues in the aged Sprague-Dawley rat. *Neuroendocrinology* **62**, 32–38.

Schaaf, M. J., de Jong, J., De Kloet, E. R., and Vreugdenhil, E. (1998). Downregulation of BDNF mRNA and protein in the rat hippocampus by corticosterone. *Brain Res*. **813**, 112–120.

Seckl, J. R., and Olsson, T. (1995). Glucocorticoid hypersecretion and the age-impaired hippocampus: cause of effect? *J. Endocrinol*. **145**, 201–211.

Seeman, T. E., McEwen, B. S., Singer, B. H., Albert, M. S., and Rowe, J. W. (1997). Increase in urinary cortisol excretion and memory declines: MacArthur studies of successful aging. *J. Clin. Endocrinol. Metab*. **82**, 2458–2465.

Seo, S. Y., Kim, E. Y., Kim, H., and Gwag, B. J. (1990). Neuroprotective effect of high glucose against NMDA, free radical, and oxygen-glucose deprivation through enhanced mitochondrial potentials. *J. Neurosci*. **19**, 8849–8855.

Sheline, Y. I., Wang, P. W., Gado, M. H., Csernansky, J. G., and Vannier, M. W. (1996). Hippocampal atrophy in recurrent major depression. *Proc. Natl. Acad. Sci. U. S. A*. **93**, 3908–3813.

Sheline, Y. I., Sanghavi, M., Mintun, M. A., and Gado, M. H. (1999). Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. *J. Neurosci*. **19**, 5034–5043.

Simic, G., Kostovic, I., Winblad, B., and Bogdanovic, N. (1997). Volume and number of neurons of the human hippocampal formation in normal aging and Alzheimer's disease. *J. Comp. Neurol*. **379**, 482–494.

Sloviter, R. S., Valiquette, G., Abrams, G. M., Ronk, E. C., Sollas, A. L., Paul, L. A., and Neubort, S. (1989). Selective loss of hippocampal granule cells in the mature rat brain after adrenalectomy. *Science* **243**, 535–538.

Sloviter, R. S., Sollas, A. L., Dean, E., and Neubort, S. (1993a). Adrenalectomy-induced granule cell degeneration in the rat hippocampal dentate gyrus: Characterization of an in vivo model of controlled neuronal death. *J. Comp. Neurol*. **330**, 324–336.

Sloviter, R. S., Dean, E., and Neubort, S. (1993b). Electron microscopic analysis of adrenalectomy-induced hippocampal granule cell degeneration in the rat: Apoptosis in the adult central nervous system. *J. Comp. Neurol*. **330**, 337–351.

Smith, D. E., Roberts, J. Gage, F. H., and Tuszynski, M. H. (1999). Age-associated neuronal atrophy occurs in the primate brain and is reversible y growth factor gene therapy. *Proc. Natl. Acad. Sci. U. S. A*. **96**, 893–898.

Smith, M. A., and Cizza, G. (1996). Stress-induced changes in brain-derived neurotrophic factor expression are attenuated in aged Fischer 344/N rats. *Neurobiol. Aging* **17**, 859–864.

Soininen, H. S., and Riekkinen, P. J., Sr. (1996). Apolipoprotein E, Memory and Alzheimer's disease. *Trends Neurosci*. **19**, 224–228.

Sonntag, W. E., Goliszek, A. G., Brodish, A., and Eldridge, J. C. (1987). Diminished diurnal secretion of adrenocorticotropin (ACTH), but not corticosterone, in old male rats: Possible relation to increased adrenal sensitivity to ACTH in vivo. *Endocrinology (Baltimore)* **120**, 2308–2315.

Sonntag, W. E., Lynch, C. D., Bennett, S. A., Khan, A. S., Thornton, P. L., Cooney, P. T., Ingram, R. L., McShane, T., and Brunso-Bechtold, J. K. (1999). Alterations in insulin-like growth factor-1 gene and protein expression and type 1 insulin-like growth factor receptors in the brains of ageing rats. *Neuroscience* **88**, 269–279.

Sousa, N., Madeira, M. D., and Paula-Barbosa, M. M. (1997). Structural alterations of the hippocampal formation of adrenalectomized rats: An unbiased stereological study. *J. Neurocytol*. **26**, 423–438.

Sousa, N., Almeida, O. F., Holsboer, F., Paula-Barbosa, M. M., and Madeira, M. D. (1998a). Maintenance of hippocampal cell numbers in young and aged rats submitted to chronic unpredictable stress. Comparison with the effects of corticosterone treatment. *Stress* **2**, 237–249.

Sousa, N., Madeira, M. D., and Paula-Barbosa, M. M. (1998b). Effects of corticosterone treatment and rehabilitation on the hippocampal formation of neonatal and adult rats. An unbiased stereological study. *Brain Res*. **794**, 199–210.

Sousa, N., Paula-Barbosa, M. M., and Almeida, O. F. (1999). Ligand and subfield specificity of corticoid-induced neuronal loss in the rat hippocampal formation. *Neuroscience* **89**, 1079–1087.

Sousa, N., Lukoyanov, N. V., Madeira, M. D., Almeida, O. F., Paula-Barbosa, M. M. (2000). Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. *Neuroscience* **97**, 253–266.

Spencer, R. L., Kim, P. J., Kalman, B. A., and Cole, M. A. (1998). Evidence for mineralocorticoid receptor facilitation of glucocorticoid receptor-dependent regulation of hypothalamic-pituitary-adrenal axis activity. *Endocrinology (Baltimore)* **139**, 2718–2726.

Starkman, M. N., Gebarski, S. S., Berent, S., and Schteingart, D. E. (1992). Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome. *Biol. Psychiatry* **32**, 756–765.

Stein-Behrens, B., Mattson, M. P., Chang, I., Yeh, M., and Sapolsky R. (1994). Stress exacerbates neuron loss and cytoskeletal pathology in the hippocampus. *J. Neurosci*. **14**, 5373–5380.

Stein, D. J., Buchsbaum, M. S., Hof, P. R., Siegel, B. V., Jr., and Shihabuddin, L. (1998). Greater metabolic rate decreases in hippocampal formation and proisocortex than in neocortex in Alzheimer's disease. *Neuropsychobiology* **37**, 10–19.

Stienstra, C. M., van der Graaf, F., Bosma, A., Karten, Y. J. G., Hesen, W., and Joëls, M. (1998). Synaptic transmission in the rat dentate gyrus after adrenalectomy. *Neuroscience* **85**, 1061–1071.

Sugaya, K., Chouinard, M., Greene, R., Robbins, M., Personett, D., Kent, C, Gallagher, M., and McKinney, M. (1996). Molecular indices of neuronal and glial plasticity in the hippocampal formation in a rodent model of age-induced spatial learnin impairment. *J. Neurosci*. **16**, 3427–3443.

Sugaya, K., Greene, R., Personett, D., Robbins, M., Kent, C., Bryan, D., Skiba, E., Gallagher, M., and McKinney, M. (1998). Septo-hippocampal cholinergic and neurotrophin markers in age-induced cognitive decline. *Neurobiol. Aging* **19**, 351–361.

Swaab, D. F. (1995). Ageing of the human hypothalamus. *Horm. Res*. **43**, 8–11.

Swaab, D. F., Raadsheer, F. C., Endert, E., Hofman, M. A., Kamphorst, W., and Ravid, R. (1994). Increased cortisol levels in aging and Alzheimer's disease in postmortem cerebrospinal fluid. *J. Neuroendocrinol*. **6**, 681–687.

Swaab, D. F., Lucassen, P. J., Salehi, A., Scherder, E. J. A., Van Someren, E. J. W., and Verwer, R. W. H. (1999). Decreased neuronal activity and therapeutic strategies directed towards reactivation in Alzheimer's disease. *In* "Alzheimer's Disease and Related Disorders" K. Iqbal, D. F. Swaab, B. Winblad, and H. M. Wisniewski, eds.), pp. 749–756. Wiley, New York.

Sze, C. I., Troncoso, J. C., Kawas, C., Mouton, P., Price, D. L., and Martin, L. J. (1997). Loss of the presynaptic vesicle protein synaptophysin in hippocampus correlates with cognitive decline in Alzheimer disease. *J. Neuropathol. Exp. Neurol*. **56**, 933–944.

Talmi, M., Carlier, E., and Soumireu-Mourat, B. (1993). Similar effects of aging and corticosterone treatment on mouse hippocampal function. *Neurobiol. Aging* **14**, 239–244.

Tanapat, P., Hastings, N. B., Reeves, A. J., and Gould, E. (1999). Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat. *J. Neurosci*. **19**, 5792–5801.

Teter, B., Xu, P. T., Gilbert, J. R., Roses, A. D., Galasko, D., and Cole, G. M. (1999). Human apolipoprotein E isoform-specific differences in neuronal sprouting in organotypic hippocampal culture. *J. Neurochem*. **73**, 2613–2616.

Tombaugh, G. C., Yang, S. H., Swanson, R. A., and Sapolsky, R. M. (1992). Glucocorticoids exacerbate hypoxic and hypoglycemic hippocampal injury in vitro: Biochemical correlates and a role for astrocytes. *J. Neurochem*. **59**, 137–146.

Touitou, Y., Sulon, J., Bogdan, A., Reinberg, A., Sodoyez, J. C., and Demey-Ponsart, E. (1983). Adrenocortical hormones, ageing and mental condition: Seasonal and circadian rhythms of plasma 18-hydroxy-11-deoxycorticosterone, total and free cortisol and urinary corticosteroids. *J. Endocrinol*. **96**, 53–64.

Uno, H., Tarara, R., Else, J., Suleman, M., and Sapolsky, R. M. (1989). Hippocampal damage associated with prolonged and fatal stress in primates. *J. Neurosci*. **9**, 1705–1711.

Uno, H., Eisele, S., Sakai, A., Shelton, S., Baker, E., DeJesus, O., and Holden, J. (1994). Neurotoxicity of glucocorticoids in the primate brain. *Horm. Behav.* **28**, 336–348.

Van Cauter, E., Leproult, R., and Kupfer, D. J. (1996). Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. *J. Clin. Endocrinol. Metab.* **81**, 2468–2473.

Van Eekelen, J. A., Rots, N. Y., Sutanto, W., Oitzl, M. S., and De Kloet, E. R. (1991). Brain corticosteroid receptor gene expression and neuroendocrine dynamics during aging. *J. Steroid Biochem. Mol. Biol*. **40**, 679–683.

Van Eekelen, J. A. M., Rots, N. Y., Sutanto, W., and De Kloet, E. R. (1992). The effect of aging on stress responsiveness and central corticosteroid receptors in the brown Norway rat. *Neurobiol. Aging* **13**, 159–170.

Van Kampen, M., and Fuchs, E. (1998). Age-related levels of urinary free cortisol in the tree shrew. *Neurobiol. Aging* **19**, 363–366.

Van Oers, H. J., De Kloet, E. R., Whelan, T., and Levine, S. (1998). Maternal deprivation effect on the infant's neural stress markers is reversed by tactile stimulation and feeding but not by suppressing corticosterone. *J. Neurosci*. **18**, 10171–10179.

Van Oers, H. J., De Kloet, E. R., and Levine, S. (1999). Persistent effects of maternal deprivation on HPA regulation can be reversed by feeding and stroking, but not by dexamethasone. *J. Neuroendocrinol*. **11**, 581–588.

Van Praag, H., Kempermann, G., and Gage, F. H. (1999). Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat. Neurosci*. **2**, 266–270.

Veinbergs, I., Jung, M. W., Young, S. J., Van Uden, E., Groves, P. M., and Masliah, E. (1998). Altered long-term potentiation in the hippocampus of apolipoprotein E-deficient mice. *Neurosci. Lett*. **249**, 71–74.

Veinbergs, I., Mallory, M., Mante, M., Rockenstein, E., Gilbert, J. R., and Masliah, E. (1999). Differential neurotrophic effects of apolipoprotein E in aged transgenic mice. *Neurosci. Lett*. **265**, 218–222.

Vekhratsky, A., and Toescu, E. C. (1998). Calcium and neuronal ageing. *Trends Neurosci*. **21**, 2–7.

Virgin, C. E., Jr., Ha, T. P., Packan, D. R., Tombaugh, G. C., Yang, S. H., Horner, H. C., and Sapolsky, R. M. (1991). Glucocorticoids inhibit glucose transport and glutamate uptake in hippocampal astrocytes: Implications for glucocorticoid neurotoxicity. *J. Neurochem.* **57**, 1422–1428.

Vollmann-Honsdorf, G. K., Flügge, G., and Fuchs, E. (1997). Chronic psychosocial stress does not affect the number of pyramidal neurons in tree shrew hippocampus. *Neurosci. Lett*. **233**, 121–124.

Vreugdenhil, E., and De Kloet, E. R. (1998). Corticosteroid hormones and neuronal vulnerability: Towards identification of candidate vulnerability genes. *Prog. Brain Res*. **117**, 9–22.

Ward, M. T., Oler, J. A., and Markus, E. J. (1999). Hippocampal dysfunction during aging I: Deficits in memory consolidation. *Neurobiol. Aging* **20**, 363–372.

West, M. (1993). Regionally specific loss of neurons in the aging human hippocampus *Neurobiol. Aging* **14**, 287–293.

West, M. J. (1999). Stereological methods for estimating the total numbers of neurons and synapses; issues of precision and bias. *Trends Neurosci*. **22**, 51–61.

White-Gbadebo, D., and Hamm, R. J. (1993). Chronic corticosterone treatment potentiates deficits following traumatic brain injury in rats: Implications for aging. *J. Neurotrauma* **10**, 297–306.

Wilkinson, C. W., Peskind, E. R., and Raskind, M. A. (1997). Decreased hypothalamic-pituitary-adrenal axis sensitivity to cortisol feedback inhibition in human aging. *Neuroendocrinology* **65**, 79–79.

Winocur, G., and Gagnon, S. (1998). Glucose treatment attenuates spatial learning and memory deficits of aged rats on tests of hippocampal function. *Neurobiol. Aging* **19**, 233–241.

Wolf, O. T., and Kirschbaum, C. (1999). Actions of dehydroepiandrosterone and its sulfate in the central nervous system: Effects on cognition and emotion in animals and humans. *Brain Res. Rev.* **30**, 264–288.

Wolf, O. T., Kudielka, B. M., Hellhammer, D. H., Hellhammer, J., and Kirschbaum, C. (1998). Opposing effects of DHEA replacement in elderly subjects on declarative memory and attention after exposure to a laboratory stressor. *Psychoneuroendocrinology* **23**, 617–629.

Woods, A. G., Guthrie, K. M., Kurlawalla, M. A., and Gall, C. M. (1998). Deafferentiation-induced increases in hippocampal insulin-like growth factor-1 messenger RNA expression are severely attenuated in middle aged and aged rats. *Neuroscience* **83**, 663–668.

Workel, J. O., Oitzl, M. S., Ledeboer, A., and De Kloet, E. R. (1997). The Brown Norway rat displays enhanced stress-induced ACTH reactivity at day 18 after 24-h maternal deprivation at day 3. *Dev. Brain Res*. **103**, 199–203.

Workel, J. O., Oitzl, M. S., Fluttert, M., Lesscher, H., and De Kloet, E. R. (2000). Persistent effects of maternal deprivation during life. Submitted for publication.

Yau, J. L., Olsson, T., Morris, R. G., Meaney, M. J., and Seckl, J. R. (1995). Glucocorticoids, hippocampal corticosteroid receptor gene expression and antidepressant treatment: Relationship with spatial learning in young and aged rats. *Neuroscience* **66**, 571–581.

Yau, J. L., Kelly, P. A., Olsson, T., Noble, J., and Seckl, J. R. (1999). Chronic amitriptyline administration increases serotonin transporter binding sites in the hippocampus of aged rats. *Neurosci. Lett*. **261**, 183–185.

Zhou, Y., Elkins, P. D., Howell, L. A., Ryan, D. H., and Harris, R. B. (1998). Apolipoprotein-E deficiency results in an altered stress responsiveness in addition to an impaired spatial memory in young mice. *Brain Res*. **788**, 151–159.

Zimmerman, M., and Coryell, W. (1987). The dexamethasone suppression test in healthy controls. *Psychoneuroendocrinology* **12**, 245–251.

Zoli, M., Ferraguti, F., Gustafsson, J. A., Toffano, G., Fuxe, K., and Agnati, L. F. (1991). Selective reduction of glucocorticoid receptor immunoreactivity in the hippocampal formation and central amygdaloid nucleus of the aged rat. *Brain Res*. **545**, 199–207.

Zoli, M., Picciotto, M. R., Ferrari, R., Cocchi, D., and Changeux, J. P. (1999). Increased neurodegeneration during ageing in mice lacking high-affinity nicotine receptors. *EMBO J*. **18**, 1235–1244.

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Phillip L. Thornton and William E. Sonntag

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Growth Hormone, Insulin-like Growth Factor-1, and the Aging Brain

I. Introduction

It is widely accepted that biological aging is a consequence of functional and morphological changes in tissues that result in an increased susceptibility to disease. Although the etiology of these age-related changes continues to be rigorously debated, recent studies have unequivocally established that a decline in several anabolic hormones occurs in aged animals and that hormone replacement therapy is capable of increasing tissue function and, in some cases, preventing the age-related decline in tissue function. These results have led to the conclusion that at least part of the aging phenotype results from hormone deficiency, especially deficiencies in anabolic hormones that regulate tissue growth, maintenance, and repair. To date, two hormones that are capable of reversing the catabolic processes associated with age include growth hormone and insulin-like growth factor-1 (IGF-1). Although the actions of these hormones on peripheral tissues of aging animals are well-established, new data suggest that these hormones may have a critical role in aging of the brain and the cerebrovasculature. In this chapter, age-related changes in the somatotropin pathway is reviewed and the potential contributions of deficiencies in growth hormone and insulin-like growth factor-1 are discussed in relation to age-related changes in the brain and brain vasculature.

II. Overview

A. History

In the early 1900s, a substance was found in blood that promoted body growth in young animals and was later demonstrated to be a secretory product of the pituitary gland (Evans and Long, 1921). However, it was not until 1944 that bovine growth hormone was isolated (Li and Evans, 1944) and subsequently human growth hormone was isolated in 1956 (Li and Papkoff, 1956). Data established that this hormone stimulated amino acid uptake into tissue, increased DNA, RNA, and protein synthesis, and had an important role in cell division and tissue hypertrophy. An assay for measuring plasma concentrations of growth hormone was developed by Utiger *et al*. (1962) and the amino acid sequence was determined in 1969 (Li *et al*., 1969; Niall, 1971).

As early as 1957, Salmon and Daughaday suggested that the growth promoting actions of growth hormone were mediated by a serum factor initially called sulfation factor. Data demonstrated that this factor was regulated by growth hormone and promoted the incorporation of sulfate into cartilage. These data subsequently led to the identification of the somatomedin family of hormones (Van Wyk *et al*., 1974), which are low-molecular-weight peptides ($\sim$7.5 kDa) that circulate in plasma at high concentrations. Because of their structural similarity to proinsulin, they are also termed the insulin-like growth factors (IGF-1 and IGF-II) (Rinderknecht and Humbel, 1978). IGF-1 binds with high affinity to the type 1 IGF receptor, which is widely distributed in tissues throughout the body and mediates the trophic actions of IGF-1. Until recently, it had been widely accepted that IGF-1 was produced only in the liver (Beach and Kostyo, 1968; Goldspink and Goldberg, 1975) and mediated all of the effects of growth hormone. However, it is now understood that IGF-1 is also produced in virtually every tissue and has important local autocrine and paracrine actions (Daughaday and Rotwein, 1989). These studies have led to the concept that growth hormone increases plasma concentrations of IGF-1 and regulates the secretion of IGF-1 at the tissue level, thereby modulating the paracrine and autocrine activities of IGF-1.

IGF-1 circulates in the blood either free ($t_{½}$ of 15–20 min) or bound to specific binding proteins. At present, six binding proteins have been identified (Binoux *et al*., 1986) and these proteins constitute a complicated transport system for the IGFs that serve to facilitate or inhibit the actions of IGF-1 at the target tissues (Kelley *et al*., 1996). Recent studies have also identified proteases that degrade IGF binding proteins, thereby modifying the availability of IGF-1 for its receptor (Lee *et al*., 1993). Thus, although growth hormone is a primary stimulus for cellular anabolic processes through its actions on IGF-1 secretion, a complex hierarchy of factors exists to

regulate the interaction of IGF-1 with its receptor (see reviews by Jones and Clemmons, 1995; Clemmons, 1997).

B. Neuroendocrine Regulation of Growth Hormone and Insulin-like Growth Factor-1

Studies revealed that secretion of growth hormone from the anterior pituitary occurs in discrete pulses throughout the day with the highest levels occurring approximately 2 hr after onset of sleep (Finkelstein *et al*., 1972). Although the precise function of this ultradian pattern remains unknown, the pulsatile release of growth hormone has been confirmed in every species studied to date and is necessary for full biological activity of the hormone. In humans, growth hormone is released in relatively low-amplitude pulses throughout the day and in a large pulse at night that occurs in close association with slow-wave sleep (Quabbe *et al*., 1966; Takahashi *et al*., 1968; Goldsmith and Glick, 1970). Conversely, the growth hormone pulsatile pattern in rodents occurs every 3.5 hr in males (Tannenbaum and Martin, 1976) and hourly in females (Terry *et al*., 1977). It was later discovered that the pulsatile nature of growth hormone release is regulated by two hypothalamic hormones: (1) growth hormone-releasing hormone (GHRH) which stimulates the release of growth hormone (Rivier *et al*., 1982; Ling *et al*., 1984), and (2) somatostatin, which inhibits release (Brazeau *et al*., 1973). The dynamic interaction of these two hypothalamic hormones is responsible for the high-amplitude, pulsatile release of growth hormone. The results of several studies suggest that both hypothalamic hormones are secreted in a phasic manner with release of GHRH and suppression of somatostatin resulting in an increase in pulse amplitude of growth hormone while suppression of GHRH and release of somatostatin contributes to the reduced growth hormone levels observed during trough periods (Tannenbaum and Ling, 1984). Growth hormone circulates in blood and binds to receptors in hepatic tissue, thereby increasing plasma levels of IGF-1. Growth hormone inhibits further growth hormone release in a typical feedback mechanism at the level of the hypothalamus and pituitary gland (Berelowitz *et al*., 1981). For instance, growth hormone administration inhibits the subsequent release of growth hormone in response to GHRH (Nakamoto *et al*., 1986) and stimulates the release of somatostatin (Wehrenberg *et al*., 1992). Similarly, IGF-1 has also been shown to feed back at both the hypothalamus and the pituitary and inhibits release of growth hormone by attenuating the growth hormone response to GHRH and by stimulating hypothalamic somatostatin release (Berelowitz *et al*., 1981; Ceda *et al*., 1987; Aguila *et al*., 1993; Bermann *et al*., 1994; Korbonits *et al*., 1996).

C. Biological Actions of Growth Hormone and Insulin-like Growth Factor-1

It was established in 1912 that growth hormone promotes longitudinal growth (Aschner, 1912) but the levels of growth hormone after the pubertal growth period suggested that this hormone had important regulatory actions in addition to body growth. It is now well-established that growth hormone has significant effects on protein, carbohydrate, and fat metabolism, and regulates body composition, cardiac function, and renal hemodynamics, both directly and indirectly through IGF-1 in an endocrine and/or paracrine manner. In addition, recent studies have demonstrated that both growth hormone and IGF-1 have significant actions in the central nervous system and may be essential to maintain neuronal function. These actions suggest that adequate levels of growth hormone and IGF-1 are critical in many tissues throughout life to maintain normal homeostasis.

In bone, growth hormone and IGF-1 have been shown to work in concert. In children, growth hormone induces differentiation of precursor cells into chondrocytes at the epiphyseal growth plate (Clark *et al*., 1985). Growth hormone also stimulates local production of and increases tissue responsiveness to IGF-1 (Isgaard *et al*., 1988). In turn, IGF-1 stimulates clonal expression of chondrocytes which are necessary for longitudinal bone growth. Growth hormone promotes retention of minerals, such as sodium, potassium, and phosphorus and produces an increased excretion of calcium as well as increased calcium absorption from the intestine (Burstein *et al*., 1983; Marcus *et al*., 1990; Binnerts *et al*., 1992; Antoniazzi *et al*., 1993; Bengtsson *et al*., 1993). More importantly, both growth hormone and IGF-1 have a direct action on osteoblasts (Langdahl *et al*., 1998). Growth hormone stimulates osteoblast formation and differentiation and IGF-1 supports osteoblast activity by increasing osteocalcin production (Kassem *et al*., 1994; Brixen *et al*., 1995). Together, these actions result in increased bone growth in children and maintenance of bone density in adults.

Growth hormone and IGF-1 are also essential for increasing amino acid uptake and protein synthesis at the cellular level. More specifically, early studies were able to show that growth hormone can stimulate amino acid transfer from the extracellular to the intracellular compartment (Noall *et al*., 1957; Riggs and Walker, 1960; Staehelin, 1962). The diminished total amino acid content of cartilage observed in hypophysectomized rats is replenished with growth hormone administration. Growth hormone also stimulates [^{3}H]leucine incorporation into muscle (Kostyo, 1968; Nutting, 1976; Nutting and Coats, 1977) and there is evidence that this effect of growth hormone may be secondary to an increase in tissue IGF-1. In addition, growth hormone has been reported to increase protein synthesis in the tibia (J. A. Martinez *et al*., 1991; D. A. Martinez *et al*., 1996), collagen content in rat skin (Jorgensen *et al*., 1989), rate of wound healing (Barbul *et al*., 1983; Jorgensen *et al*., 1989), and synthesis of erythropoietin (Merchav *et al*., 1988; Christ *et al*., 1997; Jorgensen *et al*., 1997).

Data also indicate that growth hormone has an important role in the regulation of adipose tissue. Studies indicate that growth hormone reduces adipose mass in several species (Bassett and Wallace, 1966; Lee *et al*., 1975) and similar results have been reported in older men (Rudman *et al*., 1991; Jorgensen *et al*., 1994; Thompson *et al*., 1995). It has also been demonstrated that growth hormone retards lipogenesis by decreasing enzyme activity. For instance, chronic administration of growth hormone to hypophysectomized rats results in inhibition of glyceride synthesis and decreased body fat composition (Goodman, 1963). In addition, acute growth hormone treatment decreases fatty acid synthesis in liver and facilitates mobilization of fatty acids from adipose tissue.

It is becoming increasingly clear that growth hormone and IGF-1 are important in maintaining several systems in the

body. For instance, data demonstrate that growth hormone deficiency results in impairments in immune function (Gelato, 1996; Geffner, 1997), cardiac dysfunction (Lombardi *et al*., 1997a,b), and reproductive dysfunction (Homburg and Farhi, 1995). These studies also demonstrate that administration of growth hormone reverses these deficits and thus suggest that normal somatotropin activity is necessary for adequate function of these systems throughout life.

One of the more recent areas of research on the biological actions of growth hormone and IGF-1 is their action within the CNS. Growth hormone receptors and its mRNA have been identified in the brains of rats (Zhai *et al*., 1994), rabbits (Lobie *et al*., 1993), and humans (Sara *et al*., 1982; Lai *et al*., 1993). Although expression is ubiquitous throughout the brain, the highest levels of expression are found in the frontal lobe of the cortex, cerebellum, hippocampus, amygdala, hypothalamus, choroid plexus, thalamus, and glial cells in the cingulum. As expected, it was also demonstrated that the highest level of binding in the brain is in the pituitary and hypothalamus and is important for the negative feedback regulation of its secretion. Despite the presence of growth hormone receptors, localization of growth hormone in the brain is controversial (Hojvat *et al*., 1982; Gossard *et al*., 1987). Additionally, it has not been determined whether growth hormone has direct actions in the brain. Nonetheless, patients receiving chronic, subcutaneous injections of growth hormone report improvements in mood, alertness, vitality and in the comprehensive psychological rating scale (McGauley 1989; McGauley *et al*., 1990; Bengtsson *et al*., 1993; Nyberg and Burman, 1996). Reports have also demonstrated that growth hormone administered subcutaneously has an effect on the turnover of dopamine in the brain via actions on the N-methyl-D-aspartate receptor system (Burman *et al*., 1993), increases the levels of the excitatory amino acid aspartate (Nyberg and Burman, 1996), increases cerebrospinal fluid concentrations of β-endorphin (Johansson *et al*., 1995), and increases cerebral vasculature density (Sonntag *et al*., 1997). Nevertheless, it is currently unknown if the actions of growth hormone on the brain are mediated by increasing peripheral or vascular-derived IGF-1 or whether growth hormone crosses the blood–brain barrier and has direct actions on neurons and/or glia. In this regard, growth hormone administration has been shown to increase the gene expression of IGF-1 in the brain (Lopez-Fernandez *et al*., 1996) and increases the cerebrospinal fluid concentration of IGF-1 (Johansson *et al*., 1995) suggesting that peripherally administered growth hormone may exert an influence on neuronal function by increasing IGF-1 levels. Since the cerebral vasculature has been shown to be an important source for some neurotrophic factors, including nerve growth factor and IGF-1, and these factors have the potential to have important trophic effects on surrounding neuronal tissues (Delafontaine, 1995; Sonntag *et al*., 1997), one potentially interesting hypothesis is that growth hormone acts by regulating vascular-derived growth factors, including IGF-1.

Both IGF-1 mRNA and protein are expressed in the developing and mature brain (Carlsson-Skwirut *et al*., 1986; Rotwein *et al*., 1988) and the type 1 IGF receptor is widely distributed throughout the brain (Sara *et al*., 1982). It is well known that IGF-1 is involved in several aspects of brain function including neuronal growth and differentiation during development and adulthood and brain metabolism. Data strongly suggest that IGF-1 also has an important role as a neurotrophic factor and/or neuromodulator in the CNS. For example, IGF-1 has been shown to support the differentiation of cortical neurons, proliferation of oligodendrocytes (McMorris and Dubois-Dalcq, 1988; Saneto *et al*., 1988; van der Pal *et al*., 1988), synaptogenesis (Ishii, 1989), and neuronal repair (Hansson *et al*., 1986; Sjöberg and Kanje, 1989; Nachemson *et al*., 1990). Electrophysiological and biochemical studies have shown that IGF-1 influences acetylcholine synthesis and release (Sara and Hall, 1990), glutamate activity (Castro-Alamancos and Torres-Aleman, 1993), dopamine release (Beck *et al*., 1993; Beck, 1994), dopamine D_2 receptor activity (Thornton *et al*., 1998), and NMDA receptors by regulating the subunit composition of NMDA receptors (Sonntag *et al*., 2000). Recent data have also demonstrated that the actions of neurotrophic agents (such as nerve growth factor, epidermal growth factor, and brain-derived neurotrophic factor) may be mediated, at least in part, by IGF-1 (Torres-Aleman *et al*., 1990; Han *et al*., 1992; Beck *et al*., 1993). These studies demonstrate that IGF-1 has an important role as a neurotrophic agent and may be essential in maintaining neuronal function throughout life.

III. Growth Hormone, Insulin-like Growth Factor-1, and Aging

A. Age-Related Impairments in the Somatotropin Pathway

1. Growth Hormone Pulse Amplitude

Many researchers have studied the effect of aging on growth hormone secretory dynamics. Initial studies in elderly humans found a decline in the release of growth hormone in response to several stimuli, including insulin-induced hypoglycemia and arginine administration (Laron *et al*., 1970). Subsequent studies suggested that the nocturnal surges of growth hormone are diminished in aged humans (Carlson *et al*., 1972; Finkelstein *et al*., 1972). Although several researchers initially found no change in plasma growth hormone levels in aged humans (Dudl *et al*., 1973; Elahi *et al*., 1982), further investigation revealed that serial sampling of blood is essential to assessing growth hormone secretory dynamics. Subsequent analysis of plasma growth hormone levels by obtaining plasma samples at brief intervals indicated that the pulsatile release of growth hormone is at its zenith during late puberty and gradually declines with age (Martha and Reiter, 1991; Martha *et al*., 1992; Rudman, 1985; Vermeulen, 1987). Iranmanesh *et al*. (1991) revealed through analysis of healthy, nonobese men between the ages of 21 and 71 that the synthesis rate of growth hormone declines by 14% and the half-life of growth hormone declines by 6% with each successive decade of life after 20 years of age. Furthermore, both the amplitude and number of growth hormone secretory bursts have been demonstrated to decrease in adults over 50 years of age (Carlson *et al*., 1972). Data from animal studies are consistent with the human data. For example, a significant decline in the pulse amplitude of growth hormone is found in aged male rats (Sonntag *et al*.,

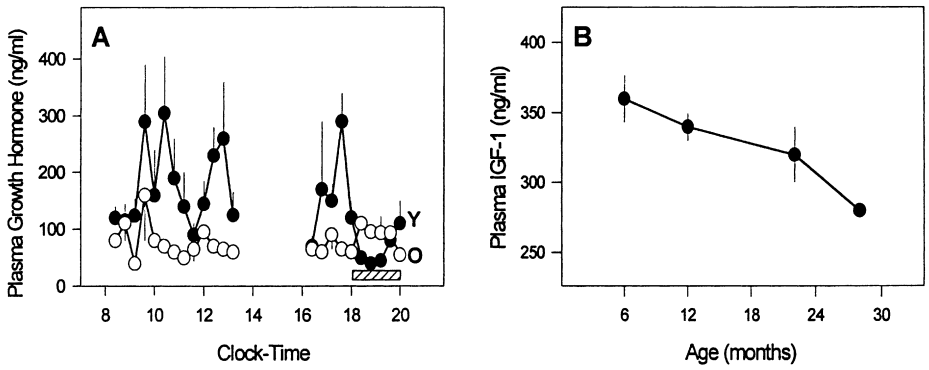

FIG. 63.1. (A) Mean plasma growth hormone concentration in young (Y) and old (O) male rats. Each point represents the mean for 2–15 animals per time point and data are presented as mean ± SEM. The dark phase of the light:dark cycle begins at 18:00 hr and is indicated by the horizontal bar. Adapted from Sonntag *et al.*, 1980, with permission. (B) Age-related decrease in plasma IGF-1.

