Handbook of Behavioral Neurobiology

14

Neurobiology of Food and Fluid Intake

Second Edition

Edited by Edward Stricker
and Stephen Woods

Handbook of

Behavioral Neurobiology

Volume 14

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KLUWER ACADEMIC PUBLISHERS

NEW YORK, BOSTON, DORDRECHT, LONDON, MOSCOW

eBook ISBN: 0-306-48643-1
Print ISBN: 0-306-48484-6

Created in the United States of America

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This Handbook is dedicated to our
scientific predecessors, who continue to be
an invaluable source of knowledge and inspiration,
and also to our students,
who carry our hopes and interests
into the future

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Preface

Like previous handbooks, the present volume is an authoritative and up-to-date compendium of information and perspective on the neurobiology of ingestive behaviors. It is intended to be stimulating and informative to the practitioner, whether neophyte or senior scholar. It is also intended to be accessible to others who do not investigate the biological bases of food and fluid ingestion, who may teach aspects of this material or simply wonder about the current state of the field. To all readers, we present this handbook as a progress report, recognizing that the present state of the field is much farther along than it was the last time a handbook was published, but mindful of the likelihood that it is not as far along as it will be when the next handbook is prepared.

This field has witnessed a spectacular accretion of scientific information since the first handbook was published in 1967. During the generation of science between then and the publication of the second handbook in 1990, numerous scientific reports have substantially changed the perspective and informational base of the field. Among the highlights of that earlier period, arranged more or less chronologically, were the following:

- hypovolemia and angiotensin II were identified as excitatory signals of thirst, which added considerations of blood volume and pressure to discussions of the biological factors that stimulate water consumption;
- inhibitory signals were identified in the control of thirst, which allowed the formulation of more complex schema than the simple presence or absence of excitatory signals;
- the intestinal peptide hormone cholecystokinin was identified as a potent factor in mediating inhibition of food intake, which helped shift the focus of investigations from signals of hunger to signals of satiety, from the control of body weight to the control of individual meals, and from the hypothalamus to the brain stem;
- other peptides were identified as important in the control of food intake, especially bombesin and insulin, which provided a physiological bridge between gastrointestinal digestion, adipose tissues lipid stores, and food consumption;

- the critical role of central dopamine-containing neurons in behavioral arousal was identified, which expanded theories of the brain's control of food and fluid ingestion by including nonspecific features of motivation;
- three circumventricular organs (i.e., the organum vasculosum of the lamina terminalis, the area postrema, and the subfornical organ) were discovered to play important roles in the control of thirst and vasopressin secretion, which helped move discussions of body fluid homeostasis into the realm of behavioral neuroscience;
- the importance of learning (e.g., conditioned taste aversions) and social influences on food intake were re-emphasized, which broadened discussions of eating to include nonhomeostatic factors;
- the distinction between suckling and independent ingestion in the development of ingestive behavior was clarified, which strengthened the ontogenetic perspective on eating;
- the roles of angiotensin II and aldosterone, and their synergetic interaction, in stimulating NaCl appetite in rats were identified, which provided a testable hypothesis to account for the activation of this motivated behavior; and
- oxytocin and atrial natriuretic peptide, two circulating hormones important for urinary fluid excretion in the service of body fluid homeostasis, were additionally identified as neurotransmitters released from brain neurons, which ultimately introduced issues of inhibition into discussions of salt appetite.

These and other discoveries greatly stimulated interest and advanced knowledge and understanding in the field of ingestive behavior. During the past 13 years, continued activity in this lively field has been highlighted by several extraordinary breakthroughs that have provided additional substance to our deliberations. Among them are the following:

- leptin was discovered, which provided another blood-borne peptide signal in the control of food intake and its integration with fat stores in adipose tissue and body weight maintenance;
- the melanocortin system in the basal hypothalamus was discovered, which provided an avenue by which leptin and insulin could exert their effects on food intake and metabolism;
- NPY-containing neurons were recognized as the other component of a modern dual control system in the hypothalamus, which provided body weight maintenance in addition to the control of individual meals; and
- overlapping signals influencing thirst and salt appetite were identified, which encouraged development of unified hypotheses that linked these two models of motivated behavior.

In addition, research in this field was greatly facilitated by technical developments that resulted from general advances in cellular and molecular neurobiology, including the following:

- the expression of immediate early genes (e.g., c-fos protein), which gained widespread use in determining the neuronal systems in the brain that were activated by experimental treatments and behaviors;
- immunocytochemical techniques, which allowed identification of chemically specific neurons in the brain; and
- genetic mutants (i.e., mice with targeted genes that had been "knocked out" or otherwise changed), which provided a new approach to removing or overexpressing ligands and receptors.

Furthermore, great advances were made in the molecular biology of taste receptors, which, when combined with behavioral measures of gustation, provided unparalleled insights into the varied role of taste in guiding ingestive behavior.

These and other striking developments stimulated preparation of the present handbook, to update the masterful chapters in the previous handbooks. Considering the subject matter in successive handbooks reveals that this dynamic field has become much more multidisciplinary over the years, which is appropriate because the biological basis of ingestive behavior is a multidisciplinary subject. A complex story is now available to describe the control of food intake, involving signals related to body fat stores and to gastrointestinal events, involving control systems in the hypothalamus and the brain stem, and involving neural signals and blood-borne peptides. This story should be of considerable interest to neuroscientists and to peptide physiologists with their interest in signals, neural circuits, and integrative neural systems. The same issues are available to investigators and scholars who are interested in the behavioral contributions to body fluid homeostasis. After all, the discovery of the prominent role of angiotensin II in thirst and NaCl appetite 20–30 years ago resembles the more recent discovery of leptin in that both developments stimulated considerable interest in the neurobiological control of ingestive behavior.