1980) (Fig. 63.1A) and a similar reduction is observed in aged female rats (Takahashi and Meites, 1987). This age-related phenomenon has been observed in every species studied to date with a decline of approximately 50% in growth hormone amplitude release occurring in aged animals when compared to levels observed in young animals.

The mechanism responsible for the age-related decline in growth hormone secretion potentially results from structural and/or functional alterations within the hypothalamic–hypophyseal axis. Since it is not technically feasible to measure the phasic release of GHRH or somatostatin in humans, the majority of studies investigating the decline in growth hormone release were completed in animal models. Studies had previously demonstrated that passive immunization with GHRH antiserum results in a complete inhibition of the pulsatile secretion of growth hormone (Wehrenberg *et al.*, 1982), whereas passive immunization with somatostatin antiserum results in an increase in the pulse amplitude of growth hormone in young animals (Arimura *et al.*, 1976), demonstrating the necessity of both hypothalamic hormones for normal growth hormone pulses. Furthermore, infusion of somatostatin antisera to old animals increased growth hormone to levels observed in young animals (Sonntag *et al.*, 1981). To date, numerous studies have supported the hypothesis that somatostatin secretion increases with age and contributes to the age-related decline in growth hormone pulse amplitude. For instance, a decline in the number of somatostatin receptors in pituitaries of aged animals has been reported and is consistent with an increased release of somatostatin (Spik and Sonntag, 1989). Researchers have also determined that an increase in total somatostatin content in the pituitary is found with age and an increased ratio of somatostatin-28 to somatostatin-14 is found in aged animals (Sonntag *et al.*, 1986). As somatostatin-28 has been shown to be more biologically potent than somatostatin-14, it was concluded that there may be age-related alterations in posttranslational processing of somatostatin that result in an increased somatostatinergic tone in the aged animal and contribute to the age-related decline in growth hormone pulse amplitude. Although many studies have identified age-related increases in somatostatin levels and activity with age, other studies also suggest that a deficiency in GHRH secretion may be equally important (Morimoto *et al.*, 1988). Studies indicated that the acute response to GHRH was reduced in aged rats (Sonntag *et al.*, 1983), dogs (Cella *et al.*, 1993) and humans (Shibasaki *et al.*, 1984), but more recent studies suggest that chronic GHRH administration restores this response (Corpas *et al.*, 1992b). Further studies revealed that a modest decline in sensitivity to GHRH occurs with age due to a decline in GHRH secretion (DeGennaro *et al.*, 1989; Abribat *et al.*, 1991; Deslauriers *et al.*, 1991). Thus, these data suggest that impairments in the regulation of and response to GHRH secretion as well as an increased level of somatostatin appear to contribute to the age-related decline in growth hormone secretory dynamics.

Several other factors regulate the release of growth hormone and many are also known to decline with age. It has been established that plasma testosterone and estradiol modulate the release of growth hormone (Martha and Reiter, 1991). Plasma estradiol levels are highly correlated with growth hormone and IGF-1 levels (Dawson-Hughes *et al.*, 1986; Ho *et al.*, 1987) and growth hormone secretion declines more rapidly in females after ovariectomy or menopause (Ho and Weissberger, 1990; De Leo *et al.*, 1993). Furthermore, administration of estradiol in postmenopausal or ovariectomized women results in an increased response to GHRH and an elevated growth hormone secretion (Friend *et al.*, 1996; Ho *et al.*, 1996). Similarly, androgen treatment increases the secretion of growth hormone in men with hypogonadrotropic hypogonadism (Martin *et al.*, 1968) and in boys with delayed puberty (Illig and Prader, 1970). Another factor that influences the release of growth hormone is obesity. While it has long been known that growth hormone decreases adiposity, only recently has an increased adiposity been demonstrated to reduce the pulsatile release of growth hormone and shorten the half-life of growth hormone (Iranmanesh *et al.*, 1991; Veldhuis *et al.*, 1991). In addition, the growth hormone secretory response to a GHRH stimulus is diminished in both men and women that exhibit increased adiposity (Kelijman and Frohman, 1988). Since aging is associated with a decline in estradiol and testosterone and an increase in adiposity, it is possible that these factors augment the decline in growth hormone secretion and contribute to the overall deficits in the somatotropin pathway with age.

2. Tissue Resistance to Growth Hormone

In addition to the age-related decline in growth hormone pulse amplitude, recent data also suggest that the response of tissues to growth hormone is diminished in the aged animals. Although a twofold increase in growth hormone receptor density has been reported in hepatic tissue with age (Takahashi and Meites, 1987), further investigation demonstrated that the activity of the growth hormone receptor, as measured by IGF-1 gene expression, declined by 40–50% with age (Xu *et al*., 1995) but no age-related changes in the affinity of the receptor were noted. Subsequent studies in our laboratory demonstrated that the decline in growth hormone receptor activity results from deficits in the signal transduction pathway of the receptor. Xu *et al*. (1995) reported that both growth hormone-induced JAK2 and growth hormone receptor phosphorylation were reduced in aged rodents in response to stimulation with growth hormone and that these impairments contribute to the suppression of MAP kinase activity and IGF-1 gene transcription and release. More recent data demonstrated that the growth hormone-induced phosphorylation and nuclear translocation of STAT3 were diminished in the aged animal (Xu and Sonntag, 1996). These impairments in the signal transduction of growth hormone are believed to result in a diminished tissue responsiveness to the hormone and contribute to the overall somatotropin deficiency.

3. Plasma and Brain Insulin-like Growth Factor-1

Previous studies indicate that plasma IGF-1 declines with age in humans and animals (Florini and Roberts, 1980; Florini *et al*., 1981; Rudman *et al*., 1981, 1990; Sonntag *et al*., 1992) (Fig. 63.1B). The primary source of plasma IGF-1 is the liver and the age-related decline in plasma IGF-1 is the direct result of a decline in hepatic IGF-1 gene expression (Breese *et al*., 1991). Since growth hormone is the major regulator of IGF-1 gene expression and secretion in the liver, the decline in growth hormone secretion and impairments in its signal transduction pathway are considered to be important, if not primary, factors contributing to the decline in hepatic IGF-1 gene expression and subsequently the reduction in plasma IGF-1 (Johanson and Blizzard, 1981; Rudman *et al*., 1990). This latter conclusion is supported by recent studies demonstrating that IGF-1 secretion in response to a growth hormone stimulus is diminished in elderly humans and animals (Lieberman *et al*., 1994; Xu *et al*., 1995). In addition to the age-related decline in plasma IGF-1, analysis of IGF binding proteins have also revealed age-related alterations. IGF binding protein-3, the major carrier of IGF-1 in plasma, has been shown to decline between 18 and 65 years of age (Baxter and Martin, 1986; Donahue *et al*., 1990; Corpas *et al*., 1992a). Frost *et al*. (1996) reported that IGF binding protein-1 increased in older individuals. Moreover, these investigators also found that IGF binding protein-1 was hyperphosphorylated, an event that significantly increases the binding affinity for IGF-1, thereby diminishing the bioavailability of IGF-1 in the elderly. Although the direct significance for tissue IGF-1 activity is unclear, serum IGF binding protein-4 was shown to increase, whereas serum IGF binding protein-5 declines with age (Mohan *et al*., 1995). These data suggest that not only does a deficit in plasma IGF-1 occur with age, but the alterations in levels of IGF binding ptoteins may reduce the bioavailability and/or $t_{1/2}$ of IGF-1.

TABLE 63.1 Insulin-like Growth Factor-1 Protein Expression in Cortex from Young (11 Months), Middle-Age (24 Months), and Old (32 Months) Male Fisher 344 × Brown Norway Rats

Age (months)	ng IGF/g tissue	% Change from young
11	37.8 ± 4.0	—
24	36.5 ± 4.9	3.4
32	$24.0 \pm 3.6^{*}$	36.5

$^{*}P < 0.05$ compared to 11- and 24-month-old animals.

Although deficiencies in the peripheral IGF-1 axis are well-known, only recently have the deficiencies in IGF-1 been studied in the brain, despite the fact that many events associated with brain aging appear to be consistent with deficits in IGF-1. Initial studies demonstrated that IGF-1 gene expression either decreases or remains unchanged in the aged animal (Lopez-Fernandez *et al*., 1996; Niblock *et al*., 1996). Nevertheless, recent studies report that IGF-1 protein expression in the cerebral cortex declines by 36.5% in the aged (32-month) rat compared to a young, adult (11-month) rat (Sonntag *et al*., 1999) (Table 63.1). Furthermore, the mRNA for the type 1 IGF receptor remains unchanged with age while the density of type 1 IGF receptors in cortical layers II/III and V/VI of rats, analyzed by [^{125}I]IGF-1 binding, increased 19.8 and 16%, respectively, between 10 and 19 months but declined by 20.7 and 27.3%, respectively, between 19 and 29 months of age. A similar age-related decrease was observed in the hippocampus (Fig. 63.2, see color insert). To date, analysis of IGF binding proteins in the brain has not been undertaken. Since age-related alterations in plasma IGF binding proteins have been found, it is likely that changes in brain expression of IGF binding proteins also occur and may have a critical role in regulating the activity of IGF-1 in the aging brain. However, it is clear that deficits in the IGF-1 axis occur in the aged brain suggesting that these deficits have the potential to contribute to brain aging.

B. Replacement of Growth Hormone and Insulin-like Growth Factor-1

Because aging has similar clinical and biochemical features to growth hormone-deficient humans and animals, it has been hypothesized that the age-related decline in growth hormone pulse amplitude and plasma IGF-1 contribute to the phenotype of aging (Marcus *et al*., 1990; Christiansen and Jorgensen, 1991; Jorgensen, 1991). The initial studies on growth hormone replacement in the aged were done in rodents and revealed that growth hormone restores tissue protein synthesis in old animals to levels observed in young animals (Sonntag *et al*., 1985) and increases plasma IGF-1 levels (Johanson and Blizzard, 1981). Replacement of growth hormone in elderly humans indicated important alterations in body composition (Rudman, 1985), including improved nitrogen balance (Marcus *et al*., 1990), increased lean body mass, increased skin thickness, increased density of lumbar vertebrae, decreased fat mass, and restored IGF-1 levels (Rudman *et al*., 1990).

Other reports demonstrated that growth hormone or IGF-1 replacement increases the expression of aortic elastin (Foster *et al*., 1990), partially restores the decline in immune function (Kelley *et al*., 1986), and increases the life span of rodents (Khansari and Gustad, 1991). In aged rodents, growth hormone and/or IGF-1 administration has been shown to reverse the age-related decline in thymic function and improve immune responses of both B and T lymphocytes (Kelley *et al*., 1988; Kelley, 1990), thereby improving immune function. To date, there are no reports of the effect of growth hormone administration on immune function in older men and women. However, there are some reports that growth hormone administration results in elevations in osteocalcin, urinary hydroxyproline, and nitrogen balance, suggesting that growth hormone may delay or reverse osteoporosis (Marcus *et al*., 1990; Kaiser *et al*., 1991).

Administration of GHRH has been shown to be an alternative method of increasing endogenous growth hormone secretion. In growth hormone-deficient children, administration of GHRH results in accelerated growth (Gelato *et al*., 1985a,b; Martha *et al*., 1988) and the diurnal profile of growth hormone secretion remains normal during long-term GHRH therapy (Martha *et al*., 1988; Vance *et al*., 1989). Furthermore, treatment with GHRH for 8–14 days in older humans was found to reverse the age-related reductions in plasma levels of growth hormone and IGF-1 and maintain normal diurnal pulsatile release of growth hormone (Corpas *et al*., 1992b). More recent studies of GHRH administration to adults suggest that nightly GHRH injections activate the growth hormone axis and improve deficits in lean body mass, insulin sensitivity, skin thickness, and psychological well-being (McGauley *et al*., 1990; Khorram *et al*., 1997).

Studies on replacement of growth hormone and/or IGF-1, correlated with the actions of these hormones, provide important indications that the decline in growth hormone secretory dynamics and plasma IGF-1 in aged animals and man has clinical significance. The deficiencies in the somatotropin pathway may be responsible, at least in part, for the generalized catabolic state that accompanies normal aging. However, only recently have growth hormone and IGF-1 been studied in the aging brain despite data that suggest that these hormones have important actions in the brain and that deficiencies associated with brain aging are consistent with a deficiency in growth hormone and/or IGF-1 (Fig. 63.3, see color insert).

IV. Memory and Age

A. Age-Related Impairments

A decline in cognitive function has been well-documented in aged animals and man. For example, nonhuman primates exhibit impairments in tasks that measure memory including delayed response and delayed non-match-to-sample tasks (Bartus *et al*., 1978; Arnsten and Goldman-Rakic, 1985; Presty *et al*., 1987; Moss *et al*., 1988; Rapp and Amaral, 1989). Age-related deficits in rodent are also observed in tasks sensitive to spatial reference memory such as the Morris water maze and the Barnes maze (Barnes 1979; Ingram *et al*., 1981; Gage *et al*., 1984; Rapp *et al*., 1987; Gallagher and Burwell, 1989) as well as tasks that emphasize spatial working memory such as the radial arm maze and alternation tasks (Barnes *et al*., 1980; Zornetzer *et al*., 1982). Furthermore, many studies indicate that elderly humans also exhibit memory deficits (Light, 1991). For instance, compared to 20-year-old adults, elderly humans exhibit impairments in free recall, cued recall, and recognition memory (Craik, 1977; Burke and Light, 1981; Poon, 1985; Guttentag and Madden, 1987; Guttentag and Hunt, 1988; Howe, 1988; Hultsch *et al*., 1990). Older adults also show deficits in other memory tasks that represent activities of daily living (West and Crook, 1990; West *et al*., 1992), including recall of information from medicine labels (Morrell *et al*., 1989), recall of topographic information near their homes (Rabbitt, 1989), appearance of common objects (Foos, 1989), activities of daily living (Kausler and Lichty, 1988), and names and faces of acquaintances (Bahrick, 1984; Maylor, 1990). While much research has been undertaken to understand the mechanisms of learning and memory and the etiology of the cognitive decline with age, the specific mechanisms for these impairments remain unknown.

Many researchers have evaluated the role of the neurotrophin family of growth factors, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin (NT) 3 and 4/5 in brain aging. Intracerebroventricular infusion of NGF has been reported to improve spatial memory in aged rats (Fischer *et al*., 1987; Fischer, 1994; Markowska *et al*., 1994, 1996; Chen *et al*., 1995). NT-3 and -4/5 infused intracerebroventricular has also been shown to improve spatial memory in rodents, but BDNF was without effect (Fischer *et al*., 1994). While it is apparent that this family of neurotrophins is important in the maintenance and survival of neurons and improves indices of behavioral performance in older animals, the clear demonstration of an age-related decline in these trophic agents is controversial. For instance, some researchers have determined that NGF mRNA levels remain constant (Alberch *et al*., 1991; Crutcher and Weingartner, 1991), increase (Hellweg *et al*., 1990; Katoh-Semba *et al*., 1991; Hasenohrl *et al*., 1997), or decrease (Larkfors *et al*., 1987) with age. Moreover, BDNF mRNA increases with age in the cortex and hippocampus while the protein levels of BDNF, NT-3, and NGF remain constant in the cortex and hippocampus when adult and aged rats are compared (Narisawa-Saito and Nawa, 1996). Together, these studies indicate that members of the neurotrophin family are capable of ameliorating age-related memory impairments but the lack of an age-related decline in these trophic factors suggest that either other aspects of growth factor function (e.g., signal transduction) are impaired with age or that these factors may not have a significant role in normal physiological and functional deficits observed in the aging brain.

B. Effects of [D-Ala^2] Growth Hormone Releasing Hormone

[D-Ala^2]GHRH has been shown to increase growth hormone pulse amplitude and plasma IGF-1 in growth hormone-deficient children and adults (Martha *et al*., 1988; Vance *et al*., 1989; Corpas *et al*., 1992b) and activate the growth hormone axis (Khorram *et al*., 1997; McGauley *et al*., 1990). Since IGF-1 has been shown to exert potent trophic effects in the

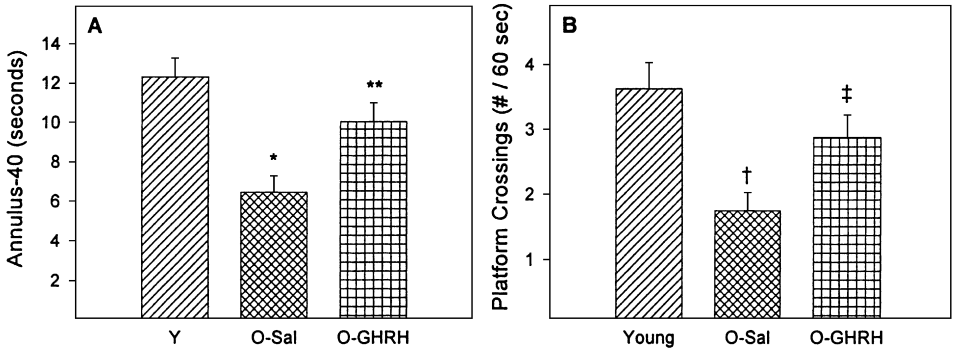

FIG. 63.4. Spatial reference memory as assessed by performance in the Morris water maze. In this task, the Annulus-40 (the time spent in a 40 cm annulus around the platform) and the Platform Crossings (number of times the animal crossed the platform) are sensitive measures of spatial reference memory. (A) An age-related decline in Annulus-40 time was observed between 9-month-old (Y) and 28-month-old (O-Sal) rats ($^{*}P < 0.001$), whereas [D-Ala^2] GHRH treatment from 9 months to 28 months (O-GHRH) prevented this decline ($^{**}P = 0.007$, comparing O-Sal and O-GHRH). (B) Number of times the platform was crossed during the probe trial, considered the most sensitive measure of spatial reference memory, declined with age ($^{\dagger}P = 0.001$ comparing 9-months-old (Y) and 28-month-old control (O-Sal) animals) and [D-Ala^2]GHRH treatment prevented this decline ($^{\ddagger}P = 0.032$ comparing O-Sal and O-GHRH animals). Data are shown as mean $\pm$ SEM.

brain and growth hormone improves cognition in growth hormone-deficient children and adults, we initially proposed that the age-related deficits in the somatotropin pathway may result in memory impairments. Initial studies, however, indicated that acute administration of GHRH did not improve memory (Schneider-Rivas *et al*., 1995). Nevertheless, long-term administration of [D-Ala^2]GHRH in rats from 9 to 28 months of age maintained a daily pulsatile release of growth hormone, partially attenuated the decline in plasma IGF-1 and prevented the age-related impairment in spatial reference memory (Thornton *et al*., 1999a) (Fig. 63.4). In fact, aged animals treated with [D-Ala^2]GHRH had a similar performance in the probe trial of the Morris water maze compared to young animals. Further analysis revealed that long-term GHRH treatment did not influence the decline in locomotion or vision, suggesting that the effects of GHRH were specific to spatial memory. Since acute administration of GHRH did not demonstrate a similar pattern, it was concluded that the effect of GHRH was mediated by an increase in pulsatile release of growth hormone and/or increasing plasma IGF-1 levels.

C. Effects of Growth Hormone

While many studies suggest that growth hormone treatment improves alertness, vitality, mood, and increases a sense of well-being (McGauley, 1989; Bengtsson *et al*., 1993), data suggesting that growth hormone administration improves memory are controversial and, in many cases, anecdotal. For instance, adults with childhood onset growth hormone deficiency are more likely to be unemployed (Ranke, 1987; Bjork *et al*., 1989) and achieve only a junior high school education (Takano *et al*., 1994). Treatment of individuals with childhood-onset growth hormone deficiency has been reported to improve measures of intelligence (Sartorio *et al*., 1995) as well as psychosocial measures (Laron *et al*., 1986). Although there is no direct evidence that growth hormone crosses the blood–brain barrier, it is known that hypophysectomy, which decreases growth hormone and plasma IGF-1, results in a decreases of IGF-1 mRNA in brain. Administration of growth hormone to hypophysectomized or aged animals results in an increase in brain levels of IGF-1 mRNA (Hynes *et al*., 1987; Lopez-Fernandez *et al*., 1996). These data suggest that growth hormone may impact brain function indirectly by increasing plasma IGF-1 or directly by regulating brain IGF-1 levels.

D. Effects of Insulin-Like Growth Factor-1

IGF-1 is well-known to influence neurotransmission and neuronal maintenance and survival which are actions similar to that observed with other neurotrophic agents. Because of its wide range of effects on neurons, it was proposed that IGF-1 contributes to synaptic plasticity and the neural mechanisms necessary for learning and memory. Markowska *et al*. (1998) subsequently demonstrated that replacement of IGF-1 in the lateral ventricle of aged animals ameliorates the age-related impairments in both spatial reference and working memory. Subsequent studies in our laboratory have confirmed this finding in another rat strain and demonstrated that IGF-1 improves learning and spatial reference memory in aged animals compared to age-matched, control animals (Fig. 63.5). In addition, antagonism of IGF-1 binding to its receptor using a specific peptide analog in young animals resulted in impairments in learning and reference memory (Thornton *et al*., 1999b). These data were the first to demonstrate that brain IGF-1 is important for learning and memory in young animals and suggested that the age-related decline in IGF-1 and type 1 IGF receptors (Sonntag *et al*., 1999) and impairments in IGF-1 activity in the brain of aged animals (D'Costa *et al*., 1995) contribute to deficits in memory that are found with increasing age.

V. Cerebrovasculature and Age

A. Age-Related Deficiencies

Despite early studies that suggested that cerebral blood flow was not influenced by age, recent studies using nitrous oxide, xenon, CT, and PET scanning techniques have clearly estab-

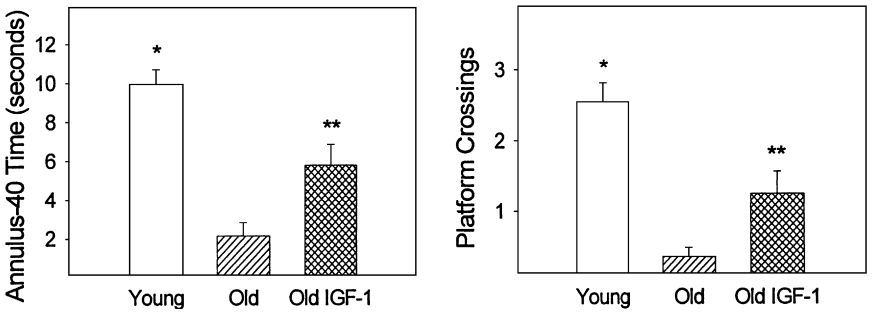

FIG. 63.5. Probe trial in the Morris water maze assessed spatial reference memory. Performance in both Annulus-40 time (left) and platform crossings (right) declined with age ($P < 0.001$ comparing young and old control animals). IGF-1 treatment for 28 days reversed this decline in both measures (left, right; $^{*}P < 0.05$ comparing young to old and old IGF-1; $^{**}P < 0.01$ in both measures comparing old control and old IGF-1 treated animals. Data are shown as mean $\pm$ SEM.

lished that a decrease in cerebral blood flow occurs in aged rodents, nonhuman primates, and man (Kety, 1956; Melamed *et al*., 1980; Amano *et al*., 1982; Shaw *et al*., 1984; Lynch *et al*., 1999). This age-related decline in cerebral blood flow has been hypothesized to result in a loss of metabolic and nutritional support for neurons and may therefore contribute to structural and functional changes that are associated with brain aging (Goldman *et al*., 1987; Bell and Ball, 1990). Although specific mechanisms for the decline in blood flow are unclear, recent studies indicate that perfusion pressure remains constant with age (Elias *et al*., 1995) leading to the conclusion that the decline in blood flow must result from alterations in vascular density. In this regard, several investigators report an age-related reduction in cerebral capillarity and an increase in intercapillary distance in aged humans and rodents (Bell and Ball, 1990; Jucker *et al*., 1990). A decline in total capillary length and capillary surface area per unit volume of tissue was also observed in humans from 26 to 96 years of age (Mann *et al*., 1986). However, other investigators report either an increase or no change in capillaries per unit volume of cerebral tissue (Bar, 1978; Hunziker *et al*., 1979; Meier-Ruge *et al*., 1980; Hughes and Lantos, 1987). The reason for this controversy results from a disproportionate decrease in the volume of cortical or subcortical structures compared to the vasculature in some animal models (Bar, 1978) or from the regulation of cerebral blood flow primarily by arteriolar and arteriole-to-arteriole anastomotic density, rather than capillary density (Zhang *et al*., 1994). More recent data demonstrate that the densities of both arterioles and arteriole-to-arteriole anastomoses on the cortical surface decrease with age (Yamaguchi *et al*., 1988; Sonntag *et al*., 1997) (Fig. 63.6, see color insert). Since vasculature on the cortical surface of the brain has been shown to mimic vascular changes throughout the brain (Rosenblum and Kontos, 1974), the loss in vessels on the cortical surface suggests that a general rarefaction of blood vessels within the brain occurs and contributes to the decrease in blood flow and neuronal function. In effect, not only is there a reduction in blood flow with age, but also a reduction in the ability to maintain homogenous flow to the brain during periods of localized ischemia which may result in an increased risk of neuronal loss in brain regions where vessel rarefaction is prominent.

It is well-known that metabolic support of neuronal tissues requires adequate blood flow. Angiogenesis has been hypothesized to be essential for neurite outgrowth and may precede neuronal growth in some models of neuronal damage (Podhajsky and Myers, 1993). In addition, studies have revealed that an increase in synaptic activity causes an angiogenic response in the cerebellar cortex (Black *et al*., 1990). Data also demonstrate that placing animals in a complex environment induces synaptogenesis and generation of new cerebral microvessels in young rats (Black *et al*., 1989b, 1991), while aged rats have deficits in the generation of new cerebral microvessels to support neuronal plasticity (Black *et al*., 1989a). These data provide important evidence to support the conclusion that an adequate vascular supply to the brain is critical for synaptic plasticity and the acquisition of memory. Thus, the age-related rarefaction of microvessels in the brain may contribute to a decline in synaptic plasticity and age-related impairments in learning and memory.

Recently, several laboratories have reported that the presence of specific growth factors that either regulate growth of the vasculature [i.e., vascular-derived endothelial growth factor (VEGF), transforming growth factor β (TGF-β), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF)] or are produced in the vasculature (i.e., IGF-1 and NGF) (Engelmann *et al*., 1989; Creedon and Tuttle, 1991; Delafontaine *et al*., 1991; Sonntag *et al*., 1999) (Fig. 63.7, see color insert). The production of IGF-1 and NGF by the vasculature not only has important implications for the paracrine regulation of surrounding tissues but may serve as an essential component in the regulation of neuronal regeneration (Delafontaine, 1995). If such a paracrine relationship between vasculature and neurons is documented by future studies, the rarefaction of the cerebral vasculature that is associated with age may be found to contribute to neuronal dysfunction, not only by diminished oxygenation of tissues and removal of metabolic waste, but also by reducing the expression of trophic agents, thus withdrawing trophic support for surrounding neurons.

B. Effects of Growth Hormone and Insulin-like Growth Factor-1

Both growth hormone and IGF-1 have been shown to be important regulatory factors in the vasculature. Studies indicate that both endothelial and smooth muscle cells have receptors for growth hormone and IGF-1 (Hansson *et al*., 1987;

Delafontaine *et al*., 1991) and IGF-1 immunoreactivity within the vasculature is increased during periods of angiogenesis (Hansson *et al*., 1989). Vascular remodeling in response to hypertension is inhibited by hypophysectomy, which decreases growth hormone and IGF-1, and is restored by growth hormone administration (Folkow *et al*., 1988). Both growth hormone and IGF-1 have been shown to stimulate endothelial cell proliferation, tube formation, and angiogenesis (Foster *et al*., 1990; Grant *et al*., 1993; Sato *et al*., 1993; Gould *et al*., 1995). Additionally, IGF-1 has been shown to increase the synthesis of elastin, thereby enhancing vascular compliance and has been reported to regulate blood flow in some tissues (Badesch *et al*., 1989; Foster *et al*., 1990).

Recent data indicate that vascular density is highly correlated with plasma IGF-1. As previously mentioned, vascular density decreases with age and injections of growth hormone in the aged animal increase cortical vascular density (Sonntag *et al*., 1997), suggesting that deficiencies in growth hormone and IGF-1, rather than aging *per se*, are important components of vascular rarefaction. In addition, it is apparent that growth hormone increases vascular supply to the brain thereby increasing vascular-derived IGF-1 and NGF to potentially act in a paracrine fashion on surrounding tissue. Thus, it appears that the decline in growth hormone that occurs in the aged contributes to the rarefaction of the cerebral vasculature thus diminishing the trophic and metabolic support to the aging brain and may have implications in structural and functional deficits associated with brain aging.

VI. Neuronal Structure, Neurotransmission, and Age

A. Deficits

1. Neuronal Size and Number

Over the past several decades, a number of morphological, functional and biochemical changes have been reported in the brain with age, but the etiology of these diverse changes remains elusive. For example, there is a decline in both glucose and oxygen utilization by the brain, a reduction in synaptic density, and functional changes in both neurons and glia. Many of the aforementioned changes appear to be both regionally and species specific. In humans, it is widely accepted that brain weight decreases with age, predominantly after 55 years of age (Terry *et al*., 1987) but the etiology of this decline has been controversial. Initial studies indicated that a significant and progressive decline in the number of neurons occurs in cortical areas of the aged, human brain (Brody, 1955; Devaney and Johnson, 1980; Henderson *et al*., 1980; Anderson *et al*., 1983), particularly in layers 2 and 4 (Brody, 1980; Hughes and Lantos, 1987). However, more recent studies suggest that neuronal number in cortex remains constant throughout the life span in humans (Terry *et al*., 1987), nonhuman primates (Vincent *et al*., 1989; Tigges *et al*., 1990), and rodents (Peinado *et al*., 1993). A significant decrease in size of neurons, specifically large neurons (>91 μm^2), has also been reported to occur in the aged animal. Furthermore, a significant increase in the number of glia has been observed (Brizzee, 1975; Brizzee *et al*., 1976; Terry *et al*., 1987; Peters *et al*., 1991; Peinado *et al*., 1993). In addition, studies by Landfield *et al*. (1981,1996) indicate a loss of neurons in the CA3 region of hippocampus. More recent analysis using stereological methods suggest that a significant loss of neurons occurs in the subiculum and the dentate gyrus of the hippocampus in elderly humans (West and Crook, 1990). It is now generally accepted that a decline in the size, but not number, of neurons occurs in the cerebral cortex, whereas the decline in hippocampal neurons appears to be regionally specific.

2. Dendritic Architecture and Synaptic Density

In addition to neuronal density, one of the most important morphological features of neurons is related to dendritic architecture and synaptic density. An age-related decline in dendritic arbors has been reported in frontal and temporal lobes of the cortex and in the limbic system (Feldman and Dowd, 1975; Scheibel *et al*., 1976; Geinisman *et al*., 1977; Vaughan, 1977; Geinisman, 1979; Brody, 1980; Cupp and Uemura, 1980; Leuba, 1983; Uemura, 1985; Nunzi *et al*., 1987; Lolova, 1989; Moroi-Fetters *et al*., 1989). The age-related decline begins with a loss of dendritic spines and is followed by alterations in the size and shape of horizontal branches resulting in a loss of basilar dendrites and/or apical branches (Nakamura *et al*., 1985). Concomitantly, an age-related decrease in the number of synapses has been reported (Geinisman *et al*., 1977; Geinisman, 1979; Bondareff, 1980; Gibson, 1983; Haug and Eggers, 1991). Although many of these changes appear to be regionally specific, it is becoming increasingly apparent that these structures have a critical role in integrating neuronal activity and that dendritic architecture, including both extent and pattern of dendrites, and the number of synapses influence neural transmission and neuronal activity (Johnston *et al*., 1996). Therefore, the deterioration of these structures observed in aged animals and man is believed to result in functional deficits including impaired neural transmission, motor function, and memory.

3. Neurotransmission

In addition to structural changes in neurons, dendrites, and synapses that occur with age, functional changes in neurons also occur in aged animals and humans, including a decline in the synthesis, release, and activity of neurotransmitters (Amenta *et al*., 1991; Strong, 1998). Since aging is associated with deficits in memory and motor function, the neurotransmitter systems that are closely associated with these functions have been of intense interest, specifically the cholinergic, glutaminergic, and dopaminergic systems. The deficits in these neuronal systems are believed to result in impairments in the aged brain and result, at least in part, in the loss of motor and cognitive function.

The hypothesis that the cholinergic system mediates learning and memory was first supported by studies demonstrating that anticholinergic agents produce memory impairments in young adults similar to impairments common in the elderly (Drachman and Leavitt, 1974). As a result of this important study which suggested that the cholinergic system mediates learning and memory, this system has been extensively studied

with age and specific declines in levels of acetylcholine noted. Specifically, choline acetyltransferase (ChAT), the rate-limiting enzyme for acetylcholine synthesis, declines by 50–60% in both the hippocampus and the cortex (Court *et al*., 1993). Similarly, acetylcholine release has been reported to decrease by 61% in the hippocampus and 73% in the striatum (Amenta *et al*., 1993). Analysis of muscarinic receptors have produced equivocal results. Muscarinic receptors have been reported to decline by as much as 50–60% in the caudate, putamen, hippocampus, and frontal cortex (Perry, 1980; Dewey *et al*., 1990; Strong *et al*., 1991; DeKosky and Palmer, 1994; Goldman *et al*., 1994), while others report a preservation of receptor density in the neocortex and hippocampus with a trend toward a decline in the striatum (Decker, 1987; Sherman and Friedman, 1990). Analysis of the levels of the specific muscarinic receptor subtypes has also produced controversial results when putative M_1 receptors were analyzed (Norman *et al*., 1986; Sirvio *et al*., 1988a,b; Watson *et al*., 1988; Biegon *et al*., 1989; Araujo *et al*., 1990). Similarly, density of the M_2 receptor has been reported to exhibit no change or increase in cortical areas and in the molecular layer of the dentate gyrus (Norman *et al*., 1986; Biegon *et al*., 1989; Araujo *et al*., 1990; Aubert *et al*., 1995) or decrease (Nordberg *et al*., 1992). In a subsequent study, a 60% decline in slow excitatory, postsynaptic cholinergic transmission in the hippocampus was reported with age using intracellular recording methods but no alterations in either presynaptic or postsynaptic inhibitory afterhyperpolarization were observed (Taylor and Griffith, 1993), suggesting an impairment in receptor function. While the age-related decline in the cholinergic system is well-founded, the consequence of these deficiencies remains unclear. For example, in diseases that result in deficiencies in ChAT expression and activity that are as profound as those observed in Alzheimer's disease (including olivopontocerebellar atrophy), no significant loss of memory occurs (Kish *et al*., 1988). In addition, recent studies suggest that the cholinergic system may have an important role in attention rather than memory in both rodents (Sarter and Bruno, 1997; Turchi and Sarter, 1997) and nonhuman primates (Voytko *et al*., 1994; Voytko, 1996), thereby influencing cognition rather than a direct effect on learning and memory.

More recently, glutaminergic neurotransmission has been shown to be essential for learning and memory. The glutaminergic system is comprised of several types of receptors, such as the NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and metabotropic glutamate receptors (Cotman *et al*., 1988, 1995; Monaghan *et al*., 1989). Most studies have focused on the NMDA receptors since: (1) NMDA receptors are expressed throughout the brain but are found in high concentrations in brain regions involved in learning and memory including the hippocampus and cortex (Mayer and Westbrook, 1987; Ishii *et al*., 1993), (2) NMDA agonists increase acquisition of memory whereas NMDA antagonists impair memory (Collingridge and Bliss, 1987; Collingridge and Singer, 1990; Ingram *et al*., 1994), and (3) induction of long-term potentiation (an event necessary for learning and memory) requires Ca^{2+} flux through NMDA receptors concentrated in the hippocampus (Artola and Singer, 1987; Collingridge and Bliss, 1987; Cotman *et al*., 1988; Ito, 1989; Collingridge and Singer, 1990). Despite the importance of glutaminergic neurotransmission for learning and memory, the assessment of age-related changes in this system have been inconsistent. For example, initial studies using MK-801 binding to the NMDA receptors demonstrated no age-related alterations in hippocampus or entorhinal cortex (Shimada *et al*., 1997). Recent studies suggest that [^{3}H]glutamate binding decreases modestly in aged animals whereas the binding of specific NMDA receptor subtype agonists and antagonists exhibit more profound decreases (Magnusson and Cotman, 1993). The efficacy of the NMDA receptor has been found to depend upon the assembly of both NMDA R1 (NR1) and R2 (NR2) subunits. Expression of the NR1 subunit in *Xenopus* oocytes demonstrated that a functional receptor is not formed with expression of NR1 alone (Chazot *et al*., 1992; Grimwood *et al*., 1996). Similarly, the expression of NR2 subunit alone does not result in a functional receptor; however, coexpression of the NR1 and NR2 subunits results in the formation of a functional NMDA receptor in both oocytes and mammalian cells (Meguro *et al*., 1992; Monyer *et al*., 1992; Ishii *et al*., 1993). The importance of this receptor heterogeneity is supported by a recent study demonstrating that transgenic mice lacking the NMDA R2 subunit exhibit impairments in spatial memory (Sakimura *et al*., 1995). In relation to aging, recent data from our own laboratory suggest that no changes in the NMDA R1 subunit occur with age but an age-related decline in both NMDA R2A and NMDA R2B subunits occur in the hippocampus compared to young animals (Sonntag *et al*., 2000). The implication of these studies is that alterations in subunit composition of the NMDA receptor with age may result in changes in physiological and pharmacological properties of the receptor resulting in impaired neuronal function.

It is well-known that deficiencies in the dopaminergic system are involved in several age-related neurological disorders such as Parkinson's disease (Hierholzer *et al*., 1998; Kelly *et al*., 1998) and stroke (Dawson *et al*., 1994) and contribute to the altered regulation of hypothalamic and hypophysiotropic hormones (Meites and Sonntag, 1981; Borowsky and Kuhn, 1992; Durham *et al*., 1997). Investigators have also reported that the dopaminergic system is involved in processes of learning and memory (Smith *et al*., 1973; Shaywitz and Pearson, 1978; Altman and Quartermain, 1983; Dubrovina and Il'iuchenok, 1990). Previous studies suggested that age-related impairments in the dopaminergic system result, at least in part, in deficits in both motor and cognitive function (Marshall and Berrios, 1979; Marshall, 1982; Morgan, 1987; Arnsten *et al*., 1995). Decreases in density of D_1 receptors (Araki *et al*., 1997), levels of striatal dopamine uptake sites (Zelnik *et al*., 1986), dopamine transporters (Bannon *et al*., 1992), dopamine content (Goldman-Rakic and Brown, 1981), and dopamine release in aged animals (Wenk *et al*., 1989) have been reported. Assessment of D_2 receptor density indicate either a decrease (Henry *et al*., 1986; Hess *et al*., 1987) or no change (De Keyser *et al*., 1990; Araki *et al*., 1997) with age. Despite controversial results on age-related changes in D_2 receptor density, recent data from our laboratory suggest that D_2 receptor activation of G-proteins declines, intrinsic efficacy of the agonist (a measure of the coupling efficiency between G-proteins and receptors in response to agonist stimulation) decreases, and receptor reserve for G-protein activation (the receptor level in excess of that required for a maximal response

to an agonist) is reduced with age in both cortex and hippocampus. These changes occurred despite the absence of changes in D_2 receptor density (Thornton *et al*., 1998). These results suggest that many aspects of the dopaminergic neurotransmission are deficient in the aged animal and contribute to deficits in both motor and cognitive function.

Although alterations in neurotransmitter levels and changes in receptor density, composition, and activity are one aspect of the decline in functional capacity of neurons, deficiencies in intracellular signaling in neurons have been reported to contribute to the decrease in neuronal function with age. For example, data demonstrate that protein kinase C (PKC), phospholipase C (PLC), protein kinase A (PKA), and calcium signaling are diminished with age (Friedman and Wang, 1989; Pisano *et al*., 1991; Undie and Friedman, 1992; Wang *et al*., 1992; Undie *et al*., 1995). It is also believed that these deficits, alone or in combination, result in impaired signal transduction in aged animals and humans and contribute to neuronal dysfunction (Roth, 1989; Pisano *et al*., 1991; Sugawa and May, 1993; Battaini *et al*., 1994; Undie *et al*., 1995). While the etiology for these general impairments remains unknown, several potential mechanisms have emerged. For instance, a reduced ability to activate receptor-linked G-proteins and/or phosphorylate critical intracellular proteins in response to receptor activation is a common finding in several neurotransmitter systems of the aged animal (Roth, 1995). It has been hypothesized that these alterations are due to age-associated changes in phospholipid content, resulting in a loss of membrane fluidity (Tacconi *et al*., 1991; Joseph *et al*., 1995). Johnson *et al*. (1995) have demonstrated that responsiveness of G-protein coupled receptors declines with age due to reductions in mRNA and protein levels of Gα subunits. However, general impairments in neuronal function are also observed in response to stimuli that produce a rapid induction of the intracellular protooncogene *c-fos*, resulting in the activation/transcription of a number of targets on the genome. Results comparing young and old animals demonstrated a >50% reduction in the number of neurons capable of activating *c-fos* in aged animals (D'Costa *et al*., 1993). Together, these studies provide convincing evidence that signal transduction is diminished in the brain of aged animals and these decreases contribute to diminished neuronal function.

B. Effects of Insulin-like Growth Factor-1

While growth hormone has been shown to influence brain levels of IGF-1 as well as other neurotrophins and the cerebral vasculature, and empirical data suggest that growth hormone may improve mood and cognition, there is no evidence to date that growth hormone has a direct action in the brain. However, IGF-1 has been demonstrated to cross the blood–brain barrier (Reinhardt and Bondy, 1994) and is produced and secreted from the cerebral vasculature, neurons and glia. In addition, IGF-1 has been shown to exert trophic actions in the brain. Nevertheless, the influence of the decline in plasma and brain levels of IGF-1 on the aging brain has only recently been studied. Data suggest that IGF-1 has a critical role in maintenance, protection, survival, and function of neurons and that the decline in brain IGF-1 may be a causative factor in deficits associated with brain aging.

As previously mentioned, IGF-1 is a potent neurotrophic agent and has been shown to stimulate DNA, RNA, and protein synthesis, cell proliferation, survival, and neurite outgrowth *in vitro* (Lenoir and Honegger, 1983; Recio-Pinto and Ishii, 1984; Recio-Pinto *et al*., 1986; Shemer *et al*., 1987; Han *et al*., 1988; Toran-Allerand *et al*., 1988; Torres-Aleman *et al*., 1989; D'Costa *et al*., 1995) and regulates synaptogenesis, myelin synthesis, and is neuroprotective *in vivo* (McMorris *et al*., 1986; McMorris and Dubois-Dalcq, 1988; Saneto *et al*., 1988; Ishii, 1989; Shinar and McMorris, 1995). In addition, recent studies using an organotypic slice preparation suggest that IGF-1 regulates the density and complexity of apical dendrites (Niblock *et al*., 1997). Deficiencies in protein synthesis, synaptic density, and dendritic architecture occur with age and these changes appear to be temporally correlated with the decline in IGF-1 and type 1 IGF receptors.

While the effect of IGF-1 on the morphological changes associated with aging are still correlative, deficits in IGF-1 have been closely linked to impaired neural transmission. IGF-1 influences acetylcholine neurotransmission by stimulating ChAT activity (Sara and Hall, 1990) and regulates K^+-induced release of acetylcholine from hippocampal neurons and cortical slices (Nilsson *et al*., 1988; Araujo *et al*., 1989), both of which decline in the aged animal. In addition, recent data from our laboratory suggest that administration of IGF-1

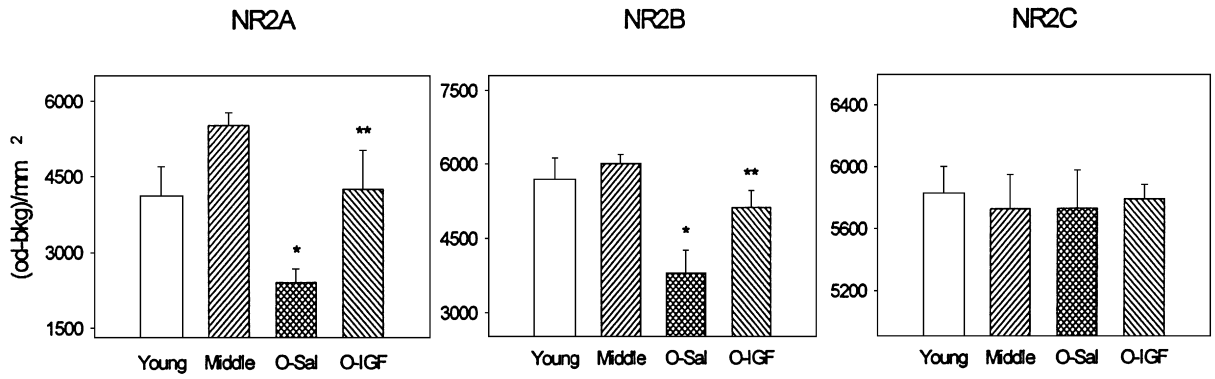

FIG. 63.8. Analysis of changes in NMDA R2 receptor subtype protein expression in hippocampus assessed by Western analysis. NMDA R2A (left) and NMDA R2B (center) indicated a significant decline between 21 and 30 months of age ($P < 0.05$) that was reversed by treatment with IGF-1 for 28 days. No alterations in protein expression of NMDA R2C were observed with age or treatment with IGF-1.

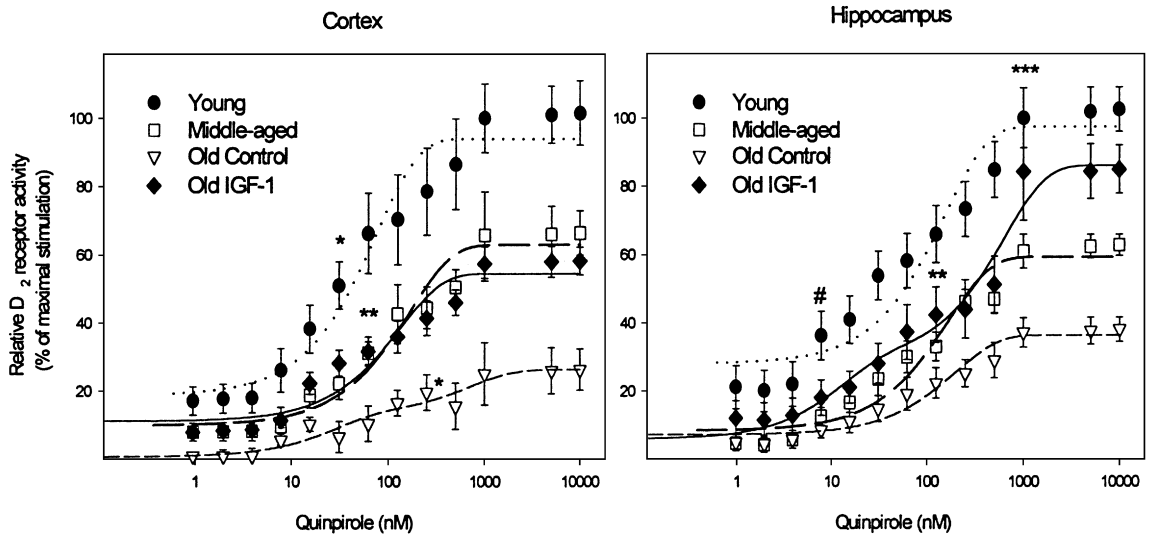

FIG. 63.9. The [^{35}S]GTPγS assay assesses the response of G-protein coupled receptors to an agonist, specifically the displacement of GTP for GDP on the α-subunit of the G-protein thereby measuring activity of the receptor. The dose–response relationship of quinpirole (a specific D_2 receptor agonist) stimulation of [^{35}S]-GTPγS binding in cortex (left) and hippocampus (right) of young (5 months), middle-age (15 months), old control (28 months), and old IGF-1 treated (28 months) Brown Norway rats demonstrates an age-related decline in agonist-stimulated G-protein activation and IGF-1 treatment for 28 days reverses this decline in both cortex and hippocampus ($^{*}P < 0.05$ compared to middle-aged, old control, and old IGF-1 treated; $^{**}P < 0.05$ compared to middle-aged and old control; $^{***}P < 0.05$ compared to old control).

icv reverses the alterations in the subunit composition of the NMDA receptors by upregulating the NMDA R2A and R2B subunits in the hippocampus (Fig. 63.8). Since the heterogeneity of the NMDA receptor is necessary for full receptor activity, it was hypothesized that IGF-1 may increase the efficacy of glutamate at the NMDA receptor, thereby modulating neural transmission of the glutaminergic system, resulting in improvements in learning and memory (Markowska *et al.*, 1998; Thornton, *et al.*, 1999b).

The effect of IGF-1 on dopaminergic neurotransmission has also recently begun to be elucidated. Biochemical studies have shown that IGF-1 is involved in the development and maintenance of dopaminergic neurons (Knusel and Hefti, 1991; Beck, 1994) and influences dopamine release (Beck *et al.*, 1993), all of which decline with age. More recent data demonstrate that icv infusion of IGF-1 in the aged animal reverses the age-related decline in D_2 receptor activated G-proteins (Thornton *et al.*, 1998). These studies demonstrate that IGF-1 improves the maximal stimulation of [^{35}S]GTPγS binding in response to a D_2 receptor agonist (Fig. 63.9), reverses the age-related decline in intrinsic efficacy of the agonist, and increases receptor reserve for G-protein activation without altering the density of D_2 receptors. Thus, IGF-1 modulates the response of dopamine D_2 receptors and the age-related decline in IGF-1 may contribute to impairments in dopaminergic neurotransmission.

IGF-1 binds to the type 1 IGF receptor and results in activation of several intracellular signaling pathways including inositol triphosphate (IP_3), insulin-related substrate-1 (IRS-1), mitogen activated protein kinase (MAPK), PKA, PKC, calcium signaling, and cAMP (Sadler and Maller, 1987; Farese *et al.*, 1989; Renganathan *et al.*, 1997). Furthermore, many of these signaling pathways are impaired with age (Roth, 1997). While studies have demonstrated that replacement of IGF-1 improves calcium flow and intracellular signaling in skeletal muscle of aged animals (Renganathan *et al.*, 1997), no studies have evaluated the actions of IGF-1 on these signaling pathways in the brain. Since IGF-1 has been shown to induce activity of these intracellular signaling proteins, it is probable that IGF-1 may also regulate neuronal function and signaling by increasing the activity of specific intracellular proteins in the brain. Similarly, deficiencies in IGF-1 similar to those observed in aged animals may induce signaling impairments and contribute to neuronal dysfunction and functional deficits.

VII. Conclusions

The prominent role of IGF-1 on the developing brain and the association between age-related changes in the brain and the actions of IGF-1 have led us to propose that decreases in plasma growth hormone and IGF-1 and/or deficiencies in the endogenous production of IGF-1 by the aging brain are contributing factors in brain aging. Certainly, previous studies support an important neuromodulatory role of IGF-1 on acetylcholine synthesis and release, protein synthesis, neurite outgrowth, synaptogenesis, and myelination, all of which are known to decrease with age. In addition, there are recent, compelling data suggesting that IGF-1 has additional actions that may influence brain aging including the regulation of subunit composition of the NMDA receptors and D_2 receptor activity. Intracerebroventricular infusion of IGF-1 into old animals was recently found to increase working and reference memory, supporting the hypothesis that age-related impairments in learning and memory result, at least in part, from hormone deficiency similar to that found in peripheral tissues. It is currently unknown if deficiencies in growth hormone or IGF-1 in early adulthood result in or facilitate functional deficits similar

to those observed in the brain of aging animals and humans. Furthermore, the etiology of the deficiencies in IGF-1 remains elusive. Furthermore, it is unknown if these hormonal deficits are a pathophysiologic response to attenuate age-related pathologies. These questions must be addressed before one can completely understand the role of growth hormone and IGF-1 in brain aging.

It is clear that we are beginning to evolve from the concept of biological aging that uses unidimensional models where aging is viewed as changes in a single system to multidimensional models where there is an interdependence and interrelationship of age-related changes across a number of systems. As a result of these evolving models, new concepts of brain aging are emerging that are the result of deficiencies in endocrine, vascular, neural, and glial components that closely interact, resulting in the aging phenotype. Although investigation of these interactions pose enormous challenges for scientists, the continued development of conditional transgenic animal models and virus-mediated gene transfer targeted to specific cell types should begin to isolate specific mechanisms contributing to brain aging and offer specific interventions to ameliorate age-related memory deficits.

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References

Abribat, T., Deslauriers, N., Brazeau, P., and Gaudreau, P. (1991). Alterations of pituitary growth hormone-releasing factor binding sites in aging rats. *Endocrinology (Baltimore)* **128**, 633–635.

Aguila, M. C., Boggaram, V., and McCann, S. M. (1993). Insulin-like growth factor I modulates hypothalamic somatostatin through a growth hormone releasing factor increased somatostatin release and messenger ribonucleic acid levels. *Brain Res*. **625**, 213–218.

Alberch, J., Perez-Navarro, E., Arenas, E., and Marsal, J. (1991). Involvement of nerve growth factor and its receptor in the regulation of the cholinergic function in aged rats. *J. Neurochem.* **57**, 1483–1487.

Altman, H. J., and Quartermain, D. (1983). Facilitation of memory retrieval by centrally administered catecholamine stimulating agents. *Behav. Brain Res*. **7**, 51–63.

Amano, T., Meyer, J. S., Okabe, T., Shaw, T., and Mortel, K. F. (1982). Stable xenon CT cerebral blood flow measurements computed by a single compartment—double integration model in normal aging and dementia. *J. Comput. Assist. Tomogr.* **6**, 923–932.

Amenta, F., Ricci, A., and Vega, J. A. (1991). Autoradiographic localization of dopamine receptors in rat cerebral blood vessels. *Eur. J. Pharmacol*. **192**, 123–132.

Amenta, F., Franch, F., Ricci, A., and Vega, J. A. (1993). Cholinergic neurotransmission in the hippocampus of aged rats: Influence of L-alpha-glycerylphosphorylcholine treatment. *Ann. N. Y. Acad. Sci*. **695**, 311–313.

Anderson, J. M., Hubbard, B. M., Coghill, G. R., and Slidders, W. (1983). The effect of advanced old age on the neurone content of the cerebral cortex. Observations with an automatic image analyser point counting method. *J. Neurol. Sci*. **58**, 235–246.

Antoniazzi, F., Radetti, G., Zamboni, G., Gambaro, G., Adami, S., and Tato, L. (1993). Effects of 1,25-dihydroxyvitamin D3 and growth hormone therapy on serum osteocalcin levels in children with growth hormone deficiency. *Bone Miner.* **21**, 151–156.

Araki, T., Kato, H., Shuto, K., Fujiwara, T., and Itoyama, Y. (1997). Effect of aging on dopaminergic receptors and uptake sites in the rat brain studied by receptor autoradiography. *J. Neurol. Sci.* **148**, 131–137.

Araujo, D. M., Lapchak, P. A., Collier, B., Chabot, J. G., and Quirion, R. (1989). Insulin-like growth factor-1 (somatomedin-C) receptors in the rat brain: Distribution and interaction with the hippocampal cholinergic system. *Brain Res*. **484**, 130–138.

Araujo, D. M., Lapchak, P. A., Meaney, M. J., Collier, B., and Quirion, R. (1990). Effects of aging on nicotinic and muscarinic autoreceptor function in the rat brain: Relationship to presynaptic cholinergic markers and binding sites. *J. Neurosci*. **10**, 3069–3078.

Arimura, A., Smith, W. D., and Schally, A. V. (1976). Blockade of the stress-induced decrease in blood GH by anti-somatostatin serum in rats. *Endocrinology (Baltimore)* **98**, 540–543.

Arnsten, A. F., and Goldman-Rakic, P. S. (1985). Alpha 2-adrenergic mechanisms in prefrontal cortex associated with cognitive decline in aged nonhuman primates. *Science* **230**, 1273–1276.

Arnsten, A. F., Cai, J. X., Steere, J. C., and Goldman-Rakic, P. S. (1995). Dopamine D_2 receptor mechanisms contribute to age-related cognitive decline: The effects of quinpirole on memory and motor performance in monkeys. *J. Neurosci*. **15**, 3429–3439.

Artola, A., and Singer, W. (1987). Long-term potentiation and NMDA receptors in rat visual cortex. *Nature (London)* **330**, 649–652.

Aschner, B. (1912). Über die Function der Hypophyse. *Pfluegers Arch.* **146**, 1–1.

Aubert, I., Rowe, W., Meaney, M. J., Gauthier, S., and Quirion, R. (1995). Cholinergic markers in aged cognitively impaired Long-Evans rats. *Neuroscience* **67**, 277–292.

Badesch, D. B., Lee, P. D., Parks, W. C., and Stenmark, K. R. (1989). Insulin-like growth factor I stimulates elastin synthesis by bovine pulmonary arterial smooth muscle cells. *Biochem. Biophys. Res. Commun.* **160**, 382–387.

Bahrick, H. P. (1984). Memory for people. *In* ''Everyday Memory, Actions and Absent-Mindedness'' (J. E. Harris and P. E. Morris, eds.), pp. 139–152. Academic Press, New York.

Bannon, M. J., Poosch, M. S., Xia, Y., Goebel, D. J., Cassin, B., and Kapatos, G. (1992). Dopamine transporter mRNA content in human substantia nigra decreases precipitously with age. *Proc. Natl. Acad. Sci. U. S. A*. **89**, 7095–7099.

Bar, T. (1978). Morphometric evaluation of capillaries in different laminae of rat cerebral cortex by automatic image analysis: Changes during development and aging. *Adv. Neurol*. **20**, 1–9.

Barbul, A., Rettura, G., Levenson, S. M., and Seifter, E. (1983). Wound healing and thymotropic effects of arginine: A pituitary mechanism of action. *Am. J. Clin. Nutr.* **37**, 786–794.

Barnes, C. A. (1979). Memory deficits associated with senescence: A neurophysiological and behavioral study in the rat. *J. Comp. Physiol. Psychol*. **93**, 74–104.

Barnes, C. A., Nadel, L., and Honig, W. K. (1980). Spatial memory deficit in senescent rats. *Can. J. Psychol*. **34**, 29–39.

Bartus, R. T., Fleming, D., and Johnson, H. R. (1978). Aging in the rhesus monkey: Debilitating effects on short-term memory. *J. Gerontol*. **33**, 858–871.

Bassett, J. M., and Wallace, A. L. (1966). Short-term effects of ovine growth hormone on plasma glucose, free fatty acids and ketones in sheep. *Metab., Clin. Exp*. **15**, 933–944.

Battaini, F., Lucchi, L., Bergamaschi, S., Ladisa, V., Trabucchi, M., and Govoni, S. (1994). Intracellular signalling in the aging brain. The role of protein kinase C and its calcium-dependent isoforms. *Ann. N. Y. Acad. Sci*. **719**, 271–284.

Baxter, R. C., and Martin, J. L. (1986). Radioimmunoassay of growth hormone-dependent insulin-like growth factor binding protein in human plasma. *J. Clin. Invest*. **78**, 1504–1512.

Beach, R. K., and Kostyo, J. L. (1968). Effect of growth hormone on the DNA content of muscles of young hypophysectomized rats. *Endocrinology (Baltimore)* **82**, 882–884.

Beck, K. D. (1994). Functions of brain-derived neurotrophic factor, insulin-like growth factor-I and basic fibroblast growth factor in the development and maintenance of dopaminergic neurons. *Prog. Neurobiol.* **44**, 497–516.

Beck, K. D., Knusel, B., and Hefti, F. (1993). The nature of the trophic action of brain-derived neurotrophic factor, des(1-3)-insulin-like growth factor-1, and basic fibroblast growth factor on mesencephalic dopaminergic neurons developing in culture. *Neuroscience* **52**, 855–866.

Bell, M. A., and Ball, M. J. (1990). Neuritic plaques and vessels of visual cortex in aging and Alzheimer's dementia. *Neurobiol. Aging* **11**, 359–370.

Bengtsson, B. A., Eden, S., Lonn, L., Kvist, H., Stokland, A., Lindstedt, G., Bosaeus, I., Tolli, J., Sjöström, L., and Isaksson, O. G. (1993). Treatment of adults with growth hormone (GH) deficiency with recombinant human GH. *J. Clin. Endocrinol. Metab.* **76**, 309–317.

Berelowitz, M., Szabo, M., Frohman, L. A., Firestone, S., Chu, L., and Hintz, R. L. (1981). Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalamus and the pituitary. *Science* **212**, 1279–1281.

Bermann, M., Jaffe, C. A., Tsai, W., DeMott-Friberg, R., and Barkan, A. L. (1994). Negative feedback regulation of pulsatile growth hormone secretion by insulin-like growth factor I. Involvement of hypothalamic somatostatin. *J. Clin. Invest.* **94**, 138–145.

Biegon, A., Hanau, M., Greenberger, V., and Segal, M. (1989). Aging and brain cholinergic muscarinic receptor subtypes: An autoradiographic study in the rat. *Neurobiol. Aging* **10**, 305–310.

Binnerts, A., Swart, G. R., Wilson, J. H., Hoogerbrugge, N., Pols, H. A., Birkenhager, J. C., and Lamberts, S. W. (1992). The effect of growth hormone administration in growth hormone deficient adults on bone, protein, carbohydrate and lipid homeostasis, as well as on body composition. *Clin. Endocrinol. (Oxford)* **37**, 79–87.

Binoux, M., Hossenlopp, P., Hardouin, S., Seurin, D., Lassarre, C., and Gourmelen, R. (1986). Somatomedin (insulin-like growth factors)-binding proteins. Molecular forms and regulation. *Horm. Res.* **24**, 141–151.

Bjork, S., Jonsson, B., Westphal, O., and Levin, J. E. (1989). Quality of life of adults with growth hormone deficiency: A controlled study. *Acta Paediatr. Scand.* **356** (Suppl.), 55–59.

Black, J. E., Polinsky, M., and Greenough, W. T. (1989a). Progressive failure of cerebral angiogenesis supporting neural plasticity in aging rats. *Neurobiol. Aging* **10**, 353–358.

Black, J. E., Sirevaag, A. M., Wallace, C. S., Savin, M. H., and Greenough, W. T. (1989b). Effects of complex experience on somatic growth and organ development in rats. *Dev. Psychobiol.* **22**, 727–752.

Black, J. E., Isaacs, K. R., Anderson, B. J., Alcantara, A. A., and Greenough, W. T. (1990). Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats. *Proc. Natl. Acad. Sci. U. S. A.* **87**, 5568–5572.

Black, J. E., Zelazny, A. M., and Greenough, W. T. (1991). Capillary and mitochondrial support of neural plasticity in adult rat visual cortex. *Exp. Neurol.* **111**, 204–209.

Bondareff, W. (1980). Changes in synaptic structure affecting neural transmission in the senescent brain. *Adv. Exp. Med. Biol.* **129**, 201–211.

Borowsky, B., and Kuhn, C. M. (1992). D_1 and D_2 dopamine receptors stimulate hypothalamo-pituitary-adrenal activity in rats. *Neuropharmacology* **31**, 671–678.

Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J., and Guillemin, R. (1973). Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science* **179**, 77–79.

Breese, C. R., Ingram, R. L., and Sonntag, W. E. (1991). Influence of age and long-term dietary restriction on plasma insulin-like growth factor-1 (IGF-1), IGF-1 gene expression, and IGF-1 binding proteins. *J. Gerontol.* **46**, B180-B187.

Brixen, K., Kassem, M., Nielsen, H. K., Loft, A. G., Flyvbjerg, A., and Mosekilde, L. (1995). Short-term treatment with growth hormone stimulates osteoblastic and osteoclastic activity in osteopenic postmenopausal women: A dose response study. *J. Bone Miner. Res.* **10**, 1865–1874.

Brizzee, K. R. (1975). Gross morphometric analysis and quantitative histology of the aging brain. *In* "Neurobiology of Aging" (J. M. Ordy and K. R. Brizzee, eds.), pp. 197–217. Plenum, New York.

Brizzee, K. R., Ordy, J. M., Hansche, J., and Kaack, B. (1976). Quantitative assessment of changes in neurons and glial cell packing density and lipofuscin accumulation in the cerebral cortex of a non-human primate (*Macaca mulatta*). *In* "Neurobiology of Aging" (R. D. Terry and S. Gershon, eds.), pp. 36–51. Raven Press, New York.

Brody, H. (1955). Organization of the cerebral cortex III: A study of aging in the human cerebral cortex. *J. Comp. Neurol.* **102**, 511–556.

Brody, H. (1980). The nervous system and aging. *Adv. Pathobiol.* **7**, 200–209.

Burke, D. M., and Light, L. L. (1981). Memory and aging: The role of retrieval processes. *Psychol. Bull.* **90**, 513–514.

Burman, P., Hetta, J., and Karlsson, A. (1993). Effect of growth hormone on brain neurotransmitters. *Lancet* **342**, 1492–1493.

Burstein, S., Chen, I. W., and Tsang, R. C. (1983). Effects of growth hormone replacement therapy on 1,25-dihydroxyvitamin D and calcium metabolism. *J. Clin. Endocrinol. Metab.* **56**, 1246–1251.

Carlson, H. E., Gillin, J. C., Gorden, P., and Snyder, F. (1972). Absence of sleep-related growth hormone peaks in aged normal subjects and in acromegaly. *J. Clin. Endocrinol. Metab.* **34**, 1102–1105.

Carlsson-Skwirut, C., Jornvall, H., Holmgren, A., Andersson, C., Bergman, T., Lundquist, G., Sjögren, B., and Sara, V. R. (1986). Isolation and characterization of variant IGF-1 as well as IGF-2 from adult human brain. *FEBS Lett.* **201**, 46–50.

Castro-Alamancos, M. A., and Torres-Aleman, I. (1993). Long-term depression of glutamate-induced gamma-aminobutyric acid release in cerebellum by insulin-like growth factor I. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 7386–7390.

Ceda, G. P., Davis, R. G., Rosenfeld, R. G., and Hoffman, A. R. (1987). The growth hormone (GH)-releasing hormone (GHRH)-GH-somatomedin axis: Evidence for rapid inhibition of GHRH-elicited GH release by insulin-like growth factors I and II. *Endocrinology (Baltimore)* **120**, 1658–1662.

Cella, S. G., Arce, V. M., Pieretti, F., Locatelli, V., Settembrini, B. P., and Muller, E. E. (1993). Combined administration of growth-hormone-releasing hormone and clonidine restores defective growth hormone secretion in old dogs. *Neuroendocrinology* **57**, 432–438.

Chazot, P. L., Cik, M., and Stephenson, F. A. (1992). Immunological detection of the NMDAR1 glutamate receptor subunit expressed in embryonic kidney 293 cells and in rat brain. *J. Neurochem.* **59**, 1176–1178.

Chen, K. S., Masliah, E., Mallory, M., and Gage, F. H. (1995). Synaptic loss in cognitively impaired aged rats is ameliorated by chronic human nerve growth factor infusion. *Neuroscience* **68**, 19–27.

Christ, E. R., Cummings, M. H., Westwood, N. B., Sawyer, B. M., Pearson, T. C., Sonksen, P. H., and Russell-Jones, D. L. (1997). The importance of growth hormone in the regulation of erythropoiesis, red cell mass, and plasma volume in adults with growth hormone deficiency. *J. Clin. Endocrinol. Metab.* **82**, 2985–2990.

Christiansen, J. S., and Jorgensen, J. O. (1991). Beneficial effects of GH replacement therapy in adults. *Acta Endocrinol. (Copenhagen)* **125**, 7–13.