The present handbook provides 11 chapters summarizing key, traditional issues in the field. The focus on food intake in many of them reflects the current emphasis in the field, which has been stimulated in large part by the discovery of leptin and the apparent epidemic of obesity and diabetes in Western cultures. More specifically, two review chapters (and in one instance three) are presented on each of five issues: neuroanatomical control systems, including their development; neural, endocrine, and substrate signals that stimulate and inhibit food intake; anorexia, including the interaction between the immune system and the central nervous system; comparative studies of the control of food intake, including those using human subjects, since most of the other chapters focus on research using rodents as experimental subjects; and body fluid homeostasis, with a focus on thirst and salt appetite. In addition, two essays are presented that provide perspective in each of three issues: motivation and reinforcement, taste, and obesity. Collectively, these 17 chapters provide an interesting mixture of emerging issues and reflective comments about the development of relevant ideas.

As with any complex and time-consuming project, our work on this handbook could not have proceeded nearly as well without the contributions and cooperation of many others, to whom we are grateful. In particular, we thank our wives, Marcy Stricker and Nancy Woods, for their continued support while we were preoccupied with an editorial enterprise that did not involve them. We also thank the senior editor of Kluwer Academic/Plenum Publishers, Kathy Lyons, for the opportunity to prepare this handbook and for her assistance throughout the process.

Since the last Handbook was published, Alan Epstein, Jacques Le Magnen, and Eliot Stellar have passed away. These prominent scientists were particularly influential to our own training and development, and their presence is greatly missed.

In behalf of our colleagues, we dedicate the volume to our scientific predecessors, who continue to be an invaluable source of knowledge and inspiration, and also to our students, who carry our hopes and interests into the future.

September 2003

Edward M. Stricker
Stephen C. Woods

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ESSAYS

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Introduction

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1

Behavioral Neurobiology of Ingestive Behaviors

Edward M. Stricker and Stephen C. Woods

The development of hunger or thirst in response to extended periods of food or water deprivation is a basic feature of motivated ingestive behavior. So, too, is the cessation of food and water intake when animals become satiated. Yet, despite the great familiarity of eating and drinking, such superficial descriptions conceal deeper questions of mechanism that have confounded scientific investigators for generations. How do animals know when they are hungry or thirsty? Why do they stop eating or drinking when they do? How is food intake associated with the maintenance of body weight? How does the brain use this information to produce sensations and motivations? These and related questions have been translated into the operational questions that have driven basic research for the past century. What stimuli elicit the sensations of hunger and thirst and satiety? How do animals keep track of body fat stores and use that information to influence food intake? Which central neural circuits control food and fluid intake, and integrate motivated ingestive behavior with complementary physiological responses that similarly promote homeostasis?

The present volume of the *Handbook of Behavioral Neurobiology* considers these and other issues of ingestive behavior. Most of the research described makes use of laboratory animals as experimental subjects, typically rodents, though the implications of this work are thought to extend to mammals generally, including humans. Disorders in human ingestive behavior are prominent symptoms of certain clinical illnesses (e.g., the polydipsia of diabetes mellitus and diabetes insipidus, the

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Neurobiology of Food and Fluid Intake, 2nd Ed., Volume 14 of *Handbook of Behavioral Neurobiology,* edited by Edward Stricker and Stephen Woods, Kluwer Academic / Plenum Publishers, New York, 2004.

hyperphagia that may be associated with obesity), and research in this field has been used to address related issues of clinical relevance. However, the work described in this volume is not intended to consider specific clinical issues but rather to provide a current assessment of the biological bases of ingestive behavior.

This volume of the *Handbook of Behavioral Neurobiology* is the third handbook to be devoted to considerations of ingestive behavior. The first volume was published in 1967 by the American Physiological Society, as part of their series of handbooks on the physiology of the gastrointestinal tract (Code, 1967). It is interesting to note that a second such volume was not included in their updated series of handbooks, published 20 years later. In truth, although hunger and thirst can be viewed as appropriate subjects of physiology, considerations of regulatory ingestive behavior never have been in the mainstream of that discipline. Indeed, all organismal considerations, not just behavior, seem to have been receiving progressively less attention by physiologists in the past 20–30 years as analyses have moved to more cellular and molecular levels. Thus, when a second handbook devoted to ingestive behavior was published in 1990 (Stricker, 1990), it was a volume in the series of handbooks of behavioral neurobiology. The present handbook is a new volume in that series, and updates the field to include the significant developments that have occurred during the past 13 years.

We believe the switch in perspective of these handbooks of ingestive behavior, from physiology to behavioral neurobiology, reflects a significant development that has occurred in the field during the past four decades. We present our views of that development in this introductory chapter. In doing so, we provide our answers to two related questions that, in conversation, we are often asked to address. First, how does contemporary work on ingestive behavior differ from the work done in the 1960s, when both of us were graduate students in departments of psychology? And second, what do we mean by the "behavioral neurobiology" of ingestive behavior? Of course, our answers to these questions necessarily reflect our individual training and subsequent experiences, and we recognize that others may hold different views. Still, we believe it will be of value to note some of the many changes in the field that have occurred during our professional lifetimes, which at the moment encompass a period roughly equal to the time between the first handbook on ingestive behavior and the present one.

Fifty years ago, ingestive behaviors were considered from the perspective of the "depletion–repletion" hypothesis. Stated simply, animals deficient in one nutrient or another were thought to be motivated to consume the needed nutrient because its depletion was associated with a specific stimulus that elicited ingestive behavior, and to experience satiety because the excitatory stimulus disappeared when nutrient repletion occurred. Thirst, for example, was recognized as a product of water deficiency, and was easily studied in the laboratory by depriving experimental animals of drinking water. By the 1950s and early 1960s, an osmoregulatory stimulus of thirst had been well established by studies in which hyperosmotic solutions were administered by systemic (Gilman, 1937; Holmes & Gregersen, 1950) or intrahypothalamic injections (Andersson, 1953). This stimulus also appeared to be the critical factor in models of thirst involving a high salt diet, renal sodium (Na^+) retention, and diabetes insipidus.

Salt appetite was similarly considered from the perspective of a depletion–repletion hypothesis, but in this case it was not possible to create Na^+ deficiency simply by depriving the animals of dietary Na^+ because urinary Na^+ loss was virtually eliminated by the renal actions of the adrenal steroid hormone, aldosterone.

Surgical removal of the adrenal gland prevented this adaptive physiological response and produced in animals an uncontrolled loss of Na^+ in urine and a compensatory salt appetite (Richter, 1936). The loss of Na^+-rich saliva through an externalized parotid fistula similarly provided a very effective model of salt appetite in intact sheep (Denton & Sabine, 1961). The paradoxical increase in NaCl intake by rats after treatment with excess mineralocorticoids, in association with renal Na^+ retention, was recognized but unexplained (Rice & Richter, 1943).

Historically, the biological bases of hunger and satiety received the most experimental attention among the ingestive behaviors (as also is true today), especially the role of the brain in the control of food intake. Studies of food intake largely involved animals that had been deprived of food, but other models were coming under increased scrutiny. For example, it was well known that food intake could be increased by insulin-induced hypoglycemia and by diabetes mellitus associated with insulin deficiency, and that both phenomena provided support for a glucostatic hypothesis of hunger (Mayer, 1955). It was also well known that daily food intake increased after animals had been food-deprived and lost body weight, and decreased after they had overeaten and gained weight; these and other observations formed the basis of a lipostatic hypothesis of hunger (Kennedy, 1953). The striking observations of hyperphagia and obesity after medial hypothalamic lesions in rats (Brobeck, Tepperman, & Long, 1943) and other experimental animals led to the concept of a satiety center in the brain (Stellar, 1954), and the existence of a complementary hunger center in the lateral hypothalamus was suggested by the remarkable findings that lateral hypothalamic lesions caused animals to stop eating completely and starve to death despite the ready availability of familiar and nutritious food (Anand & Brobeck, 1951). Perhaps even more remarkable were observations that microinjections of norepinephrine into the lateral hypothalamus elicited food intake (Grossman, 1960), which raised the possibility that behaviors were chemically coded in the brain. The elicitation of water intake by microinjections of acetylcholine into the lateral hypothalamus encouraged that perspective (Grossman, 1960). In retrospect, it is easy to recognize that a simple chemical coding of behavior was conceptually misguided. However, the general point of this idea—that the brain was not so complicated and that its organization and function ultimately could be understood—was very attractive and drew many investigators to the field.

A list of the most popular animal models of ingestive behavior under investigation in the early 1960s is presented in Table 1A. It is instructive to compare that list with the much larger number of animal models, listed in Table 1B, that are available for study at present. As discussed in various chapters in this volume, thirst is now recognized to be controlled by four excitatory signals and three or four inhibitory signals, some neural and some blood-borne, each of which can be reproduced individually by specific experimental treatments. Hunger and satiety, similarly, are now seen as the products of multiple signals related either to the consequences of individual meals or to changes in body weight; and as with the stimuli that control thirst, some of these signals are neural and some are blood-borne, and each of them can be reproduced by specific experimental treatments. Although the signals that control NaCl appetite still are controversial and unsettled, the number of experimental models being investigated has increased considerably. The hypothesis of dual hypothalamic centers controlling food intake has been replaced by a much more complex arrangement involving specific circuits in the hypothalamus and the brain stem (which retain the feature of dual controls) and nonspecific

Table 1. Animal Models of Ingestive Behavior in Common Use

A. During the early 1960s	B. During the early 21st century
Thirst	*Thirst*
Water deprivation	Water deprivation
Hypertonic saline	Hypertonic saline
Diabetes insipidus	iv, icv angiotensin II
Intracranial carbachol (rats)	sc colloidal solution
	Hypotensive agents
NaCl appetite	Caval ligation
Adrenalectomy	High salt diet
DOCA (rats)	
NaCl-deficient diet	*Hunger*
Parotid saliva loss (sheep)	Food deprivation
	Insulin-induced hypoglycemia
Hunger	Diabetes mellitus
Food deprivation	2-deoxyglucose
Insulin-induced hypoglycemia	2,5-anhydro-D-mannitol
Lateral hypothalamic lesions	Genetic mutants, transgenic animals
Diabetes mellitus	icv NPY, orexin, AgRP, MCH, opioids, galanin
Intracranial NE (rats)	Ghrelin
Satiety	*NaCl appetite*
Gastric distension	Adrenalectomy
Ventromedial hypothalamic lesions	DOCA (rats)
	NaCl-deficient diet
	Parotid saliva loss (sheep)
	sc colloid
	icv angiotensin II
	Furosemide
	Area postrema lesions
	Captopril (low dose)
	Satiety
	Gastric load
	Cholecystokinin
	Bombesin, gastrin-releasing peptide, neuromedins
	Insulin
	Leptin
	Melanocortins
	Amylin
	Genetic mutants, transgenic animals
	Apolipoprotein A-IV
	Enterostatin
	Glucagon

circuits involved in behavioral arousal and reward. Chemical coding of the brain is now a historical footnote, reminding us of an earlier era.

These animal models and the hypotheses that account for motivated ingestive behaviors are characterized by their close ties with the known physiology of organismal systems, that is, thirst and NaCl appetite with the cardiovascular system and kidneys, and hunger and satiety with the gastrointestinal tract, liver, and adipose tissue. In addition, the integration of physiology and behavior by the central nervous system is based on a broad understanding of the anatomy, physiology, and pharmacology of the brain and spinal cord, including its influence by blood-borne hormones and substrates. As might be expected, the techniques used in these

studies, and the ideas derived from experimental results, reflect the multidisciplinary nature of modern studies and their increased emphasis on biological issues. Thus, whereas investigators of ingestive behavior in the late 1950s and early 1960s often studied the effects of various treatments on eating or drinking in animals that had been given stereotaxic brain lesions (Table 2A), a huge array of experimental techniques are available for use at the beginning of the 21st century (Table 2B). Most of the contemporary techniques focus on the manipulation or measurement of the biological variables that seem to be critical in the excitation and inhibition of food and fluid ingestion.