Clark, R. G., Jansson, J. O., Isaksson, O., and Robinson, I. C. (1985). Intravenous growth hormone: Growth responses to patterned infusions in hypophysectomized rats. *J. Endocrinol.* **104**, 53–61.

Clemmons, D. R. (1997). Insulin-like growth factor binding proteins and their role in controlling IGF actions. *Cytokine Growth Factor Rev.* **8**, 45–62.

Collingridge, G. L., and Bliss, T. V. P. (1987). NMDA receptors—their role in long-term potentiation. *Trends Neurosci.* **10**, 288–293.

Collingridge, G. L., and Singer, W. (1990). Excitatory amino acid receptors and synaptic plasticity. *Trends Pharmacol. Sci*. **11**, 290–296.

Corpas, E., Harman, S. M., and Blackman, M. R. (1992a). Serum IGF-binding protein-3 is related to IGF-I, but not to spontaneous GH release, in healthy old men. *Horm. Metab. Res*. **24**, 543–545.

Corpas, E., Harman, S. M., Pineyro, M. A., Roberson, R., and Blackman, M. R. (1992b). Growth hormone (GH)-releasing hormone-(1-29) twice daily reverses the decreased GH and insulin-like growth factor-I levels in old men. *J. Clin. Endocrinol. Metab*. **75**, 530–535.

Cotman, C. W., Monaghan, D. T., and Ganong, A. H. (1988). Excitatory amino acid neurotransmission: NMDA receptors and Hebb-type synaptic plasticity. *Annu. Rev. Neurosci*. **11**, 61–80.

Cotman, C. W., Kahle, J. S., Miller, S. E., Ulas, J., and Bridges, R. J. (1995). Excitatory amino acid neurotransmission. *In* "Psychopharmacology: The Fourth Generation of Progress" (F. E. Bloom and D. J. Kupfer, eds.), pp. 7–22. Raven Press, New York.

Court, J. A., Perry, E. K., Johnson, M., Piggott, M. A., Kerwin, J. A., Perry, R. H., and Ince, P. G. (1993). Regional patterns of cholinergic and glutamate activity in the developing and aging human brain. *Brain Res. Dev. Brain Res*. **74**, 73–82.

Craik, F. I. M. (1977). Age differences in human memory. *In* "Handbook of the Psychology of aging" (J. E. Birren and K. W. Schaie, eds.), pp. 281–289. Van Nostrand-Reinhold, New York.

Creedon, D., and Tuttle, J. B. (1991). Nerve growth factor synthesis in vascular smooth muscle. *Hypertension* **18**, 730–741.

Crutcher, K. A., and Weingartner, J. (1991). Hippocampal NGF levels are not reduced in the aged Fischer 344 rat. *Neurobiol. Aging* **12**, 449–454.

Cupp, C. J., and Uemura, E. (1980). Age-related changes in prefrontal cortex of *Macaca mulatta*: Quantitative analysis of dendritic branching patterns. *Exp. Neurol*. **69**, 143–163.

Daughaday, W. H., and Rotwein, P. (1989). Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. *Endocr. Rev*. **10**, 68–91.

Dawson, V. L., Hsu, C. Y., Liu, T. H., Dawson, T. M., and Wamsley, J. K. (1994). Receptor alterations in subcortical structures after bilateral middle cerebral artery infarction of the cerebral cortex. *Exp. Neurol*. **128**, 88–96.

Dawson-Hughes, B., Stern, D., Goldman, J., and Reichlin, S. (1986). Regulation of growth hormone and somatomedin-C secretion in postmenopausal women: Effect of physiological estrogen replacement. *J. Clin. Endocrinol. Metab*. **63**, 424–432.

D'Costa, A. P., Lenham, J. E., Ingram, R. L., and Sonntag, W. E. (1993). Comparison of protein synthesis in brain and peripheral tissue during aging. Relationship to insulin-like growth factor-1 and type 1 IGF receptors. *Ann. N. Y. Acad. Sci*. **692**, 253–255.

D'Costa, A. P., Xu, X., Ingram, R. L., and Sonntag, W. E. (1995). Insulin-like growth factor-1 stimulation of protein synthesis is attenuated in cerebral cortex of aging rats. *Neuroscience* **65**, 805–813.

Decker, M. W. (1987). The effects of aging on hippocampal and cortical projections of the forebrain cholinergic system. *Brain Res*. **434**, 423–438.

DeGennaro, C. V., Zoli, M., Cocchi, D., Maggi, A., Marrama, P., Agnati, L. F., and Muller, E. E. (1989). Reduced growth hormone releasing factor (GHRF)-like immunoreactivity and GHRF gene expression in the hypothalamus of aged rats. *Peptides* **10**, 705–708.

De Keyser, J., Ebinger, G., and Vauquelin, G. (1990). Age-related changes in the human nigrostriatal dopaminergic system. *Ann. Neurol*. **27**, 157–161.

DeKosky, S. T., and Palmer, A. M. (1994). Neurochemistry of aging. *In* "Clinical Neurology of Aging" (M. L. Albert and J. E. Knoefel, eds.), 2nd ed., pp. 72–101. Oxford University Press, New York.

Delafontaine, P. (1995). Insulin-like growth factor I and its binding proteins in the cardiovascular system. *Cardiovasc. Res*. **30**, 825–834.

Delafontaine, P., Bernstein, K. E., and Alexander, R. W. (1991). Insulin-like growth factor I gene expression in vascular cells. *Hypertension* **17**, 693–699.

De Leo, V., Lanzetta, D., D'Antona, D., and Danero, S. (1993). Growth hormone secretion in premenopausal women before and after ovariectomy: Effect of hormone replacement therapy. *Fertil. Steril*. **60**, 268–271.

Deslauriers, N., Gaudreau, P., Abribat, T., Renier, G., Petitclerc, D., and Brazeau, P. (1991). Dynamics of growth hormone responsiveness to growth hormone releasing factor in aging rats: Peripheral and central influences. *Neuroendocrinology* **53**, 439–446.

Devaney, K. O., and Johnson, H. A. (1980). Neuron loss in the aging visual cortex of man. *J. Gerontol*. **35**, 836–841.

Dewey, S. L., Volkow, N. D., Logan, J., MacGregor, R. R., Fowler, J. S., Schlyer, D. J., and Bendriem, B. (1990). Age-related decreases in muscarinic cholinergic receptor binding in the human brain measured with positron emission tomography (PET). *J. Neurosci. Res*. **27**, 569–575.

Donahue, L. R., Hunter, S. J., Sherblom, A. P., and Rosen, C. (1990). Age-related changes in serum insulin-like growth factor-binding proteins in women. *J. Clin. Endocrinol. Metab*. **71**, 575–579.

Drachman, D. A., and Leavitt, J. (1974). Human memory and the cholinergic system. A relationship to aging? *Arch. Neurol. (Chicago)* **30**, 113–121.

Dubrovina, N. I., and Il'iuchenok, R. I. (1990). The role of the dopaminergic system and GABA-benzodiazepine- ionophore complex in the regulation of memory retrieval. *Fiziol. Zh. (Kiev)* **36**, 3–8.

Dudl, R. J., Ensinck, J. W., Palmer, H. E., and Williams, R. H. (1973). Effect of age on growth hormone secretion in man. *J. Clin. Endocrinol. Metab*. **37**, 11–16.

Durham, R. A., Eaton, M. J., Moore, K. E., and Lookingland, K. J. (1997). Effects of selective activation of dopamine D_2 and D_3 receptors on prolactin secretion and the activity of tuberoinfundibular dopamine neurons. *Eur. J. Pharmacol*. **335**, 37–42.

Elahi, D., Muller, D. C., Tzankoff, S. P., Andres, R., and Tobin, J. D. (1982). Effect of age and obesity on fasting levels of glucose, insulin, glucagon, and growth hormone in man. *J. Gerontol*. **37**, 385–391.

Elias, M. F., D'Agostino, R. B., Elias, P. K., and Wolf, P. A. (1995). Neuropsychological test performance, cognitive functioning, blood pressure, and age: The Framingham Heart Study. *Exp. Aging Res*. **21**, 369–391.

Engelmann, G. L., Boehm, K. D., Haskell, J. F., Khairallah, P. A., and Ilan, J. (1989). Insulin-like growth factors and neonatal cardiomyocyte development: Ventricular gene expression and membrane receptor variations in normotensive and hypertensive rats. *Mol. Cell. Endocrinol*. **63**, 1–14.

Evans, M. H., and Long, J. A. (1921). The effect of the anterior lobe administered intraperitoneally upon growth, maturity and oestrus cycles of the rat. *Anat. Rec*. **21**, 62–63.

Farese, R. V., Nair, G. P., Sierra, C. G., Standaert, M. L., Pollet, R. J., and Cooper, D. R. (1989). Insulin-like effects of epidermal growth factor and insulin-like growth factor-I on [^{3}H]2-deoxyglucose uptake, diacylglycerol generation and protein kinase C activation in BC3H-1 myocytes. *Biochem. J*. **261**, 927–934.

Feldman, M. L., and Dowd, C. (1975). Loss of dendritic spines in aging cerebral cortex. *Anat. Embryol*. **148**, 279–301.

Finkelstein, J. W., Roffwarg, H. P., Boyar, R. M., Kream, J., and Hellman, L. (1972). Age-related change in the twenty-four-hour spontaneous secretion of growth hormone. *J. Clin. Endocrinol. Metab*. **35**, 665–670.

Fischer, W. (1994). Nerve growth factor reverses spatial memory impairments in aged rats. *Neurochem. Int*. **25**, 47–52.

Fischer, W., Wictorin, K., Björklund, A., Williams, L. R., Varon, S., and Gage, F. H. (1987). Amelioration of cholinergic neuron atrophy and spatial memory impairment in aged rats by nerve growth factor. *Nature (London)* **329**, 65–68.

Fischer, W., Sirevaag, A., Wiegand, S. J., Lindsay, R. M., and Björklund, A. (1994). Reversal of spatial memory impairments in aged rats by nerve growth factor and neurotrophins 3 and 4/5 but not by brain-derived neurotrophic factor. *Proc. Natl. Acad. Sci. U. S. A*. **91**, 8607–8611.

Florini, J. R., and Roberts, S. B. (1980). Effect of rat age on blood levels of somatomedin-like growth factors. *J. Gerontol.* **35**, 23–30.

Florini, J. R., Harned, J. A., Richman, R. A., and Weiss, J. P. (1981). Effect of rat age on serum levels of growth hormone and somatomedins. *Mech. Ageing Dev.* **15**, 165–176.

Folkow, B., Isaksson, O. P., Karlstrom, G., Lever, A. F., and Nordlander, M. (1988). The importance of hypophyseal hormones for structural cardiovascular adaptation in hypertension. *J. Hypertension* **6** (Suppl.), S166–S169.

Foos, P. W. (1989). Age differences in memory for two common objects. *J. Gerontol.* **44**, 178–180.

Foster, J. A., Rich, C. B., Miller, M., Benedict, M. R., Richman, R. A., and Florini, J. R. (1990). Effect of age and IGF-I administration on elastin gene expression in rat aorta. *J. Gerontol.* **45**, B113–B118.

Friedman, E., and Wang, H. Y. (1989). Effect of age on brain cortical protein kinase C and its mediation of 5-hydroxytryptamine release. *J. Neurochem.* **52**, 187–192.

Friend, K. E., Hartman, M. L., Pezzoli, S. S., Clasey, J. L., and Thorner, M. O. (1996). Both oral and transdermal estrogen increase growth hormone release in postmenopausal women—a clinical research center study. *J. Clin. Endocrinol. Metab.* **81**, 2250–2256.

Frost, R. A., Nachman, S. A., Lang, C. H., and Gelato, M. C. (1996). Proteolysis of insulin-like growth factor-binding protein-3 in human immunodeficiency virus-positive children who fail to thrive. *J. Clin. Endocrinol. Metab.* **81**, 2957–2962.

Gage, F. H., Dunnett, S. B., and Bjorklund, A. (1984). Spatial learning and motor deficits in aged rats. *Neurobiol. Aging* **5**, 43–48.

Gallagher, M., and Burwell, R. D. (1989). Relationship of age-related decline across several behavioral domains. *Neurobiol. Aging* **10**, 691–708.

Geffner, M. (1997). Effects of growth hormone and insulin-like growth factor I on T- and B-lymphocytes and immune function. *Acta Paediatr.* **423** (Suppl.), 76–79.

Geinisman, Y. (1979). Loss of axosomatic synapses in the dentate gyrus of aged rats. *Brain Res.* **168**, 485–492.

Geinisman, Y., Bondareff, W., and Dodge, J. T. (1977). Partial deafferentation of neurons in the dentate gyrus of the senescent rat. *Brain Res.* **134**, 541–545.

Gelato, M. C. (1996). Aging and immune function: A possible role for growth hormone. *Horm. Res.* **45**, 46–49.

Gelato, M. C., Rittmaster, R. S., Pescovitz, O. H., Nicoletti, M. C., Nixon, W. E., D'Agata, R., Loriaux, D. L., and Merriam, G. R. (1985a). Growth hormone responses to continuous infusions of growth hormone-releasing hormone. *J. Clin. Endocrinol. Metab.* **61**, 223–228.

Gelato, M. C., Ross, J. L., Malozowski, S., Pescovitz, O. H., Skerda, M., Cassorla, F., Loriaux, D. L., and Merriam, G. R. (1985b). Effects of pulsatile administration of growth hormone (GH)-releasing hormone on short term linear growth in children with GH deficiency. *J. Clin. Endocrinol. Metab.* **61**, 444–450.

Gibson, P. H. (1983). EM study of the numbers of cortical synapses in the brains of ageing people and people with Alzheimer-type dementia. *Acta Neuropathol.* **62**, 127–133.

Goldman, H., Berman, R. F., Gershon, S., Murphy, S. L., and Altman, H. J. (1987). Correlation of behavioral and cerebrovascular functions in the aging rat. *Neurobiol. Aging* **8**, 409–416.

Goldman, J. E., Calingasan, N. Y., and Gibson, G. E. (1994). Aging and the brain. *Curr. Opin. Neurol.* **7**, 287–293.

Goldman-Rakic, P. S., and Brown, R. M. (1981). Regional changes of monoamines in cerebral cortex and subcortical structures of aging rhesus monkeys. *Neuroscience* **6**, 177–187.

Goldsmith, S. J., and Glick, S. M. (1970). Rhythmicity of human growth hormone secretion. *Mt. Sinai J. Med.* **37**, 501–509.

Goldspink, D. F., and Goldberg, A. L. (1975). Influence of pituitary growth hormone on DNA synthesis in rat tissues. *Am. J. Physiol.* **228**, 302–309.

Goodman, H. M. (1963). Effects of chronic growth hormone treatment on lipogenesis by rat adipose tissue. *Endocrinology (Baltimore)* **61**, 95–101.

Gossard, F., Dihl, F., Pelletier, G., Dubois, P. M., and Morel, G. (1987). *In situ* hybridization to rat brain and pituitary gland of growth hormone cDNA. *Neurosci. Lett.* **79**, 251–256.

Gould, J., Aramburo, C., Capdevielle, M., and Scanes, C. G. (1995). Angiogenic activity of anterior pituitary tissue and growth hormone on the chick embryo chorio-allantoic membrane: A novel action of GH. *Life Sci.* **56**, 587–594.

Grant, M. B., Mames, R. N., Fitzgerald, C., Ellis, E. A., Caballero, S., Chegini, N., and Guy, J. (1993). Insulin-like growth factor I as an angiogenic agent. In vivo and in vitro studies. *Ann. N. Y. Acad. Sci.* **692**, 230–242.

Grimwood, S., Gilbert, E., Ragan, C. I., and Hutson, P. H. (1996). Modulation of 45Ca2+ influx into cells stably expressing recombinant human NMDA receptors by ligands acting at distinct recognition sites. *J. Neurochem.* **66**, 2589–2595.

Guttentag, R. E., and Hunt, R. R. (1988). Adult age differences in memory for imagined and performed actions. *J. Gerontol.* **43**, 107–108.

Guttentag, R. E., and Madden, D. J. (1987). Adult age differences in the attentional capacity demands of letter matching. *Exp. Aging Res.* **13**, 93–99.

Han, V. K., Lauder, J. M., and D'Ercole, A. J. (1988). Rat astroglial somatomedin/insulin-like growth factor binding proteins: Characterization and evidence of biologic function. *J. Neurosci.* **8**, 3135–3143.

Han, V. K., Smith, A., Myint, W., Nygard, K., and Bradshaw, S. (1992). Mitogenic activity of epidermal growth factor on newborn rat astroglia: Interaction with insulin-like growth factors. *Endocrinology (Baltimore)* **131**, 1134–1142.

Hansson, H. A., Dahlin, L. B., Danielsen, N., Fryklund, L., Nachemson, A. K., Polleryd, P., Rozell, B., Skottner, A., Stemme, S., and Lundborg, G. (1986). Evidence indicating trophic importance of IGF-I in regenerating peripheral nerves. *Acta Physiol. Scand.* **126**, 609–614.

Hansson, H. A., Jennische, E., and Skottner, A. (1987). Regenerating endothelial cells express insulin-like growth factor-I immunoreactivity after arterial injury. *Cell Tissue Res.* **250**, 499–505.

Hansson, H. A., Brandsten, C., Lossing, C., and Petruson, K. (1989). Transient expression of insulin-like growth factor I immunoreactivity by vascular cells during angiogenesis. *Exp. Mol. Pathol.* **50**, 125–138.

Hasenohrl, R. U., Soderstrom, S., Mohammed, A. H., Ebendal, T., and Huston, J. P. (1997). Reciprocal changes in expression of mRNA for nerve growth factor and its receptors TrkA and LNGFR in brain of aged rats in relation to maze learning deficits. *Exp. Brain Res* **114**, 205–213.

Haug, H., and Eggers, R. (1991). Morphometry of the human cortex cerebri and corpus striatum during aging. *Neurobiol. Aging* **12**, 336–338.

Hellweg, R., Fischer, W., Hock, C., Gage, F. H., Björklund, A., and Thoenen, H. (1990). Nerve growth factor levels and choline acetyltransferase activity in the brain of aged rats with spatial memory impairments. *Brain Res.* **537**, 123–130.

Henderson, G., Tomlinson, B. E., and Gibson, P. H. (1980). Cell counts in human cerebral cortex in normal adults throughout life using an image analysing computer. *J. Neurol. Sci.* **46**, 113–136.

Henry, J. M., Filburn, C. R., Joseph, J. A., and Roth, G. S. (1986). Effect of aging on striatal dopamine receptor subtypes in Wistar rats. *Neurobiol. Aging* **7**, 357–361.

Hess, E. J., Bracha, H. S., Kleinman, J. E., and Creese, I. (1987). Dopamine receptor subtype imbalance in schizophrenia. *Life Sci.* **40**, 1487–1497.

Hierholzer, J., Cordes, M., Venz, S., Schelosky, L., Harisch, C., Richter, W., Keske, U., Hosten, N., Maurer, J., Poewe, W., and Felix, R. (1998). Loss of dopamine-D_2 receptor binding sites in Parkinsonian plus syndromes. *J. Nucl. Med.* **39**, 954–960.

Ho, K. K., O'Sullivan, A. J., Weissberger, A. J., and Kelly, J. J. (1996). Sex steroid regulation of growth hormone secretion and action. *Horm. Res*. **45**, 67–73.

Ho, K. Y., and Weissberger, A. J. (1990). Secretory patterns of growth hormone according to sex and age. *Horm. Res*. **33** (Suppl. 4), 7–11.

Ho, K. Y., Evans, W. S., Blizzard, R. M., Veldhuis, J. D., Merriam, G. R., Samojlik, E., Furlanetto, R., Rogol, A. D., Kaiser, D. L., and Thorner, M. O. (1987). Effects of sex and age on the 24-hour profile of growth hormone secretion in man: Importance of endogenous estradiol concentrations. *J. Clin. Endocrinol. Metab.* **64**, 51–58.

Hojvat, S., Baker, G., Kirsteins, L., and Lawrence, A. M. (1982). Growth hormone (GH) immunoreactivity in the rodent and primate CNS: Distribution, characterization and presence posthypophysectomy. *Brain Res.* **239**, 543–557.

Homburg, R., and Farhi, J. (1995). Growth hormone and reproduction. *Curr. Opin. Obstet. Gynecol*. **7**, 220–223.

Howe, M. L. (1988). Measuring memory development in adulthood: A model-based approach to disentangling storage-retrieval contributions. *In* "Cognitive Development in Adulthood: Progress in Cognitive Development Research" (M. L. Howe and C. J. Brainerd, eds.), pp. 94–117. Springer-Verlag, New York.

Hughes, C. C., and Lantos, P. L. (1987). A morphometric study of blood vessel, neuron and glial cell distribution in young and old rat brain. *J. Neurol. Sci*. **79**, 101–110.

Hultsch, D. F., Hertzog, C., and Dixon, R. A. (1990). Ability correlates of memory performance in adulthood and aging. *Psychol. Aging* **5**, 356–368.

Hunziker, O., Abdel'Al, S., and Schulz, U. (1979). The aging human cerebral cortex: A stereological characterization of changes in the capillary net. *J. Gerontol*. **34**, 345–350.

Hynes, M. A., Van Wyk, J. J., Brooks, P. J., D'Ercole, A. J., Jansen, M., and Lund, P. K. (1987). Growth hormone dependence of somatomedin-C/insulin-like growth factor-I and insulin-like growth factor-II messenger ribonucleic acids. *Mol. Endocrinol*. **1**, 233–242.

Illig, R., and Prader, A. (1970). Effect of testosterone on growth hormone secretion in patients with anorchia and delayed puberty. *J. Clin. Endocrinol. Metab.* **30**, 615–618.

Ingram, D. K., London, E. D., and Goodrick, C. L. (1981). Age and neurochemical correlates of radial maze performance in rats. *Neurobiol. Aging* **2**, 41–47.

Ingram, D. K., Spangler, E. L., Iijima, S., Ikari, H., Kuo, H., Greig, N. H., and London, E. D. (1994). Rodent models of memory dysfunction in Alzheimer's disease and normal aging: Moving beyond the cholinergic hypothesis. *Life Sci*. **55**, 2037–2049.

Iranmanesh, A., Lizarralde, G., and Veldhuis, J. D. (1991). Age and relative adiposity are specific negative determinants of the frequency and amplitude of growth hormone (GH) secretory bursts and the half-life of endogenous GH in healthy men. *J. Clin. Endocrinol. Metab.* **73**, 1081–1088.

Isgaard, J., Carlsson, L., Isaksson, O. G., and Jansson, J. O. (1988). Pulsatile intravenous growth hormone (GH) infusion to hypophysectomized rats increases insulin-like growth factor I messenger ribonucleic acid in skeletal tissues more effectively than continuous GH infusion. *Endocrinology (Baltimore)* **123**, 2605–2610.

Ishii, D. N. (1989). Relationship of insulin-like growth factor II gene expression in muscle to synaptogenesis. *Proc. Natl. Acad. Sci. U. S. A*. **86**, 2898–2902.

Ishii, T., Moriyoshi, K., Sugihara, H., Sakurada, K., Kadotani, H., Yokoi, M., Akazawa, C., Shigemoto, R., Mizuno, N., and Masu, M. (1993). Molecular characterization of the family of the N-methyl-D-aspartate receptor subunits. *J. Biol. Chem*. **268**, 2836–2843.

Ito, M. (1989). Long-term depression. *Annu. Rev. Neurosci*. **12**, 85–102.

Johanson, A. J., and Blizzard, R. M. (1981). Low somatomedin-C levels in older men rise in response to growth hormone administration. *Johns Hopkins Med. J*. **149**, 115–117.

Johansson, J. O., Larson, G., Andersson, M., Elmgren, A., Hynsjo, L., Lindahl, A., Lundberg, P. A., Isaksson, O. G., Lindstedt, S., and Bengtsson, B. A. (1995). Treatment of growth hormone-deficient adults with recombinant human growth hormone increases the concentration of growth hormone in the cerebrospinal fluid and affects neurotransmitters. *Neuroendocrinology* **61**, 57–66.

Johnson, M. D., Zhou, Y., Friedman, E., and Roberts, J. (1995). Expression of G protein alpha subunits in the aging cardiovascular system. *J. Gerontol.* **50**, B14–B19.

Johnston, D., Magee, J. C., Colbert, C. M., and Cristie, B. R. (1996). Active properties of neuronal dendrites. *Annu. Rev. Neurosci*. **19**, 165–186.

Jones, J. I., and Clemmons, D. R. (1995). Insulin-like growth factors and their binding proteins: Biological actions. *Endocr. Rev.* **16**, 3–34.

Jorgensen, J. O. (1991). Human growth hormone replacement therapy: Pharmacological and clinical aspects. *Endocr. Rev.* **12**, 189–207.

Jorgensen, J. O., Thuesen, L., Muller, J., Ovesen, P., Skakkebaek, N. E., and Christiansen, J. S. (1994). Three years of growth hormone treatment in growth hormone-deficient adults: Near normalization of body composition and physical performance. *Eur. J. Endocrinol*. **130**, 224–228.

Jorgensen, J. O., Vahl, N., Fisker, S., Norrelund, H., Nielsen, S., Dall, R., and Christiansen, J. S. (1997). Somatopause and adiposity. *Horm. Res*. **48** (Suppl. 5), 101–104.

Jorgensen, P. H., Andreassen, T. T., and Jorgensen, K. D. (1989). Growth hormone influences collagen deposition and mechanical strength of intact rat skin. A dose-response study. *Acta Endocrinol*. **120**, 767–772.

Joseph, J. A., Algeri, S., De-Cesare, A., Comuzio, M., Erat, S., Kelly, J., Cagnotto, A., and Mennini, T. (1995). A reduced calorie-high fiber diet retards age-associated decreases in muscarinic receptor sensitivity. *Neurobiol. Aging* **16**, 607–612.

Jucker, M., Battig, K., and Meier-Ruge, W. (1990). Effects of aging and vincamine derivatives on pericapillary microenvironment: Stereological characterization of the cerebral capillary network. *Neurobiol. Aging* **11**, 39–46.

Kaiser, F. E., Silver, A. J., and Morley, J. E. (1991). The effect of recombinant human growth hormone on malnourished older individuals. *J. Am. Geriatr. Soc*. **39**, 235–240.

Kassem, M., Mosekilde, L., and Eriksen, E. F. (1994). Growth hormone stimulates proliferation of normal human bone marrow stromal osteoblast precursor cells in vitro. *Growth Regul*. **4**, 131–135.

Katoh-Semba, R., Semba, R., Kashiwamata, S., and Kato, K. (1991). Elevated concentrations of beta-nerve growth factor in selected tissues from senescence-accelerated mice (SAM-P/8). *Mech. Ageing Dev*. **59**, 163–175.

Kausler, D. H., and Lichty, W. (1988). Memory for activities: Rehearsal-independence and aging. *In* "Language, Memory, and Aging" (L. L. Light and D. M. Burke, eds.), pp. 136–149. Cambridge University Press, New York.

Kelijman, M., and Frohman, L. A. (1988). Enhanced growth hormone (GH) responsiveness to GH-releasing hormone after dietary manipulation in obese and nonobese subjects. *J. Clin. Endocrinol. Metab*. **66**, 489–494.

Kelley, K. M., Oh, Y., Gargosky, S. E., Gucev, Z., Matsumoto, T., Hwa, V., Ng, L., Simpson, D. M., and Rosenfeld, R. G. (1996). Insulin-like growth factor-binding proteins (IGFBPs) and their regulatory dynamics. *Int. J. Biochem. Cell Biol*. **28**, 619–637.

Kelley, K. W. (1990). The role of growth hormone in modulation of the immune response. *Ann. N. Y. Acad. Sci*. **594**, 95–103.

Kelley, K. W., Brief, S., Westly, H. J., Novakofski, J., Bechtel, P. J., Simon, J., and Walker, E. B. (1986). GH3 pituitary adenoma cells can reverse thymic aging in rats. *Proc. Natl. Acad. Sci. U. S. A*. **83**, 5663–5667.

Kelley, K. W., Davila, D. R., Brief, S., Simon, J., and Arkins, S. (1988). A pituitary-thymus connection during aging. *Ann. N. Y. Acad. Sci*. **521**, 88–98.

Kelly, M. A., Rubinstein, M., Phillips, T. J., Lessov, C. N., Burkhart-Kasch, S., Zhang, G., Bunzow, J. R., Fang, Y., Gerhardt, G. A., Grandy, D. K., and Low, M. J. (1998). Locomotor activity in D_2 dopamine receptor-deficient mice is determined by gene dosage, genetic background, and developmental adaptations. *J. Neurosci*. **18**, 3470–3479.

Kety, S. S. (1956). Human cerebral blood flow and oxygen consumption as related to aging. *J. Chronic Dis*. **8**, 478–486.

Khansari, D. N., and Gustad, T. (1991). Effects of long-term, low-dose growth hormone therapy on immune function and life expectancy of mice. *Mech. Ageing Dev.* **57**, 87–100.

Khorram, O., Laughlin, G. A., and Yen, S. S. (1997). Endocrine and metabolic effects of long-term administration of [Nle^{27}]growth hormone-releasing hormone-(1-29)-NH_2 in age-advanced men and women. *J. Clin. Endocrinol. Metab.* **82**, 1472–1479.

Kish, S. J., el-Awar, M., Schut, L., Leach, L., Oscar-Berman, M., and Freedman, M. (1988). Cognitive deficits in olivopontocerebellar atrophy: Implications for the cholinergic hypothesis of Alzheimer's dementia. *Ann. Neurol*. **24**, 200–206.

Knusel, B., and Hefti, F. (1991). Trophic actions of IGF-I, IGF-II and insulin on cholinergic and dopaminergic brain neurons. *Adv. Exp. Med. Biol*. **293**, 351–360.

Korbonits, M., Little, J. A., Camacho-Hubner, C., Trainer, P. J., Besser, G. M., and Grossman, A. B. (1996). Insulin-like growth factor-I and- II in combination inhibit the release of growth hormone-releasing hormone from the rat hypothalamus in vitro. *Growth Regul*. **6**, 110–120.

Kostyo, J. L. (1968). Rapid effects of growth hormone on amino acid transport and protein synthesis. *Ann. N. Y. Acad. Sci*. **148**, 389–407.

Lai, Z., Roos, P., Zhai, O., Olsson, Y., Fholenhag, K., Larsson, C., and Nyberg, F. (1993). Age-related reduction of human growth hormone-binding sites in the human brain. *Brain Res*. **621**, 260–266.

Landfield, P. W., Braun, L. D., Pitler, T. A., Lindsey, J. D., and Lynch, G. (1981). Hippocampal aging in rats: A morphometric study of multiple variables in semithin sections. *Neurobiol. Aging* **2**, 265–275.

Landfield, P. W., McEwan, B. S., Sapolsky, R. M., and Meaney, M. J. (1996). Hippocampal cell death. *Science* **272**, 1249–1251.

Langdahl, B. L., Kassem, M., Moller, M. K., and Eriksen, E. F. (1998). The effects of IGF-I and IGF-II on proliferation and differentiation of human osteoblasts and interactions with growth hormone. *Eur. J. Clin. Invest*. **28**, 176–183.

Larkfors, L., Ebendal, T., Whittemore, S. R., Persson, H., Hoffer, B., and Olson, L. (1987). Decreased level of nerve growth factor (NGF) and its messenger RNA in the aged rat brain. *Brain Res*. **427**, 55–60.

Laron, Z., Doron, M., and Arnikan, B. (1970). Plasma growth hormone in men and woman over 70 years of age. *Med. Sports Phys. Act. Aging* **4**, 126–129.

Laron, Z., Aran, O., Nofar, E., Beit-Halachmi, N., Pertzelan, A., and Galatzer, A. (1986). Psychosocial aspects of young adult growth hormone deficient patients previously treated with human growth hormone–a preliminary report. *Acta Paediatr. Scand.* **325** (Suppl.), 80–82.

Lee, P. D., Conover, C. A., and Powell, D. R. (1993). Regulation and function of insulin-like growth factor-binding protein-1. *Proc. Soc. Exp. Biol. Med*. **204**, 4–29.

Lee, V., Ramachandran, J., and Li, C. H. (1975). Human pituitary growth hormone. Intrinsic lipolytic activity on rabbit fat cells. *Arch. Biochem. Biophys*. **169**, 669–677.

Lenoir, D., and Honegger, P. (1983). Insulin-like growth factor I (IGF I) stimulates DNA synthesis in fetal rat brain cell cultures. *Brain Res*. **283**, 205–213.

Leuba, G. (1983). Aging of dendrites in the cerebral cortex of the mouse. *Neuropathol. Appl. Neurobiol*. **9**, 467–475.

Li, C. H., and Evans, M. H. (1944). The isolation of pituitary growth hormone. *Science* **99**, 183–184.

Li, C. H., and Papkoff, H. (1956). Preparation and properties of growth hormone from human and monkey pituitary glands. *Science* **124**, 1293–1294.

Li, C. H., Dixon, J. S., and Liu, W. K. (1969). Human pituitary growth hormone. XIX. The primary structure of the hormone. *Arch. Biochem. Biophys*. **133**, 70–91.

Lieberman, S. A., Mitchell, A. M., Marcus, R., Hintz, R. L., and Hoffman, A. R. (1994). The insulin-like growth factor I generation test: Resistance to growth hormone with aging and estrogen replacement therapy. *Horm. Metab. Res*. **26**, 229–233.

Light, L. L. (1991). Memory and aging: Four hypotheses in search of data. *Annu. Rev. Psychol*. **42**, 333–376.

Ling, N., Esch, F., Bohlen, P., Brazeau, P., Wehrenberg, W. B., and Guillemin, R. (1984). Isolation, primary structure, and synthesis of human hypothalamic somatocrinin: Growth hormone-releasing factor. *Proc. Natl. Acad. Sci. U. S. A*. **81**, 4302–4306.

Lobie, P. E., Garcia-Aragon, J., Lincoln, D. T., Barnard, R., Wilcox, J. N., and Waters, M. J. (1993). Localization and ontogeny of growth hormone receptor gene expression in the central nervous system. *Dev. Brain Res*. **74**, 225–233.