These very positive developments reflect the transformation of studies of ingestive behavior from the province of Psychology to the emergent field of Neuroscience. More specifically, 30–40 years ago, ingestive behavior was studied predominantly by physiological psychologists seeking to explain ingestive behavior in biological terms but measuring behavior, like their colleagues in other branches of psychology. Their biological hypotheses often were speculative and vague compared with those of the smaller number of physiologists who were also interested in ingestive behavior but who measured biological variables and interpreted their findings from the perspective of physiological systems. Le Magnen (1971)

Table 2. Techniques in the Study of Ingestive Behavior in Common Use

A. During the early 1960s
Learned behaviors (operant equipment)
Psychophysics
Systemic injections
Electrical brain stimulation
Chemical brain stimulation
Stereotaxic brain lesions
Electrophysiological recording in anesthetized animals
Electroencephalography
Histology of perfused brain tissue (light microscopy)
B. During the early 21st century
Microanalysis of behavior
Gustometer
Immunocytochemistry
Radioimmunoassays
Neurochemical assays
Neurotoxic lesions
Intracellular recording
Extracellular recording in conscious animals
Electrophysiological recording in tissue slices
In vivo dialysis
Microscopy
Imaging
Patch clamp
Receptor binding
In situ hybridization
Complex surgical preparations
iv, icv infusions
Gene cloning
High performance liquid chromatography

characterized this situation cogently when he noted that "there was little physiology in physiological psychology." The rest of this oft-quoted remark is equally trenchant: "there was little behavior in studies of physiology." The integration of the two approaches was not commonly found in a single laboratory.

We experienced first-hand the relatively low emphasis on biology in our own graduate training. Departments of psychology at that time were preparing many graduate students to enter careers as academic psychologists, and those students had to be able to teach courses not only in their specialty areas but also in broader "service courses" such as introductory psychology, statistics, and learning. Thus, in addition to whatever courses existed on the brain and behavior, graduate students in departments of psychology typically received training in statistics and experimental design as well as in learning and memory, developmental psychology, and cognitive psychology. It was (and is) easy to question the value of such a broad education in psychology to a future investigator with interests in the biological bases of behavior. But putting that question aside, there were two additional issues that affected graduate training. First, the great amount of time that was required for the required courses in psychology precluded the possibility of taking many other courses outside the discipline. And, second, relevant biological courses often were not available on campus, or, if they were available, graduate students in psychology often were not well prepared for them and may not have been allowed to take them.

Because of these limitations in their graduate training, students in physiological psychology with focal interests in ingestive behavior often required additional training in biological sciences once their doctoral work in psychology was completed. However, there were relatively few laboratories in which a student could receive expert training of relevance compared to the large number of investigators of more popular topics in physiological psychology such as learning and memory, reproductive behavior, and sensory systems. Furthermore, postdoctoral training was not common 30–40 years ago, in part because such an experience usually was not necessary in obtaining an academic job. Thus, physiological psychologists newly hired in academia may not have been well prepared to understand, much less exploit, the revolutionary developments in the biological sciences that began to occur in the 1970s. Instead, if they were to acquire the new experimental techniques, information, and perspective that could enrich their work, they had to do so either on their own or through collegial interactions with faculty who had been trained in other disciplines.

During the past few decades, studies of the biological basis of ingestive behavior have taken place increasingly in neuroscience centers that provide broad training in relevant biological variables ranging from molecular biology and genetics to whole animal physiology and endocrinology. In addition, because these training programs commonly are located in medical centers, or associated with them, there is increased likelihood that physicians will be involved in the research programs and that clinical issues will influence the goals of the experiments and the interpretation of the results. These familiar features of contemporary research differ greatly from the setting and perspective that was common 30–40 years ago.

With this account as background, it is easy to describe the substantial transformation that has occurred from "physiological psychology" to "behavioral neuroscience" (or "behavioral neurobiology"). Physiological psychology is a branch of psychology whose goal is to understand the behavior in biological terms.

Investigations usually involved measurements of behavior, with inferences about biological variables that were not measured. In contrast, behavioral neuroscience is a branch of neuroscience (which itself is a branch of biology) whose goals are to understand the role of the brain in the control of behavior, and the influence of behavior on the signals that stimulate the brain. Investigations may involve measurements of behavior, but relevant biological variables in the nervous system or in some other organ system are always measured.

The difference between physiological psychology and behavioral neurobiology can be seen at each level of the scientific process, that is, in the goals of the experiments, the techniques used, the measurements made, and the perspectives taken in interpreting the data. Behavior now is considered from a much broader perspective than before; not only are the biological bases of behavior under investigation but also the biological consequences of behavior and the biological context in which behavior occurs. In other words, contemporary studies reflect the immense developments that have occurred in every branch of the biological sciences during the past three decades. These developments have allowed rapid progress toward an understanding that is both broader and deeper than could have been easily imagined 40 years ago.

It is worth noting the costs that have accompanied those gains. Whereas students interested in food and fluid ingestion previously received training in programs that emphasized behavior, behavior is now receiving little attention in modern graduate training programs in neuroscience. Thus, contemporary studies of ingestive behavior often measure only food and water intakes, and perhaps the latency to ingest. Of course, behavior is more complex than can be captured by such measurements. It involves motivation, it involves learning from experience, and it involves reinforcements of many different types. After all, specific stimuli do not invariably lead to predictable choices of food or fluid or amounts consumed. There has also been an accompanying decrease in the complexity of statistical analyses of obtained data. In other words, some of the unique strengths of training in departments of experimental psychology are missing from contemporary training in neuroscience. Nonetheless, the net effect of changes in the field certainly has been positive. Ingestive behavior is a multidisciplinary phenomenon that is finally receiving multidisciplinary attention. Experimental studies increasingly involve teams of investigators, sometimes including clinicians, whose backgrounds and positions are in multiple academic departments. The familiar phenomena of eating and drinking are recognized as extremely complex, and the rewards of investigating such challenging problems are recognized as substantial. Consequently, the field continues to attract new investigators from multiple disciplines, who bring their expertise, perspective, and technical skills to what has become a large communal effort. Thus, investigators are obtaining the necessary skills and knowledge not only by a series of graduate and postgraduate research training experiences, but also by supplementary "tutorials" from scientific collaborators who have been trained in neighboring disciplines.

This *Handbook* celebrates the singular achievements of the past 13 years. The relatively lengthy review chapters summarize the state of progress in some of the focal areas of greatest interest and activity, while the shorter essays provide more personal perspectives on these and related developments. We expect readers to enjoy them all and find them stimulating, provocative, and inspiring, as we have.

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Motivation/Reinforcement

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2

Accumbens Dopamine is a Physiological Correlate of the Rewarding and Motivating Effects of Food

Gerard P. Smith

Motivation and Reward: Origins and Usage

The development of law concerning property and persons stimulated an interest in psychology (Smith, 1997). "Why did he steal that?" "Why did she kill him?" Such questions focused attention on the springs to action in the individual. As early as the 16th century, motive was the English word used to refer to the inner cause of movements or actions that brought people under the scrutiny of local law.

From the beginning, motivation attributed agency to others. Motivation was bound up with desire and will—what you did was what you wanted, and what you wanted was what you willed. This implies not only the ability to choose, but also the burden of responsibility for the consequences. Thus, motivated actions were not *reflex* responses to coercive internal or external stimuli.

Motivated actions are specific and goal-directed. The kind of goal defines the motivation: there are motivations for food, water, mating, defense and fighting, for territory, for care of the young, etc. The goal is the reward, a word in use in English since the 14th century.

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Neurobiology of Food and Fluid Intake, 2nd Ed., Volume 14 of *Handbook of Behavioral Neurobiology,* edited by Edward Stricker and Stephen Woods, Kluwer Academic / Plenum Publishers, New York, 2004.

So why don't we just talk about eating instead of saying that she is motivated to eat? The reason is that motivation refers to the entire functional sequence integrated by the brain and consisting of movements varying in form, persistence, and duration that culminate in actions appropriate to the specific goal, for example, eating food. Until the person is interacting with the reward, it is difficult, frequently impossible, to infer what motivation is being expressed by observing the movements that lead the person to the reward. It is not possible to deduce the motive from the movements—this is the basis of betrayal and surprise.

When the scientific analysis of animal behavior began in the last decades of the 19th century, it became clear that some of the behaviors of vertebrates, particularly domesticated mammals, had characteristics that were similar to those of human motivated behavior. For example, under conditions of apparent environmental stability a dog or monkey would begin to move about its living space and approach food or water or another animal. Then the animal selected one of these objects and began to eat, drink, fight, or mate. This functional sequence had the three characteristics of human motivated behavior: First, the behavior could occur without a detectable change in the environment or access to the goal object. Second, the movements used to approach the goal varied in form, persistence, and duration depending on the spatial characteristics of the environment, the kind of animal, and the type of reward. Third, the goal of the sequence of movements could not be deduced from the form or intensity of the movements; it was defined by the reward selected and interacted with. Since the sequence of movements culminating in appropriate action with the goal object was not a reflex movement, it was reasonable to refer to this kind of behavior as motivated. In 1918, Craig (1918) built on Sherrington's distinction between the behavioral functions of distance and nondistance receptors (Sherrington, 1947) to divide motivated behavioral sequences into two phases—the appetitive and the consummatory. The variable movements of approach to the goal object were the appetitive phase and the interaction with the goal object was the consummatory phase. This provided a conceptual framework, still used, for assigning experimental changes to specific phases of a motivated behavioral sequence.

Although animal behavior had many of the characteristics of human motivated behavior, many investigators reserved the word "motivation" for human behavior and used "instinct" to refer to similar behavioral sequences in animals (Stellar & Stellar, 1985). In the 1950s this linguistic move was rejected in favor of experimental analysis (Beach, 1955).

Drive, on the other hand, coined by Woodworth (1918) to denote the internal change that initiated the behavioral sequence of a motivated behavior, survived and is still used as a synonym for motivation (Pfaff, 1999). But motivation is a better word than drive because its use does not connote some psychological form of mechanical energy that causes movement. This use of motion in physics to provide an explanatory model of motivated behavior is the basis of the energetic and hydraulic models of motivated behavior proposed by Freud, Lorenz, and Tinbergen. The defects of such models were made clear in a devastating critique by Hinde (1960). Hinde pointed out that energetic models were bad models because their explanations of behavior were logically circular and that using such models to explain changes in responsiveness as changes of physical energy led to serious confusion (Hinde, 1970).

Problematic language always expresses confused thinking. The fundamental requirement of thinking about motivation is to understand that it is a word for an

intervening variable(s) between internal and external stimuli and the functional behavioral sequences they elicit.

The motivated sequence consists of observable movements. This makes the sequence of movements the natural object (Pickstone, 2001) of investigation for Behavioral Neuroscience.

The functional sequence sets the problem for physiological and neuroscientific analysis. That analysis consists of delineating the sensory control of the movements and the central neural mechanisms that organize sensory information into sequential movements that attain specific goals.

The functional sequence is also the test of the adequacy of the experimental analysis. If the sensory control of the behavioral sequence cannot be deduced from the analytic results, the analysis is incomplete.