Lolova, I. (1989). Dendritic changes in the hippocampus of aged rats. *Acta Morphol. Hung*. **37**, 3–10.

Lombardi, G., Colao, A., Cuocolo, A., Longobardi, S., Di Somma, C., Orio, F., Merola, B., Nicolai, E., and Salvatore, M. (1997a). Cardiological aspects of growth hormone and insulin-like growth factor-I. *J. Pediatr. Endocrinol. Metab.* **10**, 553–560.

Lombardi, G., Colao, A., Ferone, D., Marzullo, P., Orio, F., Longobardi, S., and Merola, B. (1997b). Effect of growth hormone on cardiac function. *Horm. Res*. **48** (Suppl. 4), 38–42.

Lopez-Fernandez, J., Sanchez-Franco, F., Velasco, B., Tolon, R. M., Pazos, F., and Cacicedo, L. (1996). Growth hormone induces somatostatin and insulin-like growth factor I gene expression in the cerebral hemispheres of aging rats. *Endocrinology (Baltimore)* **137**, 4384–4391.

Lynch, C. D., Cooney, P. T., Hutchins, P. M., Bennett S. A., Thornton, P. L., Khan, A. S., Ingram, R. L., and Sonntag, W. E. (1999). Effects of moderate caloric restriction on cortical microvascular density and local cerebral blood flow in aged rats. *Neurobiol. Aging* **20**, 191–200.

Magnusson, K. R., and Cotman, C. W. (1993). Age-related changes in excitatory amino acid receptors in two mouse strains. *Neurobiol. Aging* **14**, 197–206.

Mann, D. M., Eaves, N. R., Marcyniuk, B., and Yates, P. O. (1986). Quantitative changes in cerebral cortical microvasculature in ageing and dementia. *Neurobiol. Aging* **7**, 321–330.

Marcus, R., Butterfield, G., Holloway, L., Gilliland, L., Baylink, D. J., Hintz, R. L., and Sherman, B. M. (1990). Effects of short term administration of recombinant human growth hormone to elderly people. *J. Clin. Endocrinol. Metab*. **70**, 519–527.

Markowska, A. L., Koliatsos, V. E., Breckler, S. J., Price, D. L., and Olton, D. S. (1994). Human nerve growth factor improves spatial memory in aged but not in young rats. *J. Neurosci*. **14**, 4815–4824.

Markowska, A. L., Price, D., and Koliatsos, V. E. (1996). Selective effects of nerve growth factor on spatial recent memory as assessed by a delayed nonmatching-to-position task in the water maze. *J. Neurosci*. **16**, 3541–3548.

Markowska, A. L., Mooney, M., and Sonntag, W. E. (1998). Insulin-like growth factor-1 (IGF-1) ameliorates age-related behavioral deficits. *Neuroscience* **87**, 559–569.

Marshall, J. F. (1982). Sensorimotor disturbances in the aging rodent. *J. Gerontol*. **37**, 548–554.

Marshall, J. F., and Berrios, N. (1979). Movement disorders of aged rats: Reversal by dopamine receptor stimulation. *Science* **206**, 477–479.

Martha, P. M. J., and Reiter, E. O. (1991). Pubertal growth and growth hormone secretion. *Endocrinol. Metab. Clin. North Am*. **20**, 165–182.

Martha, P. M. J., Blizzard, R. M., McDonald, J. A., Thorner, M. O., and Rogol, A. D. (1988). A persistent pattern of varying pituitary responsivity to exogenous growth hormone (GH)-releasing hormone in GH-deficient children: Evidence supporting periodic somatostatin secretion. *J. Clin. Endocrinol. Metab*. **67**, 449–454.

Martha, P. M. J., Gorman, K. M., Blizzard, R. M., Rogol, A. D., and Veldhuis, J. D. (1992). Endogenous growth hormone secretion and clearance rates in normal boys, as determined by deconvolution analysis: Relationship to age, pubertal status, and body mass. *J. Clin. Endocrinol. Metab.* **74**, 336–344.

Martin, L. G., Clark, J. W., and Connor, T. B. (1968). Growth hormone secretion enhanced by androgens. *J. Clin. Endocrinol. Metab.* **28**, 425–428.

Martinez, D. A., Orth, M. W., Carr, K. E., Vanderby, R. J., and Vailas, A. C. (1996). Cortical bone growth and maturational changes in dwarf rats induced by recombinant human growth hormone. *Am. J. Physiol*. **270**, E51–E59.

Martinez, J. A., Del Barrio, A. S., and Larralde, J. (1991). Evidence of a short-term effect of growth hormone on in vivo bone protein synthesis in normal rats. *Biochim. Biophys. Acta* **1093**, 111–113.

Mayer, M. L., and Westbrook, G. L. (1987). The physiology of excitatory amino acids in the vertebrate central nervous system. *Prog. Neurobiol*. **28**, 197–276.

Maylor, E. A. (1990). Recognizing and naming faces: Aging, memory retrieval, and the tip of the tongue state. *J. Gerontol*. **45**, 215–226.

McGauley, G. A. (1989). Quality of life assessment before and after growth hormone treatment in adults with growth hormone deficiency. *Acta Paediatr. Scand.* **356** (Suppl.), 70–72.

McGauley, G. A., Cuneo, R. C., Salomon, F., and Sonksen, P. H. (1990). Psychological well-being before and after growth hormone treatment in adults with growth hormone deficiency. *Horm. Res.* **33** (Suppl. 4), 52–54.

McMorris, F. A., and Dubois-Dalcq, M. (1988). Insulin-like growth factor I promotes cell proliferation and oligodendroglial commitment in rat glial progenitor cells developing in vitro. *J. Neurosci. Res*. **21**, 199–209.

McMorris, F. A., Smith, T. M., DeSalvo, S., and Furlanetto, R. W. (1986). Insulin-like growth factor I/somatomedin C: A potent inducer of oligodendrocyte development. *Proc. Natl. Acad. Sci. U. S. A*. **83**, 822–826.

Meguro, H., Mori, H., Araki, K., Kushiya, E., Kutsuwada, T., Yamazaki, M., Kumanishi, T., Arakawa, M., Sakimura, K., and Mishina, M. (1992). Functional characterization of a heteromeric NMDA receptor channel expressed from cloned cDNAs. *Nature (London)* **357**, 70–74.

Meier-Ruge, W., Hunziker, O., Schulz, U., Tobler, H. J., and Schweizer, A. (1980). Stereological changes in the capillary network and nerve cells of the aging human brain. *Mech. Ageing Dev.* **14**, 233–243.

Meites, J., and Sonntag, W. E. (1981). Hypothalamic hypophysiotropic hormones and neurotransmitter regulation: Current views. *Annu. Rev. Pharmacol. Toxicol*. **21**, 295–322.

Melamed, E., Lavy, S., Bentin, S., Cooper, G., and Rinot, Y. (1980). Reduction in regional cerebral blood flow during normal aging in man. *Stroke* **11**, 31–35.

Merchav, S., Tatarsky, I., and Hochberg, Z. (1988). Enhancement of erythropoiesis in vitro by human growth hormone is mediated by insulin-like growth factor I. *Br. J. Haematol*. **70**, 267–271.

Mohan, S., Farley, J. R., and Baylink, D. J. (1995). Age-related changes in IGFBP-4 and IGFBP-5 levels in human serum and bone: Implications for bone loss with aging. *Prog. Growth Factor Res*. **6**, 465–473.

Monaghan, D. T., Bridges, R. J., and Cotman, C. W. (1989). The excitatory amino acid receptors: Their classes, pharmacology, and distinct properties in the function of the central nervous system. *Annu. Rev. Pharmacol. Toxicol*. **29**, 365–402.

Monyer, H., Sprengel, R., Schoepfer, R., Herb, A., Higuchi, M., Lomeli, H., Burnashev, N., Sakmann, B., and Seeburg, P. H. (1992). Heteromeric NMDA receptors: Molecular and functional distinction of subtypes. *Science* **256**, 1217–1221.

Morgan, D. G. (1987). The dopamine and serotonin systems during aging in human and rodent brain. A brief review. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **11**, 153–157.

Morimoto, N., Kawakami, F., Makino, S., Chihara, K., Hasegawa, M., and Ibata, Y. (1988). Age-related changes in growth hormone releasing factor and somatostatin in the rat hypothalamus. *Neuroendocrinology* **47**, 459–464.

Moroi-Fetters, S. E., Mervis, R. F., London, E. D., and Ingram, D. K. (1989). Dietary restriction suppresses age-related changes in dendritic spines. *Neurobiol. Aging* **10**, 317–322.

Morrell, R. W., Park, D. C., and Poon, L. W. (1989). Quality of instructions on prescription drug labels: Effects on memory and comprehension in young and old adults. *Gerontologist* **29**, 345–354.

Moss, M. B., Rosene, D. L., and Peters, A. (1988). Effects of aging on visual recognition memory in the rhesus monkey. *Neurobiol. Aging* **9**, 495–502.

Nachemson, A. K., Lundborg, G., and Hansson, H. A. (1990). Insulin-like growth factor I promotes nerve regeneration: An experimental study on rat sciatic nerve. *Growth Factors* **3**, 309–314.

Nakamoto, J. M., Gertner, J. M., Press, C. M., Hintz, R. L., Rosenfeld, R. G., and Genel, M. (1986). Suppression of the growth hormone (GH) response to clonidine and GH-releasing hormone by exogenous GH. *J. Clin. Endocrinol. Metab.* **62**, 822–826.

Nakamura, S., Akiguchi, I., Kameyama, M., and Mizuno, N. (1985). Age-related changes of pyramidal cell basal dendrites in layers III and V of human motor cortex: A quantitative Golgi study. *Acta Neuropathol.* **65**, 281–284.

Narisawa-Saito, M., and Nawa, H. (1996). Differential regulation of hippocampal neurotrophins during aging in rats. *J. Neurochem.* **67**, 1124–1131.

Niall, H. D. (1971). Revised primary structure for human growth hormone. *Nature (London) New Biol*. **230**, 90–91.

Niblock, M. M., Lynch, C. D., Cooney, P. T., McShane, T., Brunso-Bechtold, J. K., and Sonntag, W. E. (1996). Neuronal IGF-1 mRNA expression during development and aging in Fisher 344 x Brown Norway rats. *Soc. Neurosci. Abstr.* **22**, 1236.

Niblock, M. M., Riddle, D. R., and Brunso-Bechtold, J. K. (1997). Laminar variation in neuronal viability in a slice preparation of the neonatal rat brain. *Soc. Neurosci. Abstr.* **23**, 902.

Nilsson, L., Sara, V. R., and Nordberg, A. (1988). Insulin-like growth factor 1 stimulates the release of acetylcholine from rat cortical slices. *Neurosci. Lett*. **88**, 221–226.

Noall, M. W., Riggs, T. R., Walker, L. M., and Christensen, H. N. (1957). Endocrine control of amino acid transfer. Distribution of an unmetabolizable amino acid. *Science* **126**, 1002–1004.

Nordberg, A., Alafuzoff, I., and Winblad, B. (1992). Nicotinic and muscarinic subtypes in the human brain: Changes with aging and dementia. *J. Neurosci. Res*. **31**, 103–111.

Norman, A. B., Blaker, S. N., Thal, L., and Creese, I. (1986). Effects of aging and cholinergic deafferentation on putative muscarinic cholinergic receptor subtypes in rat cerebral cortex. *Neurosci. Lett*. **70**, 289–294.

Nunzi, M. G., Milan, F., Guidolin, D., and Toffano, G. (1987). Dendritic spine loss in hippocampus of aged rats. Effect of brain phosphatidylserine administration. *Neurobiol. Aging* **8**, 501–510.

Nutting, D. F. (1976). Effects of growth hormone, thyroxine, and age on diaphragm muscle from dwarf mice. *Endocrinology (Baltimore)* **99**, 1423–1431.

Nutting, D. F., and Coats, L. J. (1977). Hormonal alterations of the sensitivity of amino acid transport to growth hormone in muscle of young rats. *Proc. Soc. Exp. Biol. Med*. **156**, 446–451.

Nyberg, F., and Burman, P. (1996). Growth hormone and its receptors in the central nervous system–location and functional significance. *Horm. Res*. **45**, 18–22.

Peinado, M. A., Martinez, M., Pedrosa, J. A., Quesada, A., and Peinado, J. M. (1993). Quantitative morphological changes in neurons and glia in the frontal lobe of the aging rat. *Anat. Rec*. **237**, 104–108.

Perry, E. K. (1980). The cholinergic system in old age and Alzheimer's disease. *Age Ageing* **9**, 1–8.

Peters, A., Josephson, K., and Vincent, S. L. (1991). Effects of aging on the neuroglial cells and pericytes within area 17 of the rhesus monkey cerebral cortex. *Anat. Rec*. **229**, 384–398.

Pisano, M. R., Wang, H. Y., and Friedman, E. (1991). Protein kinase activity changes in the aging brain. *Biomed. Environ. Sci*. **4**, 173–181.

Podhajsky, R. J., and Myers, R. R. (1993). The vascular response to nerve crush: Relationship to Wallerian degeneration and regeneration. *Brain Res*. **623**, 117–123.

Poon, L. W. (1985). Differences in human memory with aging: Nature, causes, and clinical implications. *In* "Handbook of the Psychology of Aging" (J. E. Birren and K. W. Schaie, eds.), 2nd ed., pp. 54–70. Van Nostrand-Reinhold, New York.

Presty, S. K., Bachevalier, J., Walker, L. C., Struble, R. G., Price, D. L., Mishkin, M., and Cork, L. C. (1987). Age differences in recognition memory of the rhesus monkey (*Macaca mulatta*). *Neurobiol. Aging* **8**, 435–440.

Quabbe, H. J., Schilling, E., and Helge, H. (1966). Pattern of growth hormone secretion during a 24-hour fast in normal adults. *J. Clin. Endocrinol. Metab.* **26**, 1173–1177.

Rabbitt, P. M. (1989). Inner-city decay? Age changes in structure and process in recall of familiar topographical information. *In* "Everyday Cognition in Adulthood and Late Life" (L. W. Poon, D. C. Rubin, and B. A. Wilson, eds.), pp. 82–99. Cambridge University Press, New York.

Ranke, M. B. (1987). A note on adults with growth hormone deficiency. *Acta Paediatr. Scand., Suppl*. **331**, 80–82.

Rapp, P. R., and Amaral, D. G. (1989). Evidence for task-dependent memory dysfunction in the aged monkey. *J. Neurosci*. **9**, 3568–3576.

Rapp, P. R., Rosenberg, R. A., and Gallagher, M. (1987). An evaluation of spatial information processing in aged rats. *Behav. Neurosci*. **101**, 3–12.

Recio-Pinto, E., and Ishii, D. N. (1984). Effects of insulin, insulin-like growth factor-II and nerve growth factor on neurite outgrowth in cultured human neuroblastoma cells. *Brain Res* **302**, 323–334.

Recio-Pinto, E., Rechler, M. M., and Ishii, D. N. (1986). Effects of insulin, insulin-like growth factor-II, and nerve growth factor on neurite formation and survival in cultured sympathetic and sensory neurons. *J. Neurosci*. **6**, 1211–1219.

Reinhardt, R. R., and Bondy, C. A. (1994). Insulin-like growth factors cross the blood-brain barrier. *Endocrinology (Baltimore)* **135**, 1753–1761.

Renganathan, M., Sonntag, W. E., and Delbono, O. (1997). L-type Ca2+ channel-insulin-like growth factor-1 receptor signaling impairment in aging rat skeletal muscle. *Biochem. Biophys. Res. Commun*. **235**, 784–789.

Riggs, T. R., and Walker, L. M. (1960). Growth hormone stimulation of amino acid transport into rat tissues *in vivo*. *J. Biol. Chem*. **235**, 3603–3610.

Rinderknecht, E., and Humbel, R. E. (1978). The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. *J. Biol. Chem.* **253**, 2769–2776.

Rivier, J., Spiess, J., Thorner, M., and Vale, W. (1982). Characterization of a growth hormone-releasing factor from a human pancreatic islet tumour. *Nature (London)* **300**, 276–278.

Rosenblum, W. I., and Kontos, H. A. (1974). The importance and relevance of studies of the pial microcirculation. *Stroke* **5**, 425–428.

Roth, G. S. (1989). Calcium homeostasis and aging: Role in altered signal transduction. *Ann. N. Y. Acad. Sci*. **568**, 68–72.

Roth, G. S. (1995). Changes in tissue responsiveness to hormones and neurotransmitters during aging. *Exp. Gerontol*. **30**, 361–368.

Roth, G. S. (1997). Age changes in signal transduction and gene expression. *Mech. Ageing Dev*. **98**, 231–238.

Rotwein, P., Burgess, S. K., Milbrandt, J. D., and Krause, J. E. (1988). Differential expression of insulin-like growth factor genes in rat central nervous system. *Proc. Natl. Acad. Sci. U. S. A*. **85**, 265–269.

Rudman, D. (1985). Growth hormone, body composition, and aging. *J. Am. Geriatr. Soc*. **33**, 800–807.

Rudman, D., Kutner, M. H., Rogers, C. M., Lubin, M. F., Fleming, G. A., and Bain, R. P. (1981). Impaired growth hormone secretion in the adult population: Relation to age and adiposity. *J. Clin. Invest*. **67**, 1361–1369.

Rudman, D., Feller, A. G., Nagraj, H. S., Gergans, G. A., Lalitha, P. Y., Goldberg, A. F., Schlenker, R. A., Cohn, L., Rudman, I. W., and Mattson, D. E. (1990). Effects of human growth hormone in men over 60 years old. *N. Engl. J. Med*. **323**, 1–6.

Rudman, D., Feller, A. G., Cohn, L., Shetty, K. R., Rudman, I. W., and Draper, M. W. (1991). Effects of human growth hormone on body composition in elderly men. *Horm. Res*. **36** (Suppl. 1), 73–81.

Sadler, S. E., and Maller, J. L. (1987). In vivo regulation of cyclic AMP phosphodiesterase in Xenopus oocytes. Stimulation by insulin and insulin-like growth factor 1. *J. Biol. Chem*. **262**, 10644–10650.

Sakimura, K., Kutsuwada, T., Ito, I., Manabe, T., Takayama, C., Kushiya, E., Yagi, T., Aizawa, S., Inoue, Y., and Sugiyama, H. (1995). Reduced hippocampal LTP and spatial learning in mice lacking NMDA receptor epsilon 1 subunit. *Nature (London)* **373**, 151–155.

Salmon, W. D., and Daughaday, W. H. (1957). A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage *in vitro*. *J. Lab. Clin. Med*. **49**, 825–831.

Saneto, R. P., Low, K. G., Melner, M. H., and de Vellis, J. (1988). Insulin/insulin-like growth factor I and other epigenetic modulators of myelin basic protein expression in isolated oligodendrocyte progenitor cells. *J. Neurosci. Res*. **21**, 210–219.

Sara, V. R., and Hall, K. (1990). Insulin-like growth factors and their binding proteins. *Physiol. Rev.* **70**, 591–614.

Sara, V. R., Hall, K., von Holtz, H., Humbel, R., Sjögren, B., and Wetterberg, L. (1982). Evidence for the presence of specific receptors for insulin-like growth factors 1 (IGF-1) and 2 (IGF-2) and insulin throughout the adult human brain. *Neurosci. Lett*. **34**, 39–44.

Sarter, M., and Bruno, J. P. (1997). Cognitive functions of cortical acetylcholine: Toward a unifying hypothesis. *Brain Res. Rev.* **23**, 28–46.

Sartorio, A., Molinari, E., Riva, G., Conti, A., Morabito, F., and Faglia, G. (1995). Growth hormone treatment in adults with childhood onset growth hormone deficiency: Effects on psychological capabilities. *Horm. Res*. **44**, 6–11.

Sato, Y., Okamura, K., Morimoto, A., Hamanaka, R., Hamaguchi, K., Shimada, T., Ono, M., Kohno, K., Sakata, T., and Kuwano, M. (1993). Indispensable role of tissue-type plasminogen activator in growth factor-dependent tube formation of human microvascular endothelial cells in vitro. *Exp. Cell Res*. **204**, 223–229.

Scheibel, M. E., Lindsay, R. D., Tomiyasu, U., and Scheibel, A. B. (1976). Progressive dendritic changes in the aging human limbic system. *Exp. Neurol*. **53**, 420–430.

Schneider-Rivas, S., Rivas-Arancibia, S., Vazquez-Pereyra, F., Vazquez-Sandoval, R., and Borgonio-Perez, G. (1995). Modulation of long-term memory and extinction responses induced by growth hormone (GH) and growth hormone releasing hormone (GHRH) in rats. *Life Sci*. **56**, 433–441.

Shaw, T. G., Mortel, K. F., Meyer, J. S., Rogers, R. L., Hardenberg, J., and Cutaia, M. M. (1984). Cerebral blood flow changes in benign aging and cerebrovascular disease. *Neurology* **34**, 855–862.

Shaywitz, B. A., and Pearson, D. A. (1978). Effect of phenobarbital on activity and learning in 6-hydroxydopamine treated rat pups. *Pharmacol. Biochem. Behav.* **9**, 173–179.

Shemer, J., Raizada, M. K., Masters, B. A., Ota, A., and LeRoith, D. (1987). Insulin-like growth factor I receptors in neuronal and glial cells. Characterization and biological effects in primary culture. *J. Biol. Chem.* **262**, 7693–7699.

Sherman, K. A., and Friedman, E. (1990). Pre- and post-synaptic cholinergic dysfunction in aged rodent brain regions: New findings and an interpretative review. *Int. J. Dev. Neurosci*. **8**, 689–708.

Shibasaki, T., Shizume, K., Nakahara, M., Masuda, A., Jibiki, K., Demura, H., Wakabayashi, I., and Ling, N. (1984). Age-related changes in plasma growth hormone response to growth hormone-releasing factor in man. *J. Clin. Endocrinol. Metab.* **58**, 212–214.

Shimada, A., Mukhin, A., Ingram, D. K., and London, E. D. (1997). *N*-methyl-D-aspartate receptor binding in brains of rats at different ages. *Neurobiol. Aging* **18**, 329–333.

Shinar, Y., and McMorris, F. A. (1995). Developing oligodendroglia express mRNA for insulin-like growth factor-I, a regulator of oligodendrocyte development. *J. Neurosci. Res*. **42**, 516–527.

Sirvio, J., Hervonen, A., and Riekkinen, P. J. (1988a). Cholinergic binding in the hippocampus of the aging male rat. *Comp. Biochem. Physiol*. **90**, 161–163.

Sirvio, J., Hervonen, A., Valjakka, A., and Riekkinen, P. J. (1988b). Pre- and postsynaptic markers of cholinergic neurons in the cerebral cortex of rats of different ages. *Exp. Gerontol*. **23**, 473–479.

Sjöberg, J., and Kanje, M. (1989). Insulin-like growth factor (IGF-1) as a stimulator of regeneration in the freeze-injured rat sciatic nerve. *Brain Res*. **485**, 102–108.

Smith, R. D., Cooper, B. R., and Breese, G. R. (1973). Growth and behavioral changes in developing rats treated intracisternally with 6-hydroxydopamine: Evidence for involvement of brain dopamine. *J. Pharmacol. Exp. Ther.* **185**, 609–619.

Sonntag, W. E., Steger, R. W., Forman, L. J., and Meites, J. (1980). Decreased pulsatile release of growth hormone in old male rats. *Endocrinology (Baltimore)* **107**, 1875–1879.

Sonntag, W. E., Forman, L. J., Miki, N., Steger, R. W., Ramos, T., Arimura, A., and Meites, J. (1981). Effects of CNS active drugs and somatostatin antiserum on growth hormone release in young and old male rats. *Neuroendocrinology* **33**, 73–78.

Sonntag, W. E., Hylka, V. W., and Meites, J. (1983). Impaired ability of old male rats to secrete growth hormone in vivo but not in vitro in response to hpGRF(1-44). *Endocrinology (Baltimore)* **113**, 2305–2307.

Sonntag, W. E., Hylka, V. W., and Meites, J. (1985). Growth hormone restores protein synthesis in skeletal muscle of old male rats. *J. Gerontol*. **40**, 689–694.

Sonntag, W. E., Gottschall, P. E., and Meites, J. (1986). Increased secretion of somatostatin-28 from hypothalamic neurons of aged rats in vitro. *Brain Res*. **380**, 229–234.

Sonntag, W. E., Lenham, J. E., and Ingram, R. L. (1992). Effects of aging and dietary restriction on tissue protein synthesis: Relationship to plasma insulin-like growth factor-1. *J. Gerontol*. **47**, B159–B163.

Sonntag, W. E., Lynch, C. D., Cooney, P. T., and Hutchins, P. M. (1997). Decreases in cerebral microvasculature with age are associated with the decline in growth hormone and insulin-like growth factor 1. *Endocrinology (Baltimore)* **138**, 3515–3520.

Sonntag, W. E., Lynch, C. D., Bennett, S. A., Khan, A. S., Thornton, P. L., Cooney, P. T., Ingram, R. L., McShane, T., and Brunso-Bechtold, J. K. (1999). Alterations in insulin-like growth factor-1 gene and protein expression and type 1 insulin-like growth factor receptors in the brains of ageing rats. *Neuroscience* **88**, 269–279.

Sonntag, W. E., Bennett, S. A., Thornton, P. L., Xu, X., Ingram, R. L., and Brunso-Bechtold, J. K. (2000). Age and insulin-like growth factor-1 (IGF-1) modulate *N*-Methyl-D-Aspartate (NMDA) receptor expression in hippocampus and cortex of rats. *Brain Res. Bulletin* **51**, 331–338.

Spik, K., and Sonntag, W. E. (1989). Increased pituitary response to somatostatin in aging male rats: Relationship to somatostatin receptor number and affinity. *Neuroendocrinology* **50**, 489–494.

Staehelin, M. (1962). Protein metabolism: Influence of growth hormone. *In* ''Anabolic Steroids and Nutrition in Health and Disease'' (A. Querido, ed.), pp. 288–297. Springer-Verlag, Basel.

Strong, R. (1998). Neurochemical changes in the aging human brain: Implications for behavioral impairment and neurodegenerative disease. *Geriatrics* **53** (Suppl. 1), S9–S12.

Strong, R., Wood, W. G., and Samorajski, T. (1991). Neurochemistry of aging. *In* ''Principles and Practice of Geriatric Medicine'' (M.S.J. Pathy, ed.), 2nd ed., pp. 48–60. Wiley, New York.

Sugawa, M., and May, T. (1993). Age-related alteration in signal transduction: Involvement of the cAMP cascade. *Brain Res*. **618**, 57–62.

Tacconi, M. T., Lligona, L., Salmona, M., Pitsikas, N., and Algeri, S. (1991). Aging and food restriction: Effect on lipids of cerebral cortex. *Neurobiol. Aging* **12**, 55–59.

Takahashi, S., and Meites, J. (1987). GH binding to liver in young and old female rats: Relation to somatomedin-C secretion. *Proc. Soc. Exp. Biol. Med*. **186**, 229–233.

Takahashi, Y., Kipnis, D. M., and Daughaday, W. H. (1968). Growth hormone secretion during sleep. *J. Clin. Invest*. **47**, 2079–2090.

Takano, K., Tanaka, T., and Saito, T. (1994). Psychosocial adjustment in a large cohort of adults with growth hormone deficiency treated with growth hormone in childhood: Summary of a questionnaire survey. Committee for the Study Group of Adult GH Deficiency. *Acta Paediatr.* **399** (Suppl.), 16–19.

Tannenbaum, G. S., and Ling, N. (1984). The interrelationship of growth hormone (GH)-releasing factor and somatostatin in generation of the ultradian rhythm of GH secretion. *Endocrinology (Baltimore)* **115**, 1952–1957.

Tannenbaum, G. S., and Martin, J. B. (1976). Evidence for an endogenous ultradian rhythm governing growth hormone secretion in the rat. *Endocrinology (Baltimore)* **98**, 562–570.

Taylor, L., and Griffith, W. H. (1993). Age-related decline in cholinergic synaptic transmission in hippocampus. *Neurobiol. Aging* **14**, 509–515.

Terry, L. C., Saunders, A., Audet, J., Willoughby, J. O., Brazeau, P., and Martin, J. B. (1977). Physiologic secretion of growth hormone and prolactin in male and female rats. *Clin. Endocrinol*. **6** (Suppl.), 19S–28S.

Terry, R. D., DeTeresa, R., and Hansen, L. A. (1987). Neocortical cell counts in normal human adult aging. *Ann. Neurol*. **21**, 530–539.

Thompson, J. L., Butterfield, G. E., Marcus, R., Hintz, R. L., Van Loan, M., Ghiron, H., and Hoffman, A. R. (1995). The effects of recombinant human insulin-like growth factor-I and growth hormone on body composition in elderly women. *J. Clin. Endocrinol. Metab*. **80**, 1845–1852.

Thornton, P. L., Bennett, S. A., Childers, S. R., and Sonntag, W. E. (1998). Insulin-like growth factor 1 reverses age-related D_2 receptor deficits: Activity, intrinsic efficacy and receptor reserve. (Abstract) 28th Annual Meeting Soc. for Neuroscience, Los Angeles, CA.

Thornton, P. L., Ingram, R. L., and Sonntag, W. E. (2000). Chronic [D-Ala^2]-growth hormone releasing hormone administration attenuates age-related deficits in spatial memory. *J. Gerontol. A* (Biological Sciences) **55a**, 106–112.

Thornton, P. L., Ng, A., and Sonntag, W. E. (1999). Insulin-like growth factor-1 modulates learning and memory in young and old rats. (Abstract) Soc. for Neuroscience, Miami Beach, FL.

Tigges, J., Herndon, J. G., and Peters, A. (1990). Neuronal population of area 4 during the life span of the rhesus monkey. *Neurobiol. Aging* **11**, 201–208.

Toran-Allerand, C. D., Ellis, L., and Pfenninger, K. H. (1988). Estrogen and insulin synergism in neurite growth enhancement in vitro: Mediation of steroid effects by interactions with growth factors? *Brain Res*. **469**, 87–100.

Torres-Aleman, I., Naftolin, F., and Robbins, R. J. (1989). Growth promoting effects of IGF-I on fetal hypothalamic cell lines under serum-free culture conditions. *Int. J. Dev. Neurosci*. **7**, 195–202.

Torres-Aleman, I., Naftolin, F., and Robbins, R. J. (1990). Trophic effects of basic fibroblast growth factor on fetal rat hypothalamic cells: Interactions with insulin-like growth factor I. *Dev. Brain Res*. **52**, 253–257.

Turchi, J., and Sarter, M. (1997). Cortical acetylcholine and processing capacity: Effects of cortical cholinergic deafferentation on crossmodal divided attention in rats. *Cogn. Brain Res*. **6**, 147–158.

Uemura, E. (1985). Age-related changes in the subiculum of *Macaca mulatta*: Dendritic branching pattern. *Exp. Neurol*. **87**, 412–427.

Undie, A. S., and Friedman, E. (1992). Aging-induced decrease in dopaminergic-stimulated phosphoinositide metabolism in rat brain. *Neurobiol. Aging* **13**, 505–511.

Undie, A. S., Wang, H. Y., and Friedman, E. (1995). Decreased phospholipase C-beta immunoreactivity, phosphoinositide metabolism, and protein kinase C activation in senescent F-344 rat brain. *Neurobiol. Aging* **16**, 19–28.

Utiger, R. D., Parker, M. L., and Daughaday, W. H. (1962). Studies on human growth hormone: A radioimmunoassay for human growth hormone. *J. Clin. Invest*. **41**, 254–261.

Vance, M. L., Kaiser, D. L., Martha, P. M. J., Furlanetto, R., Rivier, J., Vale, W., and Thorner, M. O. (1989). Lack of in vivo somatotroph desensitization or depletion after 14 days of continuous growth hormone (GH)-releasing hormone administration in normal men and a GH-deficient boy. *J. Clin. Endocrinol. Metab.* **68**, 22–28.

van der Pal, R. H., Koper, J. W., van Golde, L. M., and Lopes-Cardozo, M. (1988). Effects of insulin and insulin-like growth factor (IGF-I) on oligodendrocyte-enriched glial cultures. *J. Neurosci. Res*. **19**, 483–490.

Van Wyk, J. J., Underwood, L. E., Hintz, R. L., Clemmons, D. R., Voina, S. J., and Weaver, R. P. (1974). The somatomedins: A family of insulin-like hormones under growth hormone control. *Recent Prog. Horm. Res*. **30**, 259–318.

Vaughan, D. W. (1977). Age-related deterioration of pyramidal cell basal dendrites in rat auditory cortex. *J. Comp. Neurol*. **171**, 501–515.

Veldhuis, J. D., Iranmanesh, A., Ho, K. K., Waters, M. J., Johnson, M. L., and Lizarralde, A. (1991). Dual defects in pulsatile growth hormone secretion and clearance subserve the hyposomatotropism of obesity in man. *J. Clin. Endocrinol. Metab.* **72**, 51–59.

Vermeulen, A. (1987). Nyctohemeral growth hormone profiles in young and aged men: Correlation with somatomedin-C levels. *J. Clin. Endocrinol. Metab.* **64**, 884–888.

Vincent, S. L., Peters, A., and Tigges, J. (1989). Effects of aging on the neurons within area 17 of rhesus monkey cerebral cortex. *Anat. Rec*. **223**, 329–341.

Voytko, M. L. (1996). Cognitive functions of the basal forebrain cholinergic system in monkeys: Memory or attention? *Behav. Brain Res*. **75**, 13–25.

Voytko, M. L., Olton, D. S., Richardson, R. T., Gorman, L. K., Tobin, J. R., and Price, D. L. (1994). Basal forebrain lesions in monkeys disrupt attention but not learning and memory. *J. Neurosci*. **14**, 167–186.

Wang, H. Y., Pisano, M. R., and Friedman, E. (1992). Age-related alteration in G protein function in rat cortex. *Ann. N. Y. Acad. Sci*. **663**, 426–428.