Thus, motivation refers to the physiological gap between the internal or external stimulus that elicits appetitive and consummatory phases of the behavioral sequence. Although it is only a word for an important ignorance in Behavioral Neuroscience, motivation is heuristic in two distinct but related ways: First, it reminds us that reflex function cannot account for motivated behavior. Second, it stimulates the search for a neural organization beyond the reflex that is capable of integrating information from internal and external stimuli into an effective sequence of movements that attains a goal object important for the individual or species.

Experimental Identification of Motivated Eating

The experimental demonstration that eating is motivated requires two kinds of evidence. First, the movements used to gain access to the food must be various and flexible. They include running down runways, pressing levers, nose poking that breaks a detector beam, pushing a panel, touching a button, going up stairs, etc. Second, the movements of approach must culminate in the ingestion of food, not drinking water or gnawing inedible objects. Such evidence identifies the appetitive and consummatory phases, respectively. The evidence is strongest when a quantitative, monotonic relationship is demonstrated between the magnitude of the stimulus and an experimental procedure, for example, hours of deprivation, and the appetitive and consummatory movements. The shape of the functions depends on the measures of the movements. For example, when meal size is used as the measure of the consummatory movements, its relationship to deprivation, and to the appetitive movements required is distorted by the effect of satiation that occurs during ingestion and that limits the size of the meal.

Objective Correlates of Motivated Eating

The history of scientific attempts to understand motivation for food in the past century is a chronicle of the search for reliable objective correlates of motivation in the physiological gaps between internal or external stimulus and motor sequence that leads the animal to eating. It is an example of the fundamental problem of translation. Quine (1960) argued that translation is necessarily indeterminate or imperfect. The problem of translation pervades Behavioral Neuroscience. For example, there are the specific problems of translating the language and

measurements of psychology into physiology, of behavior into psychology or physiology, and of physiology into behavior or psychology. Thus, we should not expect to find a physiological correlate that captures all of the meaning of motivation or covers all of the examples of behavior that motivation refers to. That is the myth of complete reduction, that is, perfect translation, of the words and measurements of psychology or behavior into the words and measurements of the physiology of the central nervous system.

Peripheral Physiological Correlates

Instead of an ideal correlate, investigators of the motivating effects of food should be looking for a "good-enough" correlate, one that is sufficiently reliable to assist the neuroscientific analysis in a number of representative cases. As we shall see, this intellectually more modest problem can be solved by current techniques.

Peripheral physiological correlates dominated the first 40 years of this search. To measure the feelings of hunger or the desire to eat Pavlov (1910) used salivation in the dog, Cannon (Cannon & Washburn, 1912) proposed gastric contractions, and Carlson (1916) apparently confirmed the importance of Cannon's gastric contractions. Unfortunately, salivation and gastric contractions were subsequently found to be weak or inconsistent correlates with self-reports of hunger (James, 1957; Penick, Smith, Wieneke, & Hinkle, 1963).

Metabolic Measures and Ventromedial Hypothalamic Lesions

Beginning in the 1940s, and stimulated by the hyperphagia produced by bilateral lesions of the ventromedial hypothalamus (VMH), metabolic measures with a hypothalamic site of action became the new candidates for physiological correlates. The two most important were glucose utilization (Mayer, 1955) and body temperature (Brobeck, 1960). They were not only objective correlates; they were suggested to be important mechanisms of the motivation to eat. Decreases of glucose utilization and of temperature stimulated eating; thus, they were correlated with increased motivation to eat. Increases of glucose utilization and of temperature stopped eating; they were correlated with decreased motivation to eat. The effects of the reciprocal changes of glucose utilization and of temperature on eating were referred to as the glucostatic and thermostatic controls of eating, respectively.

In 1953, Kennedy (1953) proposed the lipostatic hypothesis. The core of this hypothesis was that the fat mass produced an inhibitory control on eating and body weight. The inhibitory control was mediated by an unidentified humoral signal from white adipose tissue that acted on the VMH. The lipostatic hypothesis explained the increased motivation to eat after VMH lesions by the removal of the site of action of the inhibitory signal from fat.

By the middle of the 1970s, research had achieved a critical perspective on these metabolic correlates and controls. The glucostatic and thermostatic controls operated only during unusual conditions of hypoglycemia and low temperature. When the inhibitory molecule of the lipostatic hypothesis was identified as leptin in 1994, its importance for the control of eating was shown to be due to decreases of leptin during relatively long periods of food deprivation that resulted in increased eating (Woods & Seeley, 2000). When endogenous leptin increased as fat mass increased, it was not able to restrain intake to normal. In fact, when a palatable diet increased

intake and body weight, leptin's inhibitory control decreased, a phenomenon referred to as "leptin resistance." Thus, glucose utilization, temperature, and leptin were not useful correlates of the motivation to eat under the most common laboratory conditions of free access to food and controlled environmental temperature.

Lateral Hypothalamic Lesions

Anand and Brobeck's discovery of aphagia after bilateral lesions of the lateral hypothalamus (LH) moved the search for the objective correlate from the periphery to the brain (Anand & Brobeck, 1951). The aphagia produced by LH lesions suggested that the fibers cut by the lesion or the cells killed by it mediated the motivation to eat. This suggestion was tested by electrical stimulation of the LH. At most rostro-caudal sites along the LH, stimulation increased locomotion and other exploratory behaviors, but it did not elicit eating. Stimulation of the perifornical region of the LH, however, produced flexible and effective behavioral sequences that ended in eating food. Wise (Wise, 1974) concluded that stimulation-induced eating was very similar to deprivation-induced eating—it was motivated.

To see if the sites that induced the motivation to eat would also be rewarding, Hoebel and Teitelbaum (Hoebel & Teitelbaum, 1962) investigated whether rats would self-stimulate this site. They did. And unlike many other sites of self-stimulation in the LH, the threshold for self-stimulation at the sites that stimulated eating was decreased by food deprivation and increased by postingestive preloads (Wise, Devor, Milgram, & Hoebel, 1970).