Watson, M., Roeske, W. R., and Yamamura, H. I. (1988). Decreased muscarinic receptor density in the aged rat brain. *Proc. West. Pharmacol. Soc*. **31**, 61–65.

Wehrenberg, W. B., Brazeau, P., Luben, R., Bohlen, P., and Guillemin, R. (1982). Inhibition of the pulsatile secretion of growth hormone by monoclonal antibodies to the hypothalamic growth hormone releasing factor (GRF). *Endocrinology (Baltimore)* **111**, 2147–2148.

Wehrenberg, W. B., Wiviott, S. D., Voltz, D. M., and Giustina, A. (1992). Pyridostigmine-mediated growth hormone release: Evidence for somatostatin involvement. *Endocrinology (Baltimore)* **130**, 1445–1450.

Wenk, G. L., Pierce, D. J., Struble, R. G., Price, D. L., and Cork, L. C. (1989). Age-related changes in multiple neurotransmitter systems in the monkey brain. *Neurobiol. Aging* **10**, 11–19.

West, R. L., and Crook, T. H. (1990). Age differences in everyday memory: Laboratory analogues of telephone number recall. *Psychol. Aging* **5**, 520–529.

West, R. L., Crook, T. H., and Barron, K. L. (1992). Everyday memory performance across the life span: Effects of age and noncognitive individual differences. *Psychol. Aging* **7**, 72–82.

Xu, X., and Sonntag, W. E. (1996). Growth hormone-induced nuclear translocation of Stat-3 decreases with age: Modulation by caloric restriction. *Am. J. Physiol*. **271**, E903–E909.

Xu, X., Bennett, S. A., Ingram, R. L., and Sonntag, W. E. (1995). Decreases in growth hormone receptor signal transduction contribute to the decline in insulin-like growth factor I gene expression with age. *Endocrinology (Baltimore)* **136**, 4551–4557.

Yamaguchi, S., Kobayashi, S., Murata, A., Yamashita, K., and Tsunematsu, T. (1988). Effect of aging on collateral circulation via pial anastomoses in cats. *Gerontology* **34**, 157–164.

Zelnik, N., Angel, I., Paul, S. M., and Kleinman, J. E. (1986). Decreased density of human striatal dopamine uptake sites with age. *Eur. J. Pharmacol*. **126**, 175–176.

Zhai, Q., Lai, Z., Roos, P., and Nyberg, F. (1994). Characterization of growth hormone binding sites in rat brain. *Acta Paediatr.* **406** (Suppl.), 92–95.

Zhang, W. W., Badonic, T., Hoog, A., Jiang, M. H., Ma, K. C., Nie, J. X., Olsson, Y., and Sourander, P. (1994). Structural and vasoactive factors influencing intracerebral arterioles in cases of vascular dementia and other cerebrovascular disease: A review. Immunohistochemical studies on expression of collagens, basal lamina components and endothelin-1. *Dementia* **5**, 153–162.

Zornetzer, S. F., Thompson, R., and Rogers, J. (1982). Rapid forgetting in aged rats. *Behav. Neural Biol*. **36**, 49–60.

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The Aged Sympathetic Nervous System

I. Basal Sympathetic Activity in Human Aging

Sensitive assays for the quantitation of norepinephrine (NE) and epinephrine (E) in plasma and other body fluids have now been available for nearly three decades. It is generally accepted that circulating NE originates from sympathetic nervous system (SNS) nerve terminals, while E is produced almost exclusively by adrenomedullary secretory activity (Lee *et al*., 1984). As a result, a number of earlier studies utilized these relatively noninvasive measurements when examining the potential role of the sympatho-chromaffin system in the pathophysiology of specific disease states. For example, plasma NE levels were measured in individuals who suffered from either hyperthyroidism or hypertension. SNS dysregulation was thought to play a significant role in both conditions. Nevertheless, in thyrotoxic (Christensen, 1973), as well as hypertensive (Pedersen and Christensen, 1975) individuals, plasma NE levels were similar to those measured in appropriately age-matched controls suffering from neither condition. However, plasma NE levels increased with the age of subject, irrespective of the individuals' thyroid or hypertension status (Christensen, 1973; Pedersen and Christensen, 1975). These findings have now been reproduced in a larger number of studies, with reported basal plasma NE levels as much as 50% higher in older individuals than in younger controls (Ziegler *et al*., 1976; Kuchel and Kuchel, 1993). In contrast, plasma E levels do not appear to be significantly influenced by the subjects' age (Kuchel and Kuchel, 1993). Similarly, urine and cerebrospinal fluid NE, but not E, levels tend be higher in older individuals (Kuchel and Kuchel, 1993). Moreover, recordings of sympathetic nerve activity in skeletal muscle have revealed that this activity correlates with plasma NE levels and increases with age (Wallin *et al*., 1981). Although difficult to detect, recent evidence points to dopamine as another catecholamine with important peripheral effects (Kuchel and Kuchel, 1991, 1993). In addition to being the direct precursor of NE, peripheral dopamine appears to exert significant cardiovascular and renal effects (Kuchel and Kuchel, 1991). Interestingly, in spite of significant increase in basal plasma NE levels with age, urinary dopamine excretion (Fukagawa *et al*., 1995), as well as dopamine fibers (Amenta *et al*., 1990) and receptors appear to decline with aging of the peripheral nervous system. Moreover, since dopamine promotes renal salt excretion, these changes could contribute to the increased prevalence of hypertension and of disorders in salt fluid and salt handling observed in old age (Kuchel and Kuchel, 1991).

However, caution must be exercised when interpreting human studies of basal SNS activity, particularly as related to aging (Rowe and Troen, 1980). Most importantly, a number of criteria should be met in order to be able to assume that plasma NE levels are truly reflective of SNS activity (Lee *et al*., 1984). Since plasma NE can be affected by many factors, it is essential to ensure that in each subject NE release, its diffusion into the circulation and subsequent neuronal reuptake, as well as extraction or clearance by other tissues all be in a ''steady-state'' at the time of blood sampling (Lee *et al*., 1984). Thus, efforts must be made to ensure that the subject's SNS activity is truly at a basal state. These considerations are particularly crucial in aging studies since older subjects have been shown to require a longer period of time to reach basal NE levels (Rowe and Troen, 1980). In addition to higher basal SNS activity, older individuals tend to demonstrate evidence of sympathetic dysregulation in response to a wide variety of physiologic and behavioral stimuli. As a result, following most stimuli older subjects tend to exhibit elevations in plasma NE levels which are both greater and more prolonged that those induced by equivalent stimuli in younger individuals (Rowe and Troen, 1980; Kuchel and Kuchel, 1993).

Another potential confounder in gerontologic research is the presence of disease. A disease process, either overt or subclinical, could potentially influence both SNS activity and NE levels. Thus, details of health screening in the research subjects are important. Nevertheless, age-associated increases in NE plasma levels do not appear to be related to the presence of hypertension (Lake *et al*., 1997), carbohydrate intake (Chen *et al*., 1986), thyroid hormone status (Christensen, 1973), or obesity (Sowers *et al*., 1983). However, even in the absence of identifiable disease, individuals experience a highly variable pattern of changes as they grow older. It has been proposed that it may be useful to further separate aging without disease or ''normal'' aging into two categories, usual and successful (Rowe and Kahn, 1987). Many of the differences between individuals in the two categories could then be attributed to ex-

trinsic factors such as diet, exercise, personal habits, and psychological profiles (Rowe and Kahn, 1987). Since many of these factors are potentially modifiable, addressing them raises the hope that specific lifestyle interventions could be designed with the goal of modifying the aging process and its associated disability. Studies of extremely fit elderly bicycle marathon racers have shown that their basal NE levels are similar to those obtained in older individuals who were less fit, yet healthy (Lehmann and Keul, 1981, 1986). Thus, elevated basal NE levels in old age cannot be attributed to degree of physical fitness or muscle strength (Lehmann and Keul, 1981, 1986). More recently, it has been proposed that elevated SNS activity in old age may be related to a smoking history, rather than age itself (Jensen, 1999). The significance of these preliminary observations is unclear, at least in part, due to the fact that few geriatric patients are current or past smokers. Nevertheless, it is possible that in addition to the aging process, other associated lifestyle factors may contribute to SNS dysfunction in old age.

II. Sympathetic Dysregulation in the Older Subject

Nearly all maneuvers known to induce SNS activity have been shown to result in greater and more prolonged increases in NE plasma levels in older, as compared to younger subjects (Rowel and Troen, 1980; Kuchel and Kuchel, 1993). This type of NE hyperresponsiveness with aging has been seen after standing (Young *et al*., 1980; Sowers *et al*., 1983), various types of exercise (Lehmann *et al*., 1981; Lehmann and Reul, 1981, 1986; Sowers *et al*., 1983; Fleg *et al*., 1985), oral glucose ingestion (Young, *et al*., 1980), and mental stress (Barnes *et al*., 1982). Only following a euglycemic insulin administration was the NE increase diminished in older subjects (Minaker *et al*., 1982). In contrast, similar challenges generally do not result in E hyperresponsiveness in older subjects (Kuchel and Kuchel, 1993). In spite of this type of SNS hyperresponsiveness, older individuals tend to be more likely to experience difficulty in maintaining normal blood pressure (Lipsitz, 1989) or temperature (Kenney, 1997; Young and Lee, 1997). This type of vulnerability is particularly evident in the frail elderly who suffer from significant concomitant morbidity, as well as in healthy older subjects who have been exposed to several concurrent homeostatic challenges (Shannon *et al*., 1986). Since SNS activity contributes to, among other things, the maintenance of normal blood pressure and temperature, one has to reconcile the apparent discordance between elevated SNS activity and enhanced vulnerability to such homeostatic challenges in old age.

A variety of human studies have indicated that although indicators of presynaptic SNS activity are generally elevated even under basal conditions in old age (i.e., sympathetic nerve recordings and plasma NE levels), postsynaptic responses to adrenergic tend to be diminished. Although it was once thought that the postsynaptic changes are as a result of diminished numbers of receptors (Schocken and Roth, 1977), most studies now point to a desensitization of many G-coupled receptors, including both pre- and postsynaptic receptors, while the absolute numbers of adrenergic receptors generally do not change in old age (Lakatta, 1995). These findings, together with a variety of alterations in signaling pathways, particularly involving calcium, have been used to explain well-established pharmacological phenomena such as an age-associated decline in the chronotropic cardiac response to isoproterenol (Scarpace, 1988; Lakatta, 1995).

The above observations can be explained by two different mechanisms. Sympathetic activity could be heightened due to a primary increase in sympathetic drive originating in the central nervous system and adrenergic receptor desensitization would then be the result of adrenergic hyperactivity. In contrast, it is possible that a primary deficit in the ability of receptors and tissues to respond to adrenergic stimulation would result in the activation of compensatory reflexes which would then produce secondary stimulation of SNS activity. Unfortunately, neither hypothesis completely explains all of the research findings to date (Scarpace, 1988). For example, decreased baroreceptor sensitivity which has been demonstrated in old age (Tonkin and Wing, 1994) could result in diminished suppressibility of SNS outflow in the setting of increasing blood pressure (Rowe and Troen, 1980). Nevertheless, studies showing a normal ability of centrally acting α2-agonists to suppress plasma NE and NE appearance in older individuals (Featherstone *et al*., 1987) suggest that at least these central mechanisms for SNS suppression remain intact into old age.

Although studies involving humans are not amenable to a direct assessment of synaptic function, indirect evidence exists to suggest that age-associated changes in the handling of NE at the nerve terminal level may contribute to the pathophysiology of the hyperadrenergic state of aging (Hoeldtke and Cilmi, 1985). Plasma NE concentration is determined by rates of NE release into plasma and its subsequent removal (Lee *et al*., 1984). Thus, higher NE levels in the elderly could result from increased NE entry into the plasma and/or from a reduced clearance of NE. Most studies of arterialized plasma NE kinetics have demonstrated increased appearance of NE in the plasma (Hoeldtke and Cilmi, 1985; Veith *et al*., 1986; Kuchel and Kuchel, 1993) of older subjects. NE clearance results have been less consistent with some studies reporting no change, while others reported small decreases in old age (Kuchel and Kuchel, 1993). However, the reported declines in NE clearance have been relatively modest and have not correlated with increased plasma NE levels as well as did the increased plasma appearance (Veith *et al*., 1986).

As discussed, increased appearance of NE could be due to increased NE synthesis and/or altered NE handling at the level of the nerve terminal. Catecholamine synthesis and release is increased by nerve activity, principally by regulation of tyrosine hydroxylase (TH), the enzyme involved in the rate-limiting step of catecholamine synthesis. Increased nerve activity, as has been demonstrated by sympathetic fiber recordings, could increase NE synthesis through two distinct mechanisms involving TH. Brief neural stimulation can cause TH to be activated through posttranslational (phosphorylation) modification (Zigmond *et al*., 1989), while prolonged stimulation can induce TH activity by increasing TH enzyme quantity (Zigmond, 1980; Schalling *et al*., 1989). In the absence of human data, support for a role for TH upregulation in the hyperadrenergic state of aging comes from several rodent stu-

dies demonstrating evidence of increased TH activity (Reis *et al*., 1977), as well as TH gene expression (Kedzierski and Porter, 1990; Kuchel *et al*., 1997a) in aged sympathetic superior cervical ganglia.

Hoeldtke and Cilmi (1985) have described an increase in the entry of NE into plasma without any apparent change in overall NE production in elderly subjects. They have interpreted these findings as suggesting that human aging is associated with alterations in the local disposition of sympathetic neuronal NE (Hoeldtke and Cilmi, 1985). A diminished capacity for neuronal reuptake of released NE would further increase the entry of NE into plasma (Lee *et al*., 1984). Although a direct evaluation of NE reuptake at the level of the nerve terminal is not possible in humans, animal studies do point to decreased specific NE uptake by aged sympathetic fibers (Kuchel and Zigmond, 1991). Increased synaptic spillover has been shown to result in the depletion of neuronal NE stores, as even increased catecholamine synthesis can fail to keep up with NE stores, since NE is being released, but it is not being efficiently recovered by the neuron through usual reuptake mechanisms (Bhatnager and Moore, 1971). Catecholamine fluorescence appears to be diminished in both aged human sympathetic ganglia (Hervonen *et al*., 1978), as well as in individual sympathetic fibers (Waterson *et al*., 1974) and catecholamine levels decrease in aged arteries (Neubauer and Christensen, 1978).

III. Mechanisms of Cellular Aging in Sympathetic Neurons

A. General Structural Changes

A number of changes in the sympathetic nerve supply have been described in a variety of targets in old age, including the vasculature, heart, thermoregulatory system, gut, bladder, and iris (Waterson *et al*., 1974; Neubauer and Christensen, 1978; Amenta and Mione, 1988; Baker *et al*., 1991; Abdel-Rahman *et al*., 1992; Abdel-Rahman and Cowen 1994; Andrews and Cowen, 1994a; Warburton and Santer 1994; Gavazzi *et al*., 1996). Nevertheless, the commonly held concept that aging involves the widespread degeneration and death of neurons throughout the nervous system is probably generally misleading (Morrison and Hof, 1997) and is almost certainly incorrect in relation to the sympathetic nervous system (Cowen and Gavazzi, 1998). Although human sympathetic ganglia have not been systematically investigated, studies of the rat superior cervical (Santer, 1991) and coeliac–superior mesenteric sympathetic ganglia (Baker and Santer, 1988) have provided no evidence of significant neuron loss. Pathologic studies of human sympathetic ganglia have revealed lipofuscin and neuromelanin accumulation (Hervonen *et al*., 1978), diminished histochemical staining for catecholamines (Hervonen *et al*., 1978), and evidence of neuroaxonal dystrophy (Schmidt, 1991; Schmidt *et al*., 1991). In spite of the preponderance of negative and subtle findings, the possibility that subpopulations of neurons may be selectively affected cannot at present be ruled out (see below) (Kuchel *et al*., 1997a). Furthermore, spinal neurons providing preganglionic input to the peripheral sympathetic neurons in elderly subjects may undergo cell loss, estimated at 5–8% per decade (Low *et al*., 1977).

The likelihood that sympathetic and other neurons do not die in large numbers in the aging nervous system does not mean that there are no changes in neuronal structure which might relate to significant losses of function. Morphometric light and confocal laser scanning microscopy methods have been used to characterize and quantify age-related changes in sympathetic nerve fibers and their associated neurotransmitters (see Figs. 64.1–64.3). These changes are almost invariably local in nature and neighboring neurons from the same gang-

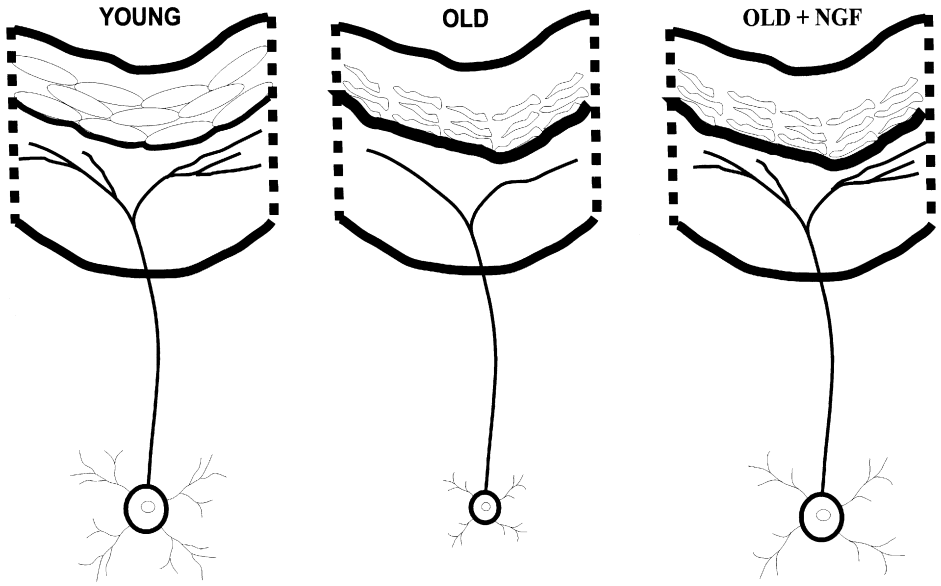

FIG. 64.1. Diagrams illustrating changes in nerve and smooth muscle in the aging blood vessel wall. In aging there is atrophy of autonomic neurons, including loss of axon collaterals and shrinkage of dendrites and the nerve cell body. Shrinkage of muscle cells also occurs, with thickening of basal lamina at the adventitial–medial border. Following NGF treatment there is regrowth of neurons, including axon collaterals, cell body, and dendrites.

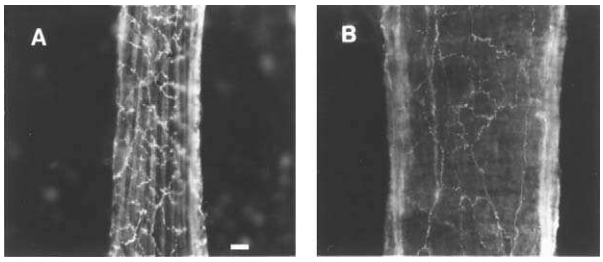

FIG. 64.2. Photomicrographs showing the sympathetic innervation to the rat middle cerebral artery through development and old age using catecholamine histochemistry (Andrews and Cowen, 1994a; Kuchel *et al*., 1997b) Nerve plexuses were investigated at 6 weeks (A) and 24 months (B). Scale bar = 25 μm.

lion but supplying different target tissues are often not affected. Where changes do occur, they may involve increases, for example in nerve fiber density or transmitter expression, as well as decreases. The changes seen are frequently sufficiently subtle to require measurement, although this should not be taken to mean that the functional outcome is insignificant. These types of studies have not been performed with human tissues. Nevertheless, the general concepts have been supported in studies where some preganglionic (Schmidt, 1991) and postganglionic (Jengeleski *et al*., 1989) sympathetic nerve fibers exhibit neuroaxonal dystrophy, while related terminals are unaffected.

B. Effects of Age on the Sympathetic Nerve Supply to Cerebral Blood Vessels

An area which has been relatively extensively studied, because of its possible relevance for the blood supply to the aging brain, is the sympathetic nerve supply to the major cerebral arteries of the circle of Willis. In humans (7th–8th decade) (Collins and Cowen, 1997) and rats (24–36 months old) (Andrews and Cowen, 1994a; Thrasivoulou and Cowen, 1995), these nerves undergo consistent age-related changes. In humans, the loss of nerve fibers affects mainly the anterior choroidal, posterior communicating, and internal carotid arteries, while in rats the changes affect the middle and posterior cerebral and basilar arteries. In rats, where the changes can be studied in more detail, sympathetic, vasoconstrictor nerves decline significantly in density between 18 and 24 months (Andrews and Cowen, 1994a), while vasodilator nerves, including sensory and parasympathetic fibers, may increase. Although sympathetic nerves in the rat cerebral vasculature decline in density with age, it appears that levels of the noradrenaline-synthesizing enzyme, tyrosine hydroxylase, may increase within these nerve fibers (Thrasivoulou and Cowen, 1995). Tracing studies using retrogradely transported dyes (Andrews *et al*., 1994) have shown that soma size and total length of dendrites of neurons projecting to different targets, including cerebral arteries, tend to reduce (or increase) in parallel with the age-changes in their peripheral axons. In addition, dystrophic changes were observed in some of these neurons of the superior cervical ganglion. More general dystrophic changes in neurites have been demonstrated using electron microscopy in sympathetic ganglia of aged human subjects and rodents. (Schmidt, 1991; Schroer *et al*., 1992; Uvelius and Gabella, 1998).

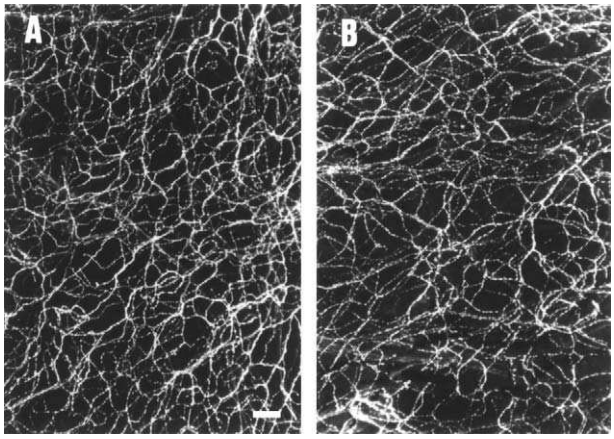

FIG. 64.3. Photomicrographs showing the sympathetic innervation to the iris through development and old age using catecholamine histochemistry (Kuchel *et al*., 1997b). Nerve plexuses were investigated at 6 weeks (A) and 24 months (B). Scale bar = 30 μm.

C. Effects of Age on the Sympathetic Nerve Supply to Sweat Glands

One of the only other systems where the effects of aging have been investigated in parallel in humans and in laboratory animals concerns the sympathetic nerve supply to cutaneous sweat glands (Abdel-Rahman *et al*., 1992; Abdel-Rahman and Cowen, 1994). In support of the evidence for impaired thermoregulation in the elderly, local deficits have been demonstrated in the structure and function of sympathetic (cholinergic) nerves around the sweat glands of elderly subjects (Abdel-Rahman *et al*., 1992). Sweat output, which is known to be a vital part of thermoregulation, declines after the seventh decade, in parallel with reduced size of glandular acini and substantially reduced density of the periacinar sympathetic nerves (Abdel-Rahman *et al*., 1992). Comparable changes were found in the structure and function of the sympathetic innervation of eccrine sweat glands of the rat footpad (Abdel-Rahman and Cowen, 1994).

D. Effects of Age on the Sympathetic Nerve Supply to Other Tissues

Other tissues have been investigated, but only in laboratory animals. Sympathetic nerve fibers from the coeliac–superior mesenteric ganglion complex of aged rats, supplying the enteric nervous system, were much reduced (Baker *et al*., 1991), as was the sympathetic nerve supply to the renal vasculature at the level of the renal artery in rabbits and around intrarenal arterioles in rats (Warburton and Santer, 1994). To emphasize the variable effects of aging on sympathetic nerves, in the iris of the aged rat, sympathetic nerves increased in density while sensory and parasympathetic nerves were reduced in density by 20–30% (Gavazzi *et al*., 1996).

A recent study has demonstrated a loss of sympathetic innervation to the aging rat pineal gland (Kuchel *et al*., 1999). Tyrosine hydroxylase-immunoreactive profiles were quantified in

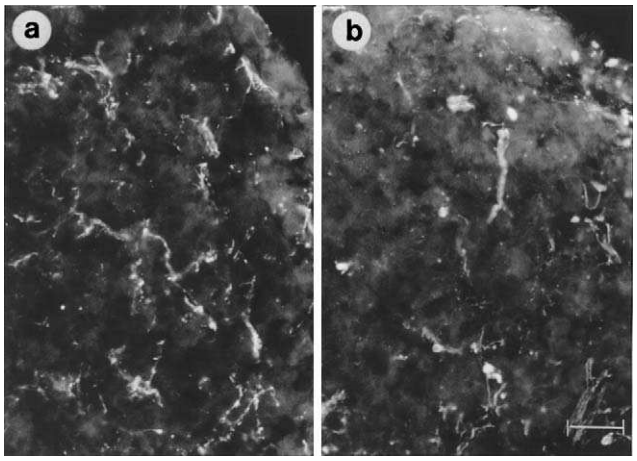

FIG. 64.4. Tyrosine hydroxylase immunoreactivity in sections of pineal glands obtained from young (a) and aged (b) animals (Kuchel *et al*., 1999). Scale bar = 50 μm.

pineal glands of young and aged male Sprague–Dawley rats. The density of TH-immunoreactive fibers was 30% lower in aged pineals, although the remaining fibers contained 20% more TH immunoreactivity, measured densitometrically (Fig. 64.4; Table 64.1) (Kuchel *et al*., 1999). Orthograde tracing of the pineal sympathetic innervation using biotinylated dextran was also performed (Fig. 64.5) (Kuchel *et al*., 1999). Quantitation of confocal images of individual labeled fibers revealed that average axon length, varicosity numbers, branch point numbers, and numbers of terminations were all decreased by approximately 50% in aged tissues (Table 64.2) (Kuchel *et al*., 1999). These findings suggest that whole branches, along with their associated varicosities are lost in old age. Although pineal melatonin production has been shown to decline dramatically in the course of mammalian aging (Kuchel and Zigmond, 1991), the primary role of melatonin in the aging process or in diseases associated with aging remains to be defined (Reppert and Weaver, 1995). Nevertheless, significant losses of sympathetic fibers to the mammalian pineal gland as described above could contribute to diminished function of this target tissue in old age.

Based on morphometric studies it seems likely that various types of growth changes (lengthening, retraction or atrophy) of axons and dendrites, combined with alterations in soma size

TABLE 64.1 Quantitation to Tyrosine Hydroxylase Immunoreactivity

Age	Area percentage	Intercept density (mm)	Gray value
Young (6)	1.65 ± 0.049	12.1 ± 0.35	67.6 ± 2.3
aged (6)	1.2 ± 0.12	8.2 ± 0.87	81.1 ± 2.14
	**	***	***

Note. Tyrosine hydroxylase immunoreactivity signal was quantified in pineal gland sections obtained from young and aged male Sprague–Dawley rats. Statistical differences in different measures of tyrosine hydroxylase immunoreactivity were compared between young and aged were examined using Student's *t* test ($^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.005$). All results are presented as the mean ± SEM (Kuchel *et al*., 1999).

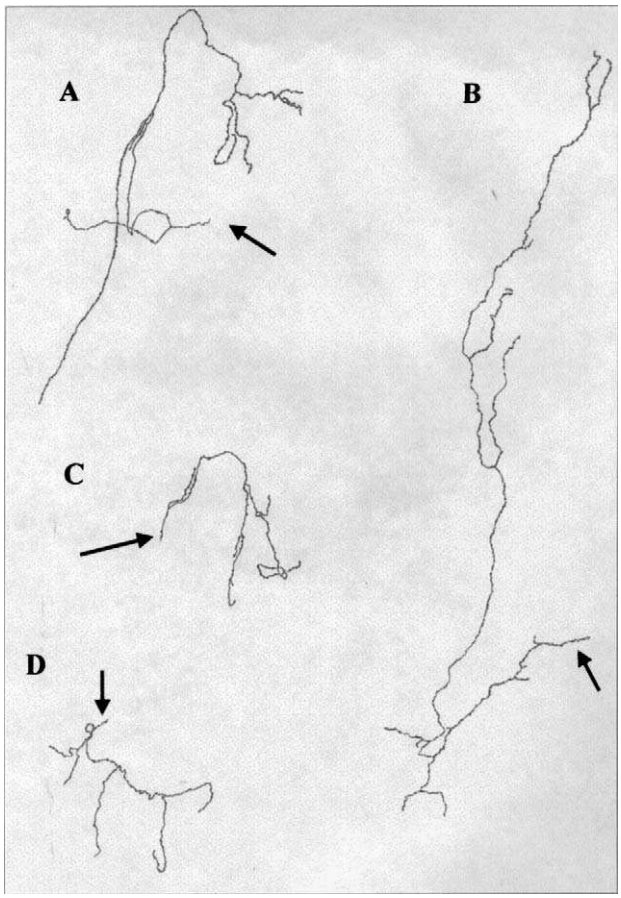

FIG. 64.5. Tracings to typical results of orthograde labeling using biotinylated dextran injections into right superior cervical ganglia demonstrating sympathetic arborizations in young (A,B) and aged (C,D) pineal glands. Note the marked reductions in extent of arborizations and numbers of varicosities in tracings from aged animals (C,D), compared to those from young animals (A,B) (Kuchel *et al*., 1999).

and neurotransmitter expression may occur in different situations. Since neurotransmitter markers are generally used to identify the peripheral fibers of particular nerve groups in tissues with multiple innervation, it is not always possible to know whether a change in staining pattern indicates altered levels of neurotransmitter, altered growth of nerve fibers, or both. The divergent changes in sympathetic neurons with age suggests that compensatory changes occur in neurons and groups of neurons as they attempt to respond to the altered demands of old age. These extra demands could contribute to the dramatic upregulation in TH mRNA expression is a small subpopulation of aged rat superior cervical ganglion neurons (Kuchel *et al*., 1997a). Moreover, additional metabolic and other stresses may contribute to the death of subsets of neurons in aged sympathetic ganglia. Alterations in the autonomic centers of the brain and spinal cord and in the dendrites of postganglionic sympathetic neurons in aging suggest altered patterns of central connectivity. The overall picture is of relatively small, quantitative changes, which, perhaps because of accumulated effects at different hierarchical levels of neural organization, lead to the rather more widespread deficits in autonomic function which have been observed clinically.

TABLE 64.2 Quantitation of Orthograde Labeling

Age	Total axon length (μm)	Total varicosities	Intervaricose distance (μm)	No. of branch points	No. of terminations	Segment length (μm)
Young (6)	1207 ± 199	110 ± 14.5	10.7 ± 1.0	8.3 ± 1.6	9.3 ± 1.6	130 ± 14.6
Aged (4)	575 ± 108	52.0 ± 7.0	10.9 ± 1.3	3.5 ± 0.6	4.8 ± 0.5	121 ± 23.3
	*	**	NS	*	*	NS

Note. Quantitation of orthograde labeling was performed in pineal glands of young and aged male Sprague–Dawley rats. Statistical differences in different measures of orthograde labeling were compared between young and aged animals using Student's *t* test ($^{*}P<0.05$; $^{**}P<0.01$). All results are presented as the mean ± SEM (Kuchel *et al*., 1999).

Two strands of thought may help us understand these diverse phenomena associated not only with the aging mammalian sympathetic nervous system, but with other aging cells and systems. The first strand, the neurotrophic theory (Purves *et al*., 1988; Gavazzi and Cowen, 1996; Cowen and Gavazzi, 1998), was originally developed to explain the rules governing survival in the embryonic sympathetic nervous system. The second strand is the network theory of aging which proposes some simple rules governing the allocation of resources in multicellular organisms (Lithgow and Kirkwood, 1996).

E. Neurotrophic Theory and Its Relation to Neuronal Aging

The neurotrophic theory states that during early development neurons compete with their neighbors for a share of limited quantities of trophic factors which are derived from the target tissues that the neurons will innervate (Purves *et al*., 1988). Success is rewarded by the survival and connectivity of an appropriate number of neurons. The first of these trophic factors, nerve growth factor (NGF), was identified when a mouse sarcoma tumor was discovered to synthesize a peptide capable of inducing extravagant growth of sympathetic nerve fibers (Levi-Montalcini and Hamburger, 1951; Levi-Montalcini *et al*., 1995). Proof of the dependent relationship of sympathetic neurons on target-derived NGF has come from experiments either depriving developing neurons of NGF, leading to extensive cell death (Bjerre *et al*., 1975), or, more recently, overexpressing NGF in target tissues in transgenic mice (Albers *et al*., 1994), which results in survival of a huge excess of sympathetic neurons. It took a further 35 years to discover that NGF was a member of a larger family—the neurotrophins—with trophic effects on many different neurons in the central and peripheral nervous systems. In addition to NGF, the neurotrophins are now known to include brain-derived neurotrophic factor (BDNF), NT3, NT4-5, and NT6, (Thoenen, 1995) of which NGF and NT3 play a crucial part in the survival of developing sympathetic neurons (Davies, 1995).

The effects of NGF on sympathetic neurons are specified by the relatively specific *trkA* receptors, and the nonspecific glycoprotein p75 receptor (Chao and Hempstead, 1995). The latter receptor binds all neurotrophins with varying affinities, but its role remains controversial (Bothwell, 1995). Interestingly, it has recently become clear that NT3 is capable of binding to *trkA*, as well as to p75 receptor with relatively high affinity (Chao and Hempstead, 1995). Although trk receptors can transduce most of the known effects of the neurotrophins, p75 NGF receptors are probably important in modulating some of these effects, particularly in later stages of development (Miller *et al*., 1994). In addition to the neurotrophins, there are a number of cytokines including CNTF (ciliary neuronotrophic factor), the FGFs (fibroblast growth factors), retinoic acid, and, more recently, GDNF which have important trophic effects on developing sympathetic neurons (Stemple *et al*., 1988; Burnham *et al*., 1994; Kobayashi *et al*., 1994; Buj-Buello *et al*., 1995).