Initially, the eating elicited by LH stimulation was considered to be the result of stimulating a "hard-wired" network of the brain that was specific for eating and the critical part of which was defined by the site of stimulation. But Valenstein showed that the ingestive behavior elicited by LH stimulation depended on what was available to ingest in the test situation (Valenstein, Cox, & Kakolewski, 1968). At certain sites, stimulation elicited eating if food was available, drinking if water was available, or gnawing wood if that was available. This important result was consistent with the idea that electrical stimulation activated a diffuse neural network for motivation for oromotor behavior that adapted to changes of the goal object. Note that in the absence of food, water or wood, stimulation induced exploratory behaviors that were useful for searching for a suitable object.

Wise (1971) demonstrated significant differences in the region of the LH where stimulation induced ingestive behavior and noted that a number of rats did not eat or drink in response to LH stimulation in the same regions. His and Valenstein's results were consistent with contributions of neural mechanisms distant from the site of stimulation to the behavior elicited by LH stimulation.

The anatomy of the LH and the neuropathology of the large LH lesions revealed extensive local and distal connections of LH neurons. Thus, neither LH lesions nor stimulation had sufficient localizing value to make an anatomical analysis of the behavioral effects possible. A more anatomically specific lesion was required to identify the function of a set of neurons with fibers or cell bodies in the LH that could serve as an objective correlate of the motivation to eat.

6-Hydroxydopamine Lesions

Such a lesion was reported by Ungerstedt in 1971. Ungerstedt (1971) used 6-hydroxydopamine (6-OHDA), a toxic substance that was taken up and

concentrated in catecholamine neurons. Bilateral microinjection of 6-OHDA into the substantia nigra (SN) produced aphagia and adipsia that was similar to the aphagia and adipsia produced by LH lesions. Thus, Ungerstedt suggested that lesion of the catecholaminergic neurons was the critical neuropathology of the LH lesions. These neurons became the new candidate for an objective correlate of the motivation to eat. The rest of this chapter traces the scientific fate of that idea.

The first step was to analyze the lesion produced by 6-OHDA. The necessary neuropathology was clarified by varying the site of bilateral injections of 6-OHDA. The most effective site was just in front of the SN and in the posterolateral hypothalamus (Smith, Strohmayer, & Reis, 1972). As the site of injection moved rostrally along the LH, aphagia continued to occur, but its duration was shorter. In contrast to the lateral injections, bilateral injections along the medial hypothalamus had no significant effect on eating (Smith, 1973; Smith *et al.*, 1972).

Pharmacological pretreatment with drugs that preferentially prevented the uptake of 6-OHDA into noradrenergic neurons revealed that lesion of the dopaminergic (DA) neurons alone was sufficient to produce the syndrome (Zigmond & Stricker, 1972). This was first shown with intraventricular injections of the uptake inhibitor and then with microinjections of it into specific sites.

The different behavioral effects of bilateral injections of 6-OHDA at different rostro-caudal sites along the LH were explained by the neuroanatomy of the DA neurons and the neuropathology of the injections. The DA neurons spanned the ventral tegmental area (VTA) in the upper mesencephalon just behind the hypothalamus (A10 cell group), clustered in the pars compacta of the SN (A9), and then tailed off into the dorsolateral region behind the SN (A8). Fibers from these neurons converged in front of the SN and then entered the hypothalamus through the neck of the posterolateral hypothalamus. The most lateral of these fibers projected to the striatum (Str); the more medial fibers continued through the anterior hypothalamus to innervate forebrain regions, such as the nucleus accumbens (Acc), septum, amygdala, medial prefrontal cortex (Pfc), olfactory tubercle, and bed nucleus of the stria terminalis. Thus, posterolateral injections of 6-OHDA lesioned nigrostriatal (NStr) and mesolimbicocortical (MLC) fibers, while anterolateral injections lesioned the MLC fibers and spared all of the NStr fibers (Fink & Smith, 1979, 1980a). The longer duration of aphagia after posterolateral than anterolateral lesions could have been due to the combined loss of NStr and MLC fibers after posterolateral lesions or to the Str damage alone.

It was shown to be the NStr damage, but 6-OHDA lesions of the NStr also produced marked deficits in many kinds of sensorimotor performances as well as aphagia and adipsia (Marshall, Berrios, & Sawyer, 1980; Marshall, Richardson, & Teitelbaum, 1974; Stricker & Zigmond, 1986). These deficits complicated the interpretation of the aphagia. Was the aphagia a motivational deficit or was it an inability to perform the necessary movements of the appetitive and consummatory phases of eating?

Dopamine Antagonists and Food Reward

While this problem was being experimentally attacked, Wise and his colleagues published two papers that related directly to the mediation of the rewarding and motivating effects of food by DA(Wise, Spindler, deWit, & Gerberg, 1978; Wise, Spindler, & Legault, 1978). They described results with pimozide, a DA antagonist. Pretreatment with pimozide produced a decrease in lever pressing and the speed

of running a runway within and across test sessions that was similar to, but not identical with, the effect of nonreward on performance. Wise *et al.*, concluded that pimozide selectively blocked the rewarding effects of ingested food.

In a third experiment Gray and Wise (1980) showed that pimozide decreased responding on a Variable Interval schedule (20 s to 5 min) in the first 20 min before the presentation of food as well as later in the test when food was obtained. The decreased responding prior to the first delivery of food was interpreted as a decrease in the potency of incentive motivational stimuli: "The performance deficit produced by pimozide is, we suggest, at least partly due to its blocking of the reward value of food, but we suggest further that pimozide also reduces the effectiveness of the incentive motivational stimuli present in the situation." These experiments implicated DA in incentive motivation for food and the rewarding effect of food, but because the antagonist was administered after the rats were trained to criterion, the relationship between the two effects of the DA antagonist could not be determined.