The relevance of neurotrophic theory does not end in early development. The theory has been extended to suggest that neuron–target interactions and neurotrophic factors are vital contributors to the regulation of plasticity in the adult and aging nervous system, including in sympathetic neurons (Gavazzi and Cowen, 1996; Cowen and Gavazzi, 1998).

F. Maintenance Programs in Neuronal Plasticity and Aging

Organismal, and perhaps cellular, longevity is regulated by the amount of energy expended by the organism on maintenance of its constituent cells and systems (Lithgow and Kirkwood, 1996). Aging, therefore, may be understood as a failure of ongoing maintenance programs—a concept that is likely to be more relevant to postmitotic cells such as neurons than aging theories based on the accumulation of defects in processes associated with cell division. Over a number of years, one of us (T.C.) has investigated the relevance of neurotrophic theory, as a paradigm of an ongoing cell maintenance program, in relation to aging processes as they affect sympathetic and other neurons.

Dynamic maintenance programs are essential for adult neurons. For example, plasticity in mature sympathetic neurons includes energy-demanding changes associated with growth (or retraction) of axons and dendrites to alter their territory and connectivity, regeneration of nerves after injury, growth or shrinkage of cell soma, and changes in expression of neurotransmitters and other markers of dynamic behavior. While plasticity is generally associated with the developing nervous system, it becomes increasingly clear that plasticity continues to be an important feature of the mature, and even aged, nervous system as it attempts to respond to altered demand.

Plastic changes in adult and aging neurons may use mechanisms "inherited" from earlier stages of development. The peripheral processes of mature autonomic and other neurons innervating target tissues continually grow and retract over

periods of a few days (Purves *et al*., 1986). The mechanism that has been proposed to control this form of plasticity is the synthesis and release by the target tissue of limiting amounts of neurotrophic factors. Adult sympathetic and other neurons appear thus to remain intimately related to their targets, providing them with appropriate signals for their activation, and receiving, in return, trophic messages required for their maintenance and continued plasticity.

Trophic interactions with target tissues are therefore important elements of plasticity at all stages of life. However, the nature of the relationship may change with age. As we have already stated, in early life, neurotrophic factors control survival of sympathetic, as well as the establishment of appropriate patterns of growth, connectivity (Voyvodic, 1987, 1989) and neurotransmitter phenotype (Landis, 1990). In later periods of life, the survival of sympathetic neurons becomes independent of NGF (Orike and Cowen, unpublished data). However, interactions between sympathetic neurons and their targets remain important for the regulation of growth of axons, soma and dendrites (Purves *et al*., 1990), and neurotransmitter expression (Schotzinger *et al*., 1994). Studies of experimentally induced hypertrophy of smooth muscle in gut and bladder have shown that the associated sympathetic and other autonomic neurons undergo a parallel hypertrophy (Gabella *et al*., 1992), providing strong evidence of the ongoing influence that target tissues can have on their innervating neurons during adulthood. Trophic interactions between nerves and end organs are therefore a vital feature of maintenance and plasticity in the mature nervous system and alterations in this relationship are likely to be critical determinants in the aging process.

G. Sympathetic Neuron–Target Interactions in Aging

Transplantation studies have been used to explore the capacity of target tissues to regulate neuronal phenotype during aging. Target tissues with known age-changes in their sympathetic innervation have been removed from old donor rats and transplanted into young host animals, making contact with the axon terminals of host sympathetic neurons (Gavazzi *et al*., 1992; Cowen *et al*., 1996a). The donor tissues are denervated on removal, providing new territory over which host nerves can grow. Transplants of cerebral arteries and sweat glands, two target tissues where the density of sympathetic innervation is reduced by about 50% with age, become reinnervated by strikingly fewer host nerves than transplants of comparable young tissues. Similar studies have attempted to find out whether target tissues can influence age-changes in neurotransmitter expression, in addition to peripheral nerve fiber density. Eccrine seat glands are innervated by sympathetic neurons, which are unusual in using acetylcholine as their peripheral neurotransmitter. Evidence from early development shows that these neurons switch from a catecholaminergic to a cholinergic neurotransmitter phenotype at the time when their peripheral axons first contact their target sweat glands (Schotzinger *et al*., 1994). Whereas sweat glands from aged rats were, like rat cerebral arteries, less attractive to host nerves, they remained competent to invoke cholinergic characteristics in the reinnervating host neurons (Cowen *et al*., 1996a), suggesting that different target-associated factors regulate nerve growth and transmitter expression.

Transplantation has also been used to find out whether neurons retain regenerative capacities in old age. Aged sympathetic neurons from the rat superior cervical ganglion, transplanted into young hosts have generally shown unimpaired plasticity, in terms of survival as well as extent of neurite outgrowth (Gavazzi and Cowen, 1993). However, in the few studies in which transplants have been made into old host rats, both young and old neurons are impaired in their capacity for neurite outgrowth, suggesting that changes in the aging environment as well as changes in neurons themselves in old age, could contribute to altered plasticity (Gavazzi *et al*., 1992). Denervation studies also show that collateral sprouting (the capacity of axons to expand their territory) is impaired in sympathetic neurons of the aged rat (Crutcher, 1990; Kuchel and Zigmond, 1991; Kuchel, 1993). These observations demonstrate that sympathetic neurons remain to some extent dependent on their target tissues for signals which regulate both the pattern and the density of their peripheral arborizations and also their patterns of neurotransmitter expression. This ongoing influence appears to extend throughout mature life and into old age. In addition, there are hints that intrinsic changes in neurons and alterations in their environment contribute to age-related changes in plasticity, presumably allowing more-or-less continuous adjustment of neuronal phenotype to altered functional demands.

H. Molecular Influences on Plasticity of Aging Sympathetic Neurons

As we have tried to show, trophic support from targets continues to be important for the maintenance and plasticity of mature sympathetic neurons. Mature neurons ''inherit'' dependence on particular factors from earlier stages of life but respond in different ways, for reasons which are often unclear. Thus, mature autonomic neurons remain dependent on NGF for growth (Ruit *et al*., 1990), collateral sprouting (Gloster and Diamond, 1992), and neurotransmitter expression (see above). However, unlike developing neurons, they are not dependent on neurotrophic factors for their survival (Orike and T. Cowen, unpublished data). In addition, regeneration of sympathetic (and sensory) nerves in mature animals is probably also not NGF-dependent (Diamond et al., 1992; Gloster and Diamond, 1992).

That adult sympathetic neurons remain dependent for their growth on NGF raises the possibility that NGF treatment could rescue those neurons that exhibit selective vulnerability from age-related neurodegeneration. Local infusion of relatively high doses of NGF with miniosmotic pumps into the third ventricle or subdural space of aged rats induces regrowth of a pattern of innervation around the internal carotid and middle cerebral (Andrews and Cowen, 1994a) arteries that resembles that seen in young rats. Sprouting of fibers mainly involved the sympathetic nerves, whereas sensory nerves also responded to NGF infusion with elevated transmitter levels (Isaacson *et al*., 1990). Dendrites of the sympathetic neurons projecting to cerebral blood vessels were also found to be capable of regrowth following local treatment of their peripheral axons with NGF (Andrews and Cowen, 1994b). Withdrawal of treatment predictably resulted in atrophy, indicat-

ing the need for a continuous supply of NGF for neuronal maintenance.

It is not clear at present whether the effects of treatment with NGF on growth of mature autonomic nerves are evidence of a pharmacological effect or whether NGF treatment supplements target-derived, endogenous NGF in the regulation of neuronal plasticity. In early postnatal life, target levels of either the NGF protein or its messenger RNA tend to correlate with the density of sympathetic innervation (Shelton and Reichardt, 1984; Weskamp and Otten, 1987), as predicted by the neurotrophic theory. However, attempts to extend this concept to the aging nervous system (Cowen, 1993) have not so far been successful. For example, sympathetic nerves are induced to sprout into the adult rat hippocampus following its denervation by lesions of the fimbria-fornix and this response is reduced in aging animals (Crutcher, 1988). However, the same group was unable to demonstrate an age-related loss of hippocampal NGF levels which could have caused the failed response (Crutcher and Weingartner, 1991). Studies of the target tissues supplied by those subpopulations of sympathetic neurons which are selectively vulnerable to aging, such as cerebral arteries (Cowen *et al*., 1996b) and the pineal gland (Kuchel *et al*., 1999) have also failed to demonstrate any alterations in NGF levels which might underpin age-related neuronal atrophy or cell death. Because targets are known to remain important in the maintenance of phenotype of adult and ageing sympathetic neurons, this lack of correlation between NGF levels and local neuronal atrophy implies that the key target-associated regulators of plasticity in adult and aging neurons may be different from those which are required by developing neurons. A further related possibility is that neuronal responsiveness to neurotrophins alters in adulthood and old age.

I. Target-Associated Factors in the Extracellular Matrix

Whilst studies of NGF have yielded important information about plasticity in mature and aged autonomic neurons, it is probable that we are only beginning to identify the different trophic factors that affect aging neurons. The extracellular matrix contains a number of insoluble, bound factors with important neurotrophic activities, to which sympathetic and other neurons respond. Extracellular matrix elements are found in the basal lamina at sites of neuroeffector junctions of somatic and autonomic (including sympathetic) neurons and are therefore ideally positioned to influence dynamic growth processes of the kind described previously through contact with terminal nerve fibers. Although the role of extracellular matrix molecules in aging has not been extensively investigated, recent studies implicate laminin and extracellular matrix in sympathetic nerve fiber atrophy in aged rats. For example, levels of laminin are reduced in the basal lamina associated with nerve terminals in cerebral arteries of aging rats (Gavazzi *et al*., 1995). *In vitro* studies have shown that laminin and NGF act synergistically to promote growth of adult and aging sympathetic neurons and that aging neurons become significantly less responsive to this combination of factors (Cowen *et al*., 1997). These results suggest an interesting scenario of coactivation of two sets of tyrosine kinases by target associated factors: one associated with integrin receptors (for laminin) and the other with *trk* receptors for the neurotrophins. In addition, they suggest that altered neuronal responsiveness to growth- and survival-promoting factors may regulate age-associated loss of plasticity.

J. Neuronal Responsiveness to Trophic Factors

Recent evidence indicates developmental shifts in the responsiveness of sympathetic neurons to NGF, some of which have been described (see above). Later in life, further changes occur that may require a revision of our understanding of the role of NGF in the mature nervous system. Aged sympathetic neurons express lower levels of both *trkA* and p75 (Kuchel *et al*., 1997a), while demonstrating fewer NGF binding sites (Uchida and Tomonaga, 1985). Thus, they may become less able to scavenge and utilize available NGF, making them vulnerable to trophic factor deprivation and providing a possible explanation for the reduced capacity for collateral sprouting previously described (Crutcher, 1990; Kuchel and Zigmond, 1991; Kuchel, 1993). *In vitro* studies also indicate reduced responsiveness of aging sympathetic neurons to NGF (Uchida and Tomonaga, 1985). Whereas NGF binding is reduced in aged sympathetic ganglia, it is not yet known for certain which NGF receptors are involved in these changes. Reduced NGF-responsiveness of aging sympathetic neurons projecting to the iris, in the absence of changes in irideal NGF levels, suggests that NGF receptor expression is no longer as tightly coupled to levels of NGF at the target as it was during early development (Gavazzi *et al*., 1996). The search for alternative influences on the responsiveness of aged autonomic neurons to trophic factors forms an important area for further study.

By virtue of their large size and surface area and high metabolic requirements, neurons become exposed to a number of local and systemic changes during the aging process which may influence plasticity. For example, circulating growth hormone levels decline substantially with age, with undetectable serum levels of growth hormone in 50% of all subjects by their seventh decade (Borst *et al*., 1994). Declining growth hormone levels have been proposed as a major influence on aging, including in the nervous system. A related pathway which may be important in the maintenance of adult sympathetic neurons is that mediated by the insulin-like growth factors (IGF-1 and IGF-2). Circulating IGF levels decline in parallel with, and perhaps in response to, growth hormone during aging and are known to affect growth in developing cultured sympathetic neurons (Recio-Pinto *et al*., 1986). Treatment of growth hormone-deficient patients with the recombinant hormone partially rescues the sweating responses, perhaps as a result of regenerative effects on sudomotor nerves (Hasan *et al*., 2000).

Altered levels of sex hormones may also affect targets of autonomic neurons such as sweat glands (Rees and Shuster, 1981) and thereby influence their autonomic innervation. The high metabolic rate of neurons means that during aging neurons are increasingly likely to encounter the damaging effects of partial ischemia or raised levels of free radicals. These changes are relatively specific to old age and introduce a negative influence, not present at earlier stages of development, on neuronal plasticity. In relation to the role of free radicals in aging, it is interesting to note that neurons which synthesize and use the free radical, nitric oxide, as a neurotransmitter

appear to be relatively protected from the cell death that affects their neighbors (Santer, 1994; Belai *et al*., 1995), although how they achieve this apparent invulnerability is not understood.

References

Abdel-Rahman, T. A., and Cowen, T. (1994). Neurodegeneration in sweat glands and skin of aged rats. *J. Auton. Nerv. Syst*. **46**, 55–63.

Abdel-Rahman, T. A., Collins, K. J., Cowen, T., and Rustin, M. (1992). Immunohistochemical, morphological and functional changes in the peripheral sudomotor neuro-effector system in elderly people. *J. Auton. Nerv. Syst*. **37**, 187–198.

Albers, K. M., Wright, D. E., and Davis, B. M. (1994). Overexpression of nerve growth factor in epidermis of transgenic mice causes hypertrophy of the peripheral nervous system. *J. Neurosc*. **14**, 1422–1432.

Amenta, F., and Mione, M. C. (1988). Age-related changes in the noradrenergic innervation of the coronary arteries in old rats: A fluorescent histochemical study. *J. Auton. Nerv. Syst*. **22**, 247–251.

Amenta, F., Collier, W. L., Coppola, L., and De Michele, M. (1990). Senescence of the peripheral dopaminergic system. *In* "Peripheral Dopamine Pathophysiology" (F. Amenta, ed.), pp. 243–249. CRC Press, Boca Raton, FL.

Andrews, T. J., and Cowen, T. (1994a). *In vivo* infusion of NGF induces the organotypic regrowth of perivascular nerves following their atrophy in aged rats. *J. Neurosci*. **14**. 3048–3058.

Andrews, T. J., and Cowen, T. (1994b). Nerve growth factor enhances the dendritic arborization of sympathetic ganglion cells undergoing atrophy in aged rats. *J. Neurocytol*. **23**, 234–241.

Andrews, T. J., Li, D., Halliwell, J., and Cowen, T. (1994). The effect of age on dendrites in the rat superior cervical ganglion. *J. Anat*. **184**, 111–117.

Baker, D. M., and Santer, R. M. (1988). Morphometric studies on pre- and paravertebral sympathetic neurons in the rat: Changes with age. *Mech. Ageing Dev*. **42**, 139–145.

Baker, D. M., Watson, S. P., and Santer, R. M. (1991). Evidence for a decrease in sympathetic control of intestinal function in the aged rat. *Neurobiol. Aging* **12**, 363–365.

Barnes, R. F., Raskind, M., Gumbrecht, G., and Halter, J. B. (1982). The effects of age on the plasma catecholamine response to mental stress in man. *J. Clin. Endocrinol. Metab*. **54**, 64–69.

Belai, A., Cooper, S., and Burnstock, G. (1995). Effect of age on NADPH-diaphorase-containing myenteric neurones of rat ileum and proximal colon. *Cell Tissue Res*. **279**, 379–383.

Bhatnagar, B. K., and Moore, K. E. (1971). Effects of electrical stimulation, α-methyltyrosine and desmethylimipramine on the norepinephrine contents of neuronal cell bodies and terminals. *J. Pharmacol. Exp. Ther*. **178**, 450–463.

Bjerre, B., Wiklund, L., and Edwards, D. C. (1975). A study of the de- and regenerative changes in the sympathetic nervous system of the adult mouse after treatment with the antiserum to nerve growth factor. *Brain Res*. **92**, 257–278.

Bleys, R. L., Cowen, T., Groen, G. J., and Hillen, B. (1996). Perivascular nerves of the human basal cerebral arteries: II. Changes in aging and Alzheimer's disease. *J. Cereb. Blood Flow Metab*. **16**, 1048–1057.

Borst, S. E., Millard, W. L., and Lowenthal, D. T. (1994). Growth hormone, exercise, and aging: The future of therapy for the frail elderly. *J. Am. Geriat. Soci*. **42**, 528–535.

Bothwell, M. (1995). Functional interactions of neurotrophins and neurotrophin receptors. *Ann. Rev. Neurosci*. **18**, 223–253.

Buj-Bello, Buchman, V. L., Horton, A., Rosenthal, A., and Davies, A. M. (1995). GDNF is an age-specific survival factor for sensory and autonomic neurons. *Neuron* **15**, 821–828.

Burnham, P., Louis, J.-C., Magal, E., and Varon,S. (1994). Effects of ciliary neurotrophic factor on the survival and response to nerve growth factor of cultured rat sympathetic neurons. *Dev. Biol*. **161**, 96–106.

Chao, M. V., and Hempstead, B. L. (1995). P75 and trk: A two-receptor system. *Trends Neurosci*. **18**, 321–326.

Chen, M., Halter, J. B., and Porte, D., Jr. (1986). Plasma catecholamines, dietary carbohydrate, and glucose intolerance: A comparison between young and old men. *J. Clin. Endocrinol. Metab*. **62**, 1193–1198.

Christensen, N. J. (1973). Plasma noradrenaline and adrenaline in patients with thyrotoxicosis and myxoedema. *Clin. Sci. Mol. Med*. **45**, 163–171.

Collins, K. J., and Cowen, T. (1997). Disorders of the autonomic nervous system. *In* "Textbook of Geriatric Medicine and Gerontology" (J. C. Brocklehurst, R. C. Tallis, H. M. Fillit, ed.), pp. 539–564. Churchill-Livingstone, New York.

Cowen, T. (1993). Ageing in the autonomic nervous system: A result of nerve- target interactions? A review. *Mech. Ageing Dev*. **68**, 163–173.

Cowen, T., and Gavazzi, I. (1998). Plasticity in adult and ageing sympathetic neurons. *Prog. Neurobiol*. **54**, 249–288.

Cowen, T., Haven, A. J., Wen-Qin, C., Gallen, D. D., Franc, F., and Burnstock, G. (1982). Development and ageing of perivascular adrenergic nerves in the rabbit. A quantitative fluorescence histochemical study using image analysis. *J. Auton. Nerv. Sys*. **5**, 317–336.

Cowen, T., Thrasivoulou, C., Shaw, S. A., and Abdel-Rahman, T. A. (1996a). Transplanted sweat glands from mature and aged donors determine cholinergic phenotype and altered density of host sympathetic nerves. *J. Auton. Nerv. Syst*. **58**, 153–162.

Cowen, T., Gavazzi, I., Weingarten, J., and Crutcher, K. A. (1996b). Levels of NGF protein do not correlate with changes in innervation of the rat iris in old age. *NeuroReport* **7**, 2216–2220.

Cowen, T., Jenner, C., Song, G. X., Santoso, A. W., and Gavazzi, I. (1997). Responses of mature and aged sympathetic neurons to laminin and NGF: An *In vitro* study. *Neurochem. Res*. **22**, 1003–1011.

Crutcher, K. A. (1988). Sympathetic sprouting in the central nervous system: A model for studies of axonal growth in the mature mammalian brain. *Brain Res. Rev*. **12**, 203–233.

Crutcher, K. A. (1990). Age-related decrease in sympathetic sprouting is primarily due to decreased target receptivity: Implications for understanding brain aging. *Neurobiol. Aging* **11**, 175–183.

Crutcher, K. A., and Weingartner, J. (1991). Hippocampal NGF levels are not reduced in the aged Fischer 344 rat. *Neurobiol. Aging* **12**, 449–454.

Davies, A. M. (1997). Neurotrophin switching: Where does it stand? *Curr. Opin. Neurobiol*. **7**, 110–118.

Diamond, J., Foerster, A., Holmes, M., and Coughlin, M. (1992). Sensory nerves in adult rats regenerate and restore sensory function to the skin independently of endrogenous NGF. *J. Neurosci*. **12**, 1467–1476.

Edgar, D. (1989). Neuronal laminin receptors. *Trends Neurosci*. **12**, 248–251.

Featherstone, J. A., Veith, R. C., Flatness, D., Murburg, M. M., Villacres, E. C., and Halter, J. B. (1987). Age and alpha-2 adrenergic regulation of plasma norepineprhine kinetics in humans. *J. Gerontol*. **42**, 271–276.

Fleg, J. L., Tzankoff, S. P., and Lakatta, E. G. (1985). Age-related augmentation of plasma catecholamines during dynamic exercise in healthy males. *J. Appl. Physiol*. **59**, 1033–1039.

Fukagawa, N. K., Bandini, L. G., Lee, M. A., and Young, J. B. (1995). Effect of age on dopaminergic responses to protein feeding. *Am. J. Physiol*. **268**, F613–F625.

Gabella, G., and Uvelius, B. (1990). Urinary bladder of rat: Fine structure of normal and hypertrophic musculature. *Cell Tissue Res*. **262**, 67–79.

Gabella, G., Berggren, T., and Uvelius, B. (1992). Hypertrophy and reversal of hypertrophy in rat pelvic ganglion neurons. *J. Neurocytol*. **21**, 649–662.

Gavazzi, I., and Cowen, T. (1993). Axonal regeneration from transplanted sympathetic ganglia is not impaired by age. *Exp. Neurol*. **122**, 57–64.

Gavazzi, I., and Cowen, T. (1996). Can the neurotrophic hypothesis explain degeneration and loss of plasticity in mature and ageing autonomic nerves. *J. Auton. Nerv. Syst*. **58**, 1–10.

Gavazzi, I., Andrews, T. J., Thrasivoulou, C., and Cowen, T. (1992). Influence of target tissues on their innervation in old age: A transplanation study. *NeuroReport* **3**, 717–720.

Gavazzi, I., Boyle, K. S., Edgar, D., and Cowen, T. (1995). Reduced laminin immunoreactivity in the blood vessel wall of ageing rats correlates with reduced innervation in vivo and following transplantation. *Cell Tissue Res*. **281**, 23–32.

Gavazzi, I., Canavan, R. E. M., and Cowen, T. (1996). Influence of age and anti-nerve growth factor treatment on the sympathetic and sensory innervation of the rat iris. *Neuroscience* **73**, 1069–1079.

Gloster, A., and Diamond, J. (1992). Sympathetic nerves in adult rats regenerate normally and restore pilomotor function during an anti-NGF treatment that prevents their collateral sprouting. *J. Comp. Neurol*. **326**, 363–374.

Hasan, W., Cowen, T., Barnett, P. S., Elliot, E., Coskeran, P., and Bouloux, P. M. G. (2000). The sweating apparatus in growth hormone deficiency, following treatment with r-hGH and in acromegaly. Submitted for publication.

Hervonen, A., Vaalasti, A., Partanen, M., Kanerva, L., and Hervonen, H. (1978). Effects of ageing on the histochemically demonstrable catecholamines and acetylcholinesterase of human sympathetic ganglia. *J. Neurocytol*. **7**, 11–23.

Hoeldtke, R. D., and Cilmi, K. M. (1985). Effects of aging on catecholamine metabolism. *J. Clin. Endocrinol. Metab*. **60**, 479–484.

Isaacson, L. G., Saffran, B. N., and Crutcher, K. A. (1990). Intracerebral NGF infusion induces hyperinnervation of cerebral blood vessels. *Neurobiol. Aging* **11**, 51–55.

Jengeleski, C. A., Powers, R. E., O'Conner, D. T., and Price, D. L. (1989). Noradrenergic innervation of human of pineal gland: Abnormalities in aging and Alzheimer's disease. *Brain Res.* **481**, 378–382.

Jensen, E. W. (1999). Sympathetic activity and aging: Relationship to smoking habits, physical fitness, blood volume, and intracellular signalling. *Dan. Med. Bull*. **46**, 106–117.

Kedzierski, W., and Porter, J. C. (1990). Quantitative study of tyrosine hydroxylase mRNA in catecholaminergic neurons and adrenals during development and aging. *Mol. Brain Res*. **7**, 45–51.

Kenney, W. L. (1997). Thermoregulation at rest and during exercise in healthy older adults. *Exercise Sport Sci. Rev.* **25**, 41–76.

Kobayashi, M., Kurihara, K., and Matsuoka, I. (1994). Retinoic acid induces BDNF responsiveness of sympathetic neurons by alteration of Trk neurotrophin receptor expression. *FEBS Lett*. **356**, 60–65.

Kuchel, G. A. (1993). Alterations in target innervation and collateral sprouting in the aging sympathetic nervous system. *Exp. Neurol*. **124**, 380–385.

Kuchel, G. A., and Zigmond, R. E. (1991). Functional recovery and collateral neuronal sprouting examined in young and aged rats following a partial neural lesion. *Brain Res*. **540**, 195–203.

Kuchel, G. A., Rowe, W., Meaney, M. J., and Richard, C. (1997a). Neurotrophin receptor and tyrosine hydroxylase gene expression in aged sympathetic neurons. *Neurobiol. Aging* **18**, 67–79.

Kuchel, G. A., Poon, T., Irshad, K., Richard, C., Julien, J. P., and Cowen, T. (1997b). Decreased neurofilament gene expression is an index of selective axonal hypotrophy in aging. *NeuroReport* **8**, 799–805.

Kuchel, G. A., Crutcher, K. A., Naheed, U., Thrasivoulou, C., and Cowen, T. (1999). NGF expression is unchanged in a target exhibiting a significant loss of sympathetic axonal branching and varicosities in aging. *Neurobiol. Aging* **20**, 685–693.

Kuchel, O. G., and Kuchel, G. A. (1991). Peripheral dopamine in pathophysiology of hypertension: Interaction with aging and lifestyle. *Hypertension* **18**, 709–721.

Kuchel, O. G., and Kuchel, G. A. (1993). Peripheral catecholamines and aging. *In* "Aging of the Autonomic Nervous System" (F. Amenta, ed.), pp. 71–93. CRC Press, Boca Raton, FL.

Lakatta, E. G. (1995). Cardiovascular system. *In* "Aging" (E. J. Masoro, ed.) Handbook of Physiology, pp. 413–474. Oxford University Press, Oxford.

Lake, C. R., Ziegler, M. G., Coleman, M. D., and Kopin, I. J. (1977). Age-adjusted plasma norepinephrine levels are similar in normotensive and hypertensive subjects. *N. Engl. J. Med*. **296**, 208–209.

Landis, S. C. (1990). Target regulation of neurotransmitter phenotype. *Trends Neurosci*. **13**, 344–350.

Lee, C. R., Chernow, B., Feuerstein, G., Goldstein, D. B., and Ziegler, M. G. (1984). The sympathetic nervous system in man: Its evaluation and the measurement of plasma NE. *In* "Norepinephrine" (M. G., and Ziegler, C. R. Lake, ed.), pp. 1–26. Williams & W. Ikins, Baltimore, MD.

Lehmann, M., and Keul, J. (1981). The age- and training-dependent effects of physical work on plasma catecholamines and lactate acidosis. *Dsch. Zschr. Sportmed*. **12**, 320–328.

Lehmann, M., and Keul, J. (1986). Age-associated changes of exercise-induced plasma catecholamine responses. *Eur. J. Appl. Physiol*. **55**, 302–306.

Lehmann, M., Keul, J., Huber, G., Bachl, N., and Simon, G. (1981). Alters- und belastungsbedingtes Verhalten der Plasmacatecholamine. *Klin. Wochenschr.* **59**, 19–25.

Levi-Montalcini, R., and Hamburger, V. (1951). Selective growth-stimulating effects of mouse sarcoma on the sensory and sympathetic nervous system of the chick embryo. *J. Exp. Zool*. **116**, 321–361.

Levi-Montalcini, R., Dal Toso, R., Della Valle, F., Skaper, S. D., and Leon, A. (1995). Update of the NGF saga. *J. Neurol. Sci*. **130**, 119–127.

Lipsitz, L. A. (1989). Orthostatic hypotension in the elderly. *N. Engl. J. Med*. **321**, 952–957.

Lithgow, G. J., and Kirkwood, T. B. (1996). Mechanisms and evolution of aging. *Science* **273**, 80.

Low, P. A., Okazaki, H., and Dyck, P. J. (1977). Splanchnic preganglionic neurons in man. I. Morphometry of preganglionic cytons. *Acta Neuropathol*. **40**, 55–61.

Miller F. D., Speelman, A., Mathew, T. C., Fabian, J., Chang, E., Pozniak, C., and Toma, J. G. (1994). Nerve growth factor derived from terminals selectively increases the ratio of p75 to trkA NGF receptors on mature sympathetic neurons. *Dev. Biol.* **161**, 206–217.

Minaker, K. L., Rowe, J. W., Young, J. B., Sparrow, D., Pallotta, J. A., and Landsberg, L. (1982). Effect of age on insulin stimulation of sympathetic nervous system activity in man. *Metab. Clin. Exp*. **31**, 1181–1184.

Morrison, J. H., and Hof, P. R. (1997). Life and death of neurons in the aging brain. *Science* **278**, 412–418.

Neubauer, B., and Christensen, N. J. (1978). The decrease in noradrenaline concentration in the posterior tibial artery with age. *Gerontology* **24**, 299–303.

Pedersen, E. B., and Christensen, N. J. (1975). Catecholamines in plasma and urine in patients with essential hypertension determined by double-isotope derivative techniques. *Acta Med. Scand.* **198**, 373–377.

Purves, D., Hadley, R. D., and Voyvodic, J. T. (1986). Dynamic changes in the dendritic geometry of individual neurons visualized over periods of up to three months in the superior cervical ganglia of living mice. *J. Neurosci*. **6**, 123–128.

Purves, D., Snider, W. D., and Voyvodic, J. T. (1988). Trophic regulation of nerve cell morphology and innervation in the autonomic nervous system. *Nature (London)* **336**, 123–128.

Purves, D., Voyvodic, J. T., Harris, L., and Pomeroy, S. L. (1990). Dynamic aspects of innervation in the peripheral nervous system of adult mammals. *Signal Sense* 589–605.

Recio-Pinto, E., Rechler, M. M., and Ishii, D. N. (1986). Effects of insulin, insulin-like growth factor-II, nerve growth factor on neurite formation and survival in cultured sympathetic and sensory neurons. *J. Neurosci.* **6**, 1211–1219.

Rees, J., and Shuster, S. (1981). Pubertal induction of sweat gland activity. *Clin. Sci.* **60**, 689–692.

Reis, D. J., Ross, R. A., and Joh, T. H. (1977). Changes in the activity and amounts of enzymes synthesizing catecholamines and acetylcholine in brain, adrenal medulla, and sympathetic ganglia of aged rat and mouse. *Brain Res*. **136**, 465–474.

Reppert, S. M., and Weaver, D. R. (1995). Melatonin madness. *Cell (Cambridge, Mass.)* **83**, 1059–1062.

Rowe, J. W., and Kahn, R. L. (1987). Human aging: Usual and successful. *Science* **237**, 143–149.

Rowe, J. W., and Troen, B. R. (1980). Sympathetic nervous system and aging in man. *Endocrinol. Rev.* **1**, 167–179.

Ruit, K. G., Osborne, P. A., Schmidt, R. E., Johnson, E. M., Jr., and Snider, W. D. (1990). Nerve growth factor regulates sympathetic ganglion cell morphology and survival in the adult mouse. *J. Neurosci.* **10**, 2412–2419.

Santer, R. M. (1991). Morphological evidence for the maintenance of the cervical sympathetic system in aged rats. *Neurosci. Lett.* **130**, 248–250.

Santer, R. M. (1994). Survival of the population of NADPH-diaphorase stained myenteric neurons in the small intestine of aged rats. *J. Auton. Nerv. Syst.* **49**, 115–121.

Scarpace, P. J. (1988). Decreased receptor activation with age. Can it be explained by desensitization? *J. Am. Geriatr. Soc.* **36**, 1067–1071.

Schalling, M., Stieg, P. E., Lindquist, C., Goldstein, M., and Hokfelt, T. (1989). Rapid increase in enzyme and peptide mRNA in sympathetic ganglia after electrical stimulation in humans. *Proc. Natl. Acad. Sci. U. S. A.* **86**, 4302–4305.

Schmidt, R. E. (1991). Pathology of the sympathetic nervous system. *In* "The pathology of the Aging Human Nervous System" (S. Duckett, ed.), pp. 431–442. Lea & Febiger, Philadelphia.

Schmidt, R. E., Spencer, S. A., Coleman, B. D., and Roth, K. A. (1991). Immunohistochemical localization of GAP-43 in rat and human sympathetic nervous system—Effects of aging and diabetes. *Brain Res.* **552**, 190–197.

Schocken, D. D., and Roth, G. S. (1977). Reduced beta adrenergic receptor concentration in ageing man. *Nature (London)* **267**, 856–858.

Schotzinger, R., Yin, X., and Landis, S. (1994). Target determination of neurotransmitter phenotype in sympathetic neurons. *J. Neurobiol.* **25**, 620–639.

Schroer, J. A., Plurad, S. B., and Schmidt, R. E. (1992). Fine structure of presynaptic axonal terminals in sympathetic autonomic ganglia of aging and diabetic human subjects. *Synapse* **12**, 1–13.

Shannon, R. P., Wei, J. Y., Rosa, R. M., Epstein, F. H., and Rowe, J. W. (1986). The effect of age and sodium depletion on cardiovascular response to orthostasis. *Hypertension* **8**, 438–443.

Shelton, D. L., and Reichardt, L. F. (1984). Expression of the β-nerve growth factor gene correlates with the density of sympathetic innervation in effector organs. *Proc. Natl. Acad. Sci. U. S. A.* **81**, 7951–7955.

Sowers, J. R., Rubenstein, L. Z., and Stern, N. (1983). Plasma norepinephrine responses to posture and isometric exercise increase with age in the absence of obesity. *J. Gerontol.* **38**, 315–317.

Stemple, D. L., Mahanhtappa, N. K., and Anderson, D. J. (1988). Basic FGF induces neuronal differentiation, cell division, and NGF dependence in chromaffin cells: A sequence of events in sympathetic development. *Neuron* **1**, 517–525.

Thoenen, H. (1995). Neurotrophins and neuronal plasticity. *Science* **270**, 593–598.

Thrasivoulu, C., and Cowen, T. (1995). Regulation of rat sympathetic nerve density by target tissues and NGF in maturity and old age. *Eur. J. Neurosci.* **7**, 381–387.

Tonkin, A. L., and Wing, L. M. H. (1994). Effects of age and isolated systolic hypertension on cardiovascular reflexes. *J. Hypertension* **12**, 1083–1088.

Uchida, Y., and Tomonaga, M. (1985). Effects of nerve growth factor and heart cell conditioned medium on neurite regeneration of aged sympathetic neurons in culture. *Brain Res.* **348**, 100–106.

Uchida, Y., and Tomonaga, M. (1987). Loss of nerve growth factor receptors in sympathetic ganglia from aged mice. *Biochem. Biophys. Res. Commun.* **146**, 797–801.

Uvelius, B., and Gabella,G. (1998). The distribution of intramural nerves in urinary bladder after partial denervation in the female rat. *Urol. Res.* **26**, 291–297.

Veith, R. C., Featherstone, J. A., Linares, O. A., and Halter, J. B. (1986). Age differences in plasma norepinephrine kinetics in humans. *J. Gerontol.* **41**, 319–324.

Voyvodic, J. T. (1987). Development and regulation of dendrites in the rat superior cervical ganglion. *J. Neurosci.* **7**, 904–912.

Voyvodic, J. T. (1989). Target size regulates calibre and myelination of sympathetic axons. *Nature* (London) **342**, 430–433.

Wallin, B. G., Sundlof, G., Eriksson, B. M., Dominiak, P., Grobecker, H., and Lindblad, L. E. (1981). Plasma noradrenaline correlates to sympathetic muscle nerve activity in normotensive man. *Acta Physiol. Scand.* **111**, 69–73.

Warburton, A. L., and Santer, R. M. (1994). Sympathetic and sensory innervation of the urinary tract in young adult and aged rats: A semi-quantitative histochemical and immunohistochemical study. *Histochem. J.* **26**, 127–133.

Waterson, J. G., Frewin, D. B., and Soltys, J. S. (1974). Age-related differences in catecholamine fluorescence of human vascular tissue. *Blood Vessels* **11**, 79–85.

Weskamp, G., and Otten, U. (1987). An enzyme-linked immunoassay for nerve growth factor (NGF): A tool for studying regulatory mechanisms in NGF production in brain and in peripheral tissues. *J. Neurochem.* **48**, 1779–1786.

Young, A. J., and Lee, D. T. (1997). Aging and human cold tolerance. *Exp. Aging Res.* **23**, 45–67.

Young, J. B., Rowe, J. W., Pallotta, J. A., Sparrow, D., and Landsberg, L. (1980). Enhanced plasma norepinephrine response to upright posture and oral glucose administration in elderly human subjects. *Metab. Clin. Exp.* **29**, 532–539.

Ziegler, M. G., Lake, C. R., and Kopin, I. J. (1976). Plasma noradrenaline increases with age. *Nature (London)* **261**, 333–335.

Zigmond, R. E. (1980). The long-term regulation of ganglionic tyrosine hydroxylase by preganglionic nerve activity. *Fed. Proc. Fed.* **39**, 3003–3008.

Zigmond, R. E., Schwarzschild, M. A., and Rittenhouse, A. R. (1989). Acute regulation of tyrosine hydroxylase by nerve activity and by neurotransmitters via phosphorylation. *Annu. Rev. Neurosci.* **12**, 415–461.

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Sandra L. McFadden

Appendix

Basic Genetic Concepts

I. Chromosomes and Genes

Chromosomes and their functional units, genes, are the basic units of inheritance. They consist of chromatin, long strands of deoxyribonucleic acid (DNA) packed around a scaffold of proteins called histones (Fig. A.1). When a cell is quiescent, the DNA strands are loose in the cell nucleus. When the cell is preparing to divide (mitosis or meiosis), the chromatin packs tightly into chromatids. Sister chromatids attach together at the centromere, and the chromosome appears as a bottom-heavy X-shaped structure (Fig. A.1). The short arm of the chromosome is referred to as the ''p'' segment, and the long arm is the ''q'' segment.

Chromosomes and genes come in pairs. One member of each pair is maternally inherited; the other is paternally inherited. Humans have 46 chromosomes: 22 pairs of autosomes and one pair of sex chromosomes. Although many people assume that more complex organisms must have more chromosomes than simpler organisms, there is actually no correspondence between chromosome number and the complexity of an organism. For example, the crayfish (*Astacus trowbridgei*) has 188 pairs of chromosomes, the dog (*Canis familiaris*) has 39 pairs, and the mouse (*Mus musculus*) has 20 pairs.

Chromosomes vary in size, depending on how many genes they have, and how many bases comprise each gene. There are about 3000–4000 genes on each chromosome. The largest human chromosome, Chromosome 1, consists of 250 million bases. In contrast, the smallest chromosome, the Y chromosome, consists of 50 million bases. Altogether, the human genome consists of approximately 3 billion bases, on about 100,000 genes.

Gene pairs determine different traits. When both genes at a locus are the same, the individual is said to be homozygous at that locus. If the two genes differ, the individual is heterozygous at that locus. A gene is said to be dominant if the phenotype depends on the presence of this gene alone. A gene is recessive if homology is necessary for its phenotype to be expressed.

II. DNA and RNA Are Long Chains of Nucleotides

In gross terms, DNA is a set of instructions for making ribonucleic acid (RNA) and proteins. In molecular terms, DNA and RNA are polymers of nucleotides. As shown in Fig. A.2, nucleotides are units consisting of three components: a nitrogenous base, a sugar molecule, and a phosphate molecule. In DNA, the sugar is deoxyribose, and the nitrogenous base may be adenine (A), guanine (G), thymine (T), or cytosine (C). In RNA, the sugar is ribose. The bases are the same as in DNA, with one exception: in RNA, uracil (U) substitutes for T. A and G are purine bases; T and C and U are pyrimidine bases. The sugar and the phosphate molecules link together in repeating units, forming the backbone of the nucleic acid. The bases are attached to the sugar molecules.

DNA is a two-stranded molecule, whereas RNA consists of a single strand. In the DNA molecule, the two strands are held together by weak hydrogen bonds between pairs of bases (Fig. A.2). The strands are complementary rather than identical, because a long purine base on one strand always links to a shorter pyrimidine base on the other strand. The pairing of bases on the two strands of DNA is very specific. A always pairs with T, and G always pairs with C. When RNA is replicated, or when it attaches to a single strand of DNA during the process of transcription, U always pairs with A. Except during transcription or replication, the double-stranded DNA molecule is coiled into a helix (see Fig. A.1).

A different kind of DNA is found in specialized intracellular organelles called mitochondria. Mitochondrial DNA (mtDNA) is arranged in a double-stranded ring rather than a helix, and its genetic code differs from that of nuclear DNA (nDNA). More will be said about mtDNA later.

III. Each Gene Codes for a Specific Polypeptide

Every cell of the body has the same set of genes and nDNA, yet cells differ in structure and function. This is due to differ-

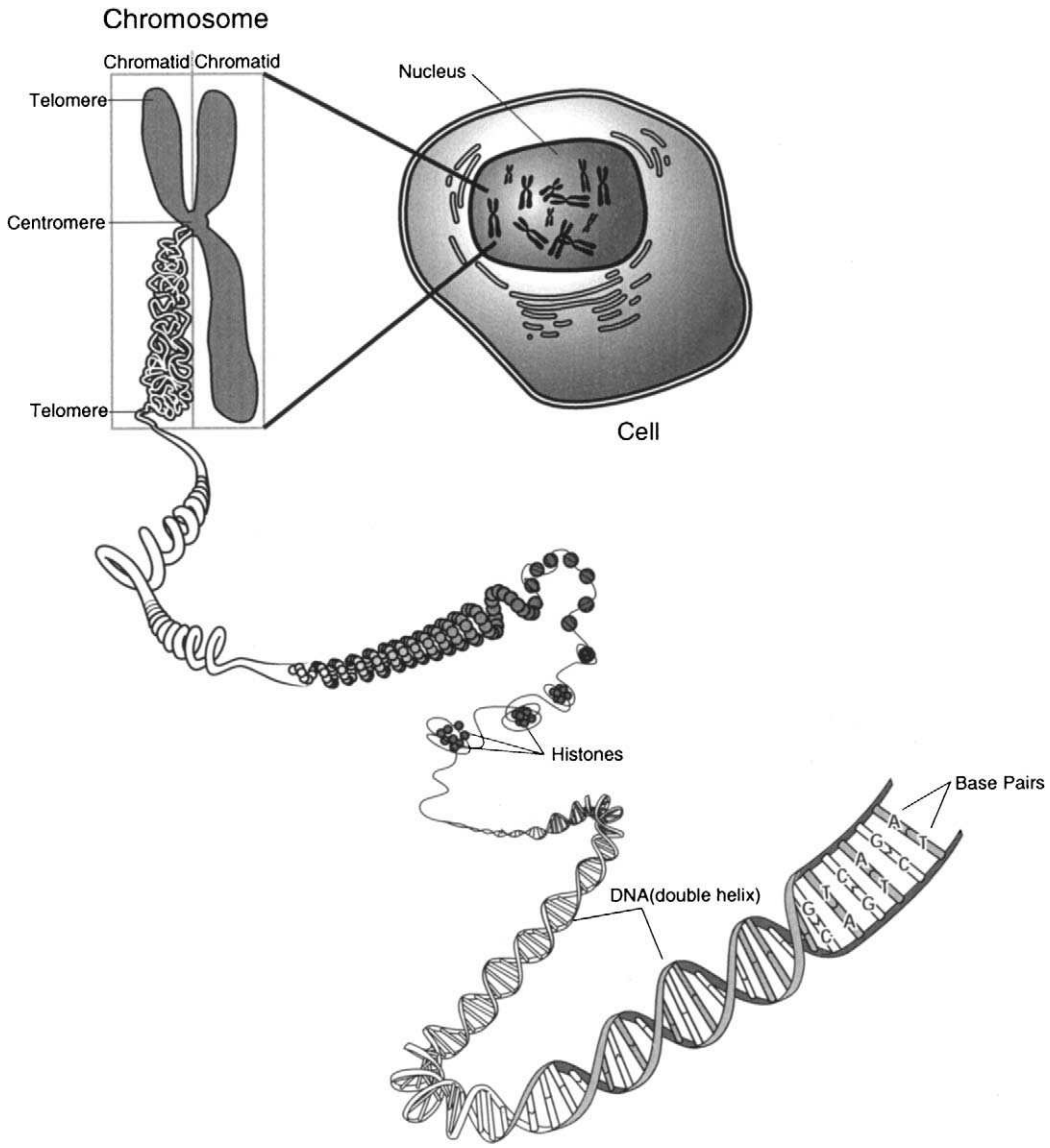

FIG. A.1. Several views of a nuclear chromosome. Sister chromatids are connected at the centromere. Each chromatid consists of tightly coiled strands of DNA and histones. (Illustration created by Darryl Leja, and obtained from the National Human Research Genome Institute, URL: www.nhgri.hih.gov.)

ential expression of proteins, related to the activation of different genes. Once a gene is activated by a cellular or environmental event, the processes of transcription and translation are set into motion. The final product is a protein, which does most of the work of the cell.

Each gene codes for a particular polypeptide, or chain of amino acids (Fig. A.3). The instructions for assembling the amino-acid chain are represented by the sequence of bases comprising a gene. The amino-acids in a chain are linked together with peptide bonds. Two linked amino acids form a unit called a ''dipeptide,'' and three linked amino acids form a ''tripeptide.'' When many (generally hundreds to thousands) amino acids are linked together, the functional unit is a protein. Proteins and their constituent amino acids perform many functions essential to life. Amino acids can act as neurotransmitters and antioxidants; proteins can act as structural elements, antibodies, enzymes, membrane channels, receptors, regulators of gene function, and molecular transporters. Proteins can also combine with nonproteins to form glycoproteins, lipoproteins, and nucleoproteins, all of which are essential to cell integrity and function.

IV. Proteins Are the End Product of Gene Expression

Gene expression involves transcription and translation (Fig. A.4). Transcription refers to the process of making a copy of the genetic code carried by the DNA of the gene. This is the function of messenger RNA (mRNA). Translation refers to the process whereby amino acids are assembled into polypeptide chains, as specified by the genetic code. This pro-

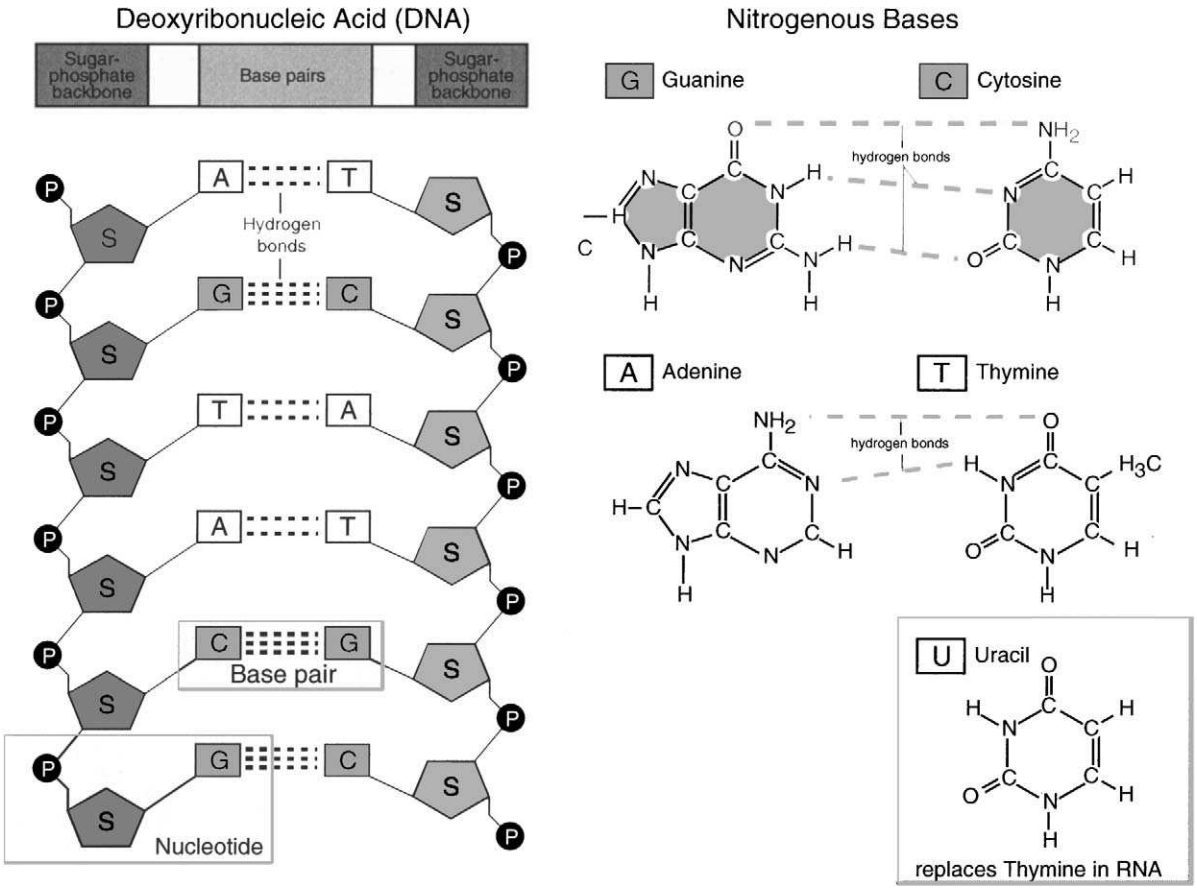

FIG. A.2. The structure of DNA. Four nitrogenous bases (adenine, A; thymine, T; cytosine, C; guanine, G) are linked like rungs of a ladder to a sugar-phosphate backbone. The two strands of DNA are held together by weak hydrogen bonds between pairs of bases. The sequence of bases contains the information necessary for protein synthesis. (Illustration created by Darryl Leja, and obtained from the National Human Research Genome Institute, URL: www.nhgri.hih.gov.)

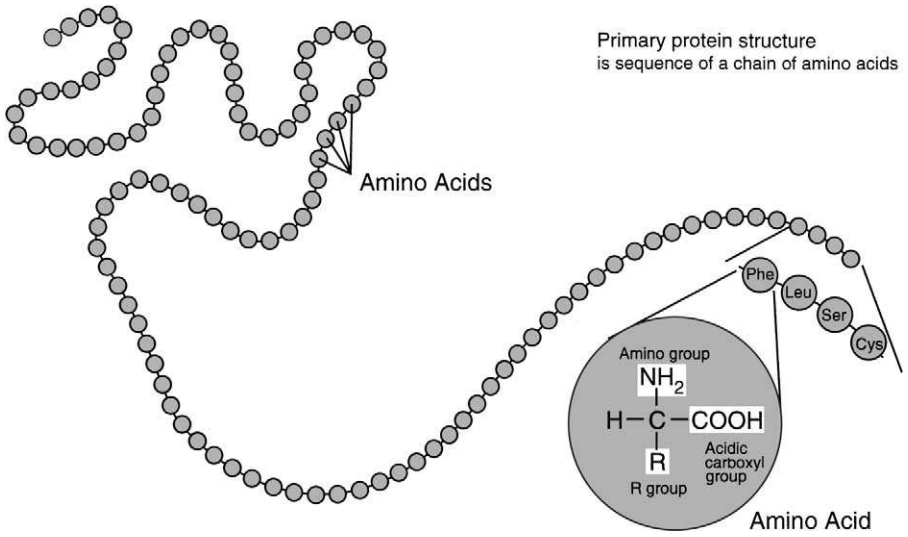

FIG. A.3. Primary structure of a protein. Proteins are long chains of amino acids, linked together with peptide bonds. Every amino acid has an amino group (NH_2) and a carboxyl group (COOH). Differences in the R group define specific amino acids. Functional proteins are characterized by a complex three-dimensional shape that makes them biologically active. (Illustration created by Darryl Leja, and obtained from the National Human Research Genome Institute, URL: www.nhgri.hih.gov.)

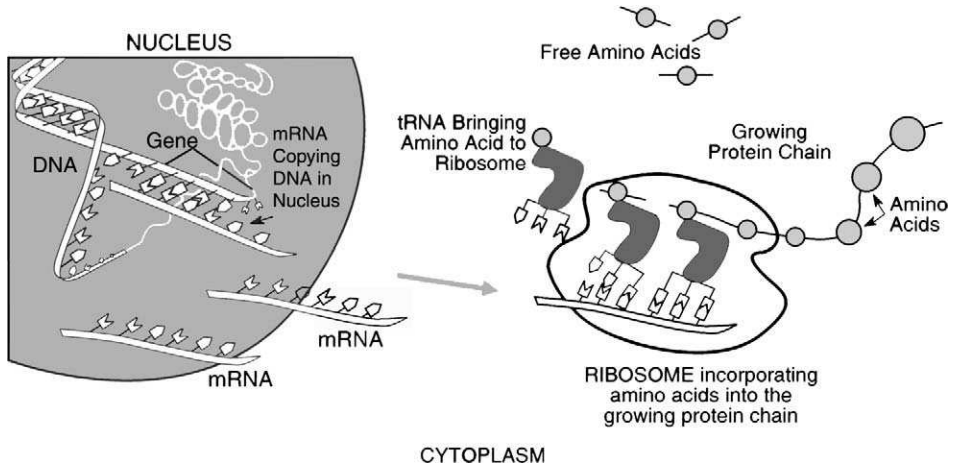

FIG. A.4. A summary of gene expression (transcription and translation). When a gene is activated, the two strands of DNA separate, and the base sequence of a strand is transcribed (copied) to messenger RNA (mRNA). The mRNA molecules are processed to remove introns (see text), then travel from the nucleus to the ribosomes in the cytoplasm of the cell. The ribosomes translate the genetic code, specified by triplets of bases called codons, and construct proteins from amino acids supplied by transfer RNA (tRNA). A protein is the final product of gene expression. (Illustration courtesy of the U.S. Department of Energy Human Genome Program, from the Primer on Molecular Genetics, which may be downloaded from www.ornl.gov/hgmis.)

cess requires ribosomes, ribosomal RNA (rRNA), amino acids, and transfer RNA (tRNA).

The transcription process begins when the gene is activated. As shown in Fig. A.4, the two strands of DNA separate, and a copy of the DNA strand is made by mRNA, a transient intermediary molecule similar to a single strand of DNA. In eukaryotic cells, only about 10% of the bases on the DNA molecule code for proteins; these coding sections are called exons. Noncoding portions are called introns. The mRNA is processed before it leaves the nucleus to remove all introns and to add ''start'' and ''stop'' sequences to the ends of the mRNA molecule. The start sequence is added to one end, called the 5′ end, and a ''poly(A)tail,'' or end sequence, is attached to the opposite (3′) end.

After the mRNA has been processed, it travels to the ribosomes in the cytoplasm of the cell, where translation occurs (Fig. A.4). Amino acids are carried to the ribosomes by tRNA, and the ribosomes incorporate them into a polypeptide chain. The sequence of amino acids is specified by the sequence of bases on the mRNA. Each three bases forms a unit called a codon, as illustrated in Fig. A.5. Three codons are ''stop'' codons that signal the end of gene transcription. Other codons represent particular amino acids, as summarized in Table A.1. For example, the DNA base sequence ATG codes for the amino acid methionine. The corresponding base sequence for RNA is AUG. Since there are 64 possible codons and 20 amino acids, there is considerable redundancy in the genetic code. For example, glycine, the simplest amino acid, is represented by four different codons: GAA, GCC, GGG, and GGT (or GGU for RNA).

V. Gene Mutations Can Take Many Forms

A mutation is a change in the original DNA code for a protein. Mutations can arise through mistakes in DNA replication or DNA damage caused by chemical mutagens or radiation. Mutations of somatic DNA will affect only that individual, whereas mutations in the DNA of a reproductive cell (gamete) will be passed on to the individual's offspring. If this occurs, every cell of the offspring's body will contain that particular mutation. Assuming that the mutation is not lethal, the off-

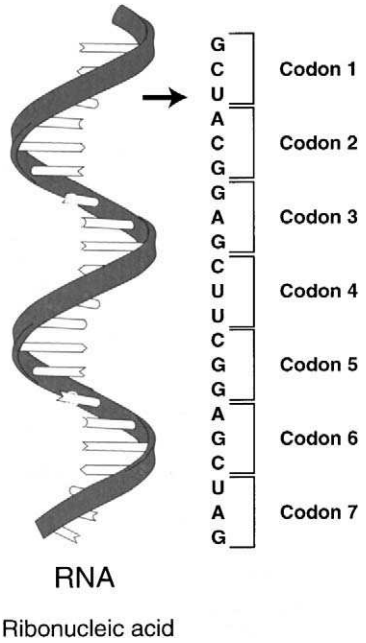

FIG. A.5. Codons on a segment of RNA. Every three bases form a codon, which is the code for a particular amino acid. Codons 1–6 represent the amino acids alanine, threonine, glutamate, leucine, arginine, and serine, respectively. Codon 7 is a ''stop'' codon signal the end of the RNA chain. (Illustration created by Darryl Leja, and obtained from the National Human Research Genome Institute, URL: www.nhgri.hih.gov.)

TABLE A.1 DNA Codons for 20 Amino Acids

Amino acid	Codons
Alanine	GCA, GCC, GCG, GCT
Arginine	AGA, AGG, CGA, CGC, CGG, CGT
Asparagine	AAC, AAT
Aspartic acid	GAC, GAT
Cysteine	TGT, TGC
Glutamic acid	GAA, GAG
Gutamine	CAA, CAG
Glycine	GGA, GGC, GGG, GGT
Histadine	CAT, CAC
Isoleucine	ATT, ATC, ATA
Leucine	CTA, CTC, CTG, CTT, TTA, TTG
Lysine	AAA, AAG
Methionine	ATG
Phenylalanine	TTT, TTC
Proline	CCA, CCC, CCG, CCT
Serine	TCA, TCC, TCG, TCT, AGC, AGT
Threonine	ACT, ACC, ACA, ACG
Tryptophan	TGG
Tyrosine	TAC, TAT
Valine	GTA, GTC, GTG, GTT

spring will in turn pass the mutation on to future generations. Individuals with single recessive mutations are known as carriers.

DNA mutations can take many forms. These include point mutations (alterations in a single base or nucleotide), single and multiple deletions or insertions of small DNA segments, and duplications of entire coding sequences. A change in a codon that results in the wrong amino acid being incorporated into the polypeptide chain is referred to as a missense mutation. A change in a codon that leads to premature termination of translation is referred to as a nonsense mutation. Hundreds of genes control auditory system development and function. Mutations of genes required for the maintenance of cochlear homeostasis through life would be expected to render the cochlea more vulnerable than normal to environmental insults or to cause hearing loss directly.

VI. Mitochondria

Mitochondria are bacteria-sized intracellular organelles (Fig. A.6) that play a pivotal role in cellular energy production through synthesis of adenosine triphosphate (ATP). They are also involved in other important cellular functions, such as synthesis of lipids, heme, and some amino acids. Each mitochondrion consists of an external membrane that is freely permeable to low molecular mass ions and molecules, and an internal membrane that is highly folded and relatively impermeable, especially to ions. The inside of the mitochondrion, the matrix, contains a variety of enzymes and fiber-like structures that are involved in the production of ATP in the oxidative phosphorylation pathway. The matrix is also the location of 2–10 molecules of double-stranded mtDNA. Unlike nDNA, mtDNA is arranged in a closed loop.

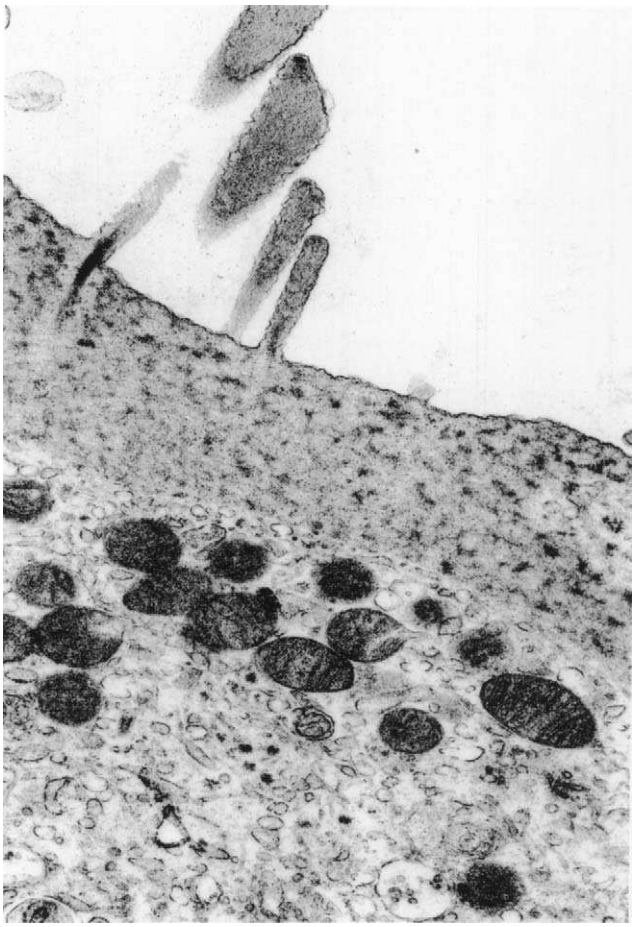

FIG. A.6. Photomicrograph showing mitochondria gathered beneath the cuticular plate of an outer hair cell. Mitochondria vary in shape and size, but each has a highly permeable external membrane, and a highly invaginated, nonpermeable internal membrane. The internal membrane forms the boundary around the internal matrix. The matrix is the location of 2–10 mitochondrial chromosomes, consisting of mitochondrial DNA (mtDNA). The matrix is also the site of oxidative phosphorylation. Toxic reactive oxygen species produced as a by-product of oxidative phosphorylation can cause damage to the mtDNA. (Photo courtesy of Dalian Ding, Center for Hearing and Deafness, SUNY at Buffalo.)

A. Genetics of mtDNA

Mitochondrial DNA has 37 genes, and it is 16,569 base pairs long. The outer strand codes for 12 proteins, 14 tRNAs, and 2 rRNAs, while the inner strand codes for 1 protein and 8 tRNAs. The 13 mRNAs are translated on mitochondrion-specific ribosomes into 13 of the proteins required for making the five enzyme complexes that perform oxidative phosphorylation. The rest of the 60 or so protein subunits required for synthesis of the respiratory chain enzymes are produced by

nuclear DNA and imported into the mitochondrion. It is notable that the genetic code of mtDNA differs from the nDNA code, and there is only one noncoding sequence in the compact mtDNA molecule.

Mitochondrial DNA is transmitted exclusively through mothers. Thus, when a disease has a maternal pattern of inheritance, mtDNA mutations are suspect. The precise mechanisms governing mtDNA replication are poorly understood, but it is clear that mtDNA transcription is not closely restricted by the cell cycle. mtDNA may replicate at any time, independent of the cell cycle, and mitochondria have a relatively rapid turnover rate (weeks). This is one reason why mtDNA is far more susceptible to mutation than is nuclear DNA. There are several other reasons as well. First, mtDNA is not protected by a histone coat, and DNA repair mechanisms are lacking relative to nDNA. Of the five eukaryotic DNA polymerases known, only one is found in mitochondria, and this polymerase is inefficient in protecting DNA from inaccurate replications. (Stable mutations of nuclear DNA are actually rare, because the cell nucleus has many repair mechanisms.) Second, since mtDNA is present in high copy number within a cell, mutations are numerically more likely. Most importantly, mtDNA is located in the matrix of the mitochondrion, where it is exposed to high levels of free radicals and reactive oxygen species produced during oxidative phosphorylation. During normal aerobic respiration, mitochondria consume oxygen, reducing it in a stepwise fashion to water. Energy is harnessed in the high-energy bonds of ATP. Reactive oxygen species, particularly superoxide and hydroxyl radicals, are by-products of the process. These highly reactive molecules can attack proteins and membranes, damage DNA and trigger cell death through apoptosis if they are not ''neutralized'' by free radical scavengers and antioxidants, such as mitochondrial manganese-superoxide dismutase (Mn-SOD).

As with nDNA, mtDNA mutations can arise through mistakes in mtDNA replication or exposure to environmental agents (including certain medications such as AZT). Some autosomal recessive and autosomal dominant inherited genetic disorders can lead to mtDNA pathology. Cells and tissues with high energy demands (e.g., the central nervous system and the inner ear) are the most vulnerable to mitochondrial dysfunction due to reactive oxygen species damage.

When a mtDNA mutation arises within a cell, the cell will have two populations of mtDNA, one wild type (normal) and the other mutant. This situation is termed heteroplasmy. Since mtDNA may segregate unevenly to daughter cells during meiotic or mitotic cell division, heteroplasmy ratios may vary from one daughter cell to another. Over many divisions, the percentage of mutant and normal mtDNA molecules drifts toward homoplasmy, with the mtDNA within the cell being either purely mutant or purely normal. This process is known as replicative segregation. Rapidly dividing cells such as leucocytes often have low levels of heteroplasmy. In contrast, cells that are postmitotic, such as mammalian hair cells and neuronal cells, accumulate mutant mtDNA molecules and have a high ratio of heteroplasmy. As the percentage of mutant molecules increases, the ability of the mitochondria to carry out their normal functions becomes increasingly impaired. When the energetic threshold of the cell is exceeded, the cell will die. When the energetic threshold of an organ is exceeded, clinical manifestations will emerge.

B. Biology of mtDNA

Evidence is accumulating that mitochondrial pathology plays a role in the pathogenesis of many late-onset neurodegenerative disorders, including Parkinson's disease, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis. Mitochondrial dysfunction has even been implicated in the degenerative processes of aging itself (see reviews by Wallace *et al*., 1995; Simon and Johns, 1999; Wallace, 1999). The ATP-generating capacity of a tissue declines with age, and this decline is correlated with an age-related increase in somatic mtDNA damage in postmitotic tissue (Brown and Wallace, 1994). The decline in energetic capacity with age may exacerbate disease expression, or make disease expression more likely. Some mtDNA mutations might lead to disease only in the presence of a specific nuclear genotype or environmental agent. Other mtDNA pathology may have direct effects on tissue and organ function. For example, the relationship between mtDNA defects and presbycusis is entirely speculative at this time. However, mutations in mitochondrial genes have recently been found to be associated with a variety of hearing defects, and it is plausible that they contribute to presbycusis as well (Fischel-Ghodsian, 1998).

References

Brown, M. D., and Wallace, D. C. (1994). Molecular basis of mitochondrial DNA disease. *J. Bioenerg. Biomembr.* **26**, 273–289.

Fischel-Ghodsian, N. (1998). Mitochondrial genetics and hearing loss: The missing link between genotype and phenotype. *Proc. Soc. Exp. Biol. Med.* **218**, 1–6.

Simon, D. K., and Johns, D. R. (1999). Mitochondrial disorders: Clinical and genetic features. *Annu. Rev. Med.* **50**, 111–127.

Wallace, D. C. (1999). Mitochondrial diseases in man and mouse. *Science* **283**, 1482–1488.

Wallace, D. C., Shoffner, J. M., Trounce, I., Brown, M. D., Ballinger, S. W., Corral-Debrinski, M., Horton, T., Jun, A. S., and Lott, M. T. (1995). Mitochondrial DNA mutations in human degenerative diseases and aging. *Biochim. Biophys. Acta* **1271**, 141–151.

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