Note that the effects of pimozide in these experiments had no localizing value within the central DA system because pimozide was given peripherally. Localization required microinjection of DA antagonists into specific DA terminal fields.

Mogenson, Motivation, and the Ventral Striatum

Meanwhile the late Gordon Mogenson organized a research program to find the neurological substrate of motivation under the title of "From Motivation to Action" (Mogenson, Jones, & Yim, 1980). Mogenson reasoned that the limbic forebrain, so important for motivational and emotional behavior, must have access to motor mechanisms in order to produce locomotion and other exploratory behaviors that were organized to form functional sequences of appetitive behavior. The first locus for interaction between limbic and locomotor mechanisms was in the upper brainstem in the region of the pediculopontine nucleus. Electrical or chemical stimulation of this region produced forward locomotion through its caudal projections to hindbrain and spinal pattern generators for walking and running.

The path from limbic regions, such as the amygdala, to this upper brainstem region was clarified by the discovery of ventral Str by Heimer and Wilson in 1975 (Heimer, Alheid, & Zahm, 1993). The key fact was that the ventral part of the Str that lay beneath the anterior commissure had different afferent inputs and efferent projections than the dorsal Str. The nucleus Acc, a major component of the ventral Str received afferent inputs from limbic structures, such as hippocampus, amygdala, and medial and orbital frontal cortex whereas the dorsal Str received neocortical inputs. Some of the efferent fibers of the ventral Str descend through the LH and project to the ventral pallidum. Other descending fibers terminate in the upper brainstem in the region of the pediculopontine nucleus and in the VTA and SN including those regions enriched with DA cells. A third group of efferent fibers projects to limbic structures, such as the septum, bed nucleus of the stria terminalis, and the preoptic and lateral hypothalamus.

Mogenson quickly showed that the electrophysiological effect on the Acc of stimulating the amygdala could be changed by pharmacological manipulation of the Acc (Mogenson, Brudzynski, Wu, Yang, & Yim, 1993; Mogenson *et al.*, 1980). The importance of the Acc for appetitive behaviors was strengthened by two other observations: First, it was densely innervated by DA terminals, most of which came

from A10 cells in the VTA. This was in contrast to the dorsal Str that received most of its DA innervation from the A9 cells in the pars compacta of the SN.

Second, local injection of DA into the Acc produced locomotion (Pijnenburg & VanRossum, 1973) and local injection of haloperidol, a DA antagonist, into the Acc blocked the locomotor response to systemic amphetamine that was mediated by the release of endogenous DA (Pinjenburg, Honig, & VanRossum, 1975). Thus, DA had a necessary local circuit function in this transformation of drug into behavior.

The fact that amphetamine produced locomotion and other exploratory behaviors focused attention on the Acc as a likely site for the transformation of motivational information into appetitive motor sequences.

Progress and Problems 1980–1995

The possibility that a neurochemical circuit of motivation had been discovered in which the release of DA was an objective correlate and a necessary mechanism attracted considerable experimental attention. The extensive multidisciplinary work was summarized in three reviews.

In a chapter in the *Handbook of Physiology*, Stricker and Zigmond (1986) focused on the recovery of feeding, drinking, and other movements in rats after extensive DA denervation of the Str, particularly the dorsal Str. Their main observations were as follows. First, at least 90–95% of DA neurons had to be destroyed to produce behavioral deficits under usual laboratory conditions.

Second, the recovery of function after DA loss in the Str was correlated with dynamic changes in the surviving DA neurons and their specific receptors. The synthesis of DA increased due to increased activity of tyrosine hydroxylase. The removal of released DA was less than normal because released DA is removed primarily by uptake into DA terminals and distal axons and these were markedly reduced after 6-OHDA lesions. The number of DA receptors, most of which were on non-DA neurons, increased significantly. This combination of increased release, decreased removal, and increased number of postsynaptic receptors amplified the local effect of DA released from the small number of surviving neurons so that recovery of function could occur. They referred to these adaptations to loss of DA terminals as synaptic homeostasis.

Third, pretreatment with L-DOPA to increase synthesis and release of DA or administration of apomorphine to stimulate DA receptors could restore behavioral function temporarily.

Stricker and Zigmond interpreted their results as evidence for a nonspecific arousal function of DA in many motivated behaviors. They emphasized that DA was not associated with any specific motivated behavior.

On the basis of the results with DA agonists and antagonists, they concluded that a certain amount of DA had to be present for a neural circuit to function optimally. If there were too little, the range of adequate stimuli narrowed and behavioral deficits occurred. If there were too much, stereotypical movements displaced normal behaviors. This arousal function of DA was based on the investigation of Str DA.

If the effects of 6-OHDA lesions of the MLC DA system produced similar behavioral results, it would support their interpretation. It became possible to investigate the MLC DA system separately from the Str DA system when our

laboratory showed that bilateral microinjections of 6-OHDA into the anterolateral hypothalamus produced essentially total loss of MLC fibers and terminals, but spared all of the striatal DA innervation except for a small anteromedial region (Fink & Smith, 1980a). MLC lesioned rats were aphagic and adipsic (Smith *et al.*, 1972). The duration of aphagia was much shorter after lesions of the MLC fibers than after lesions of the NStr fibers. Five other behaviors, however, appeared to be permanently impaired in MLC rats. These were visual placing (Sechzer, Ervin, & Smith, 1973), conditioned active avoidance response to foot shock (Smith, Levin, & Ervin, 1975), the locomotor response to amphetamine (Fink & Smith, 1980b), exploration of a novel open field (Fink & Smith, 1980a), and investigation of a novel object (Fink & Smith, 1980a). Visual placing and conditioned avoidance behavior (acquisition or performance) were abolished; the responses to amphetamine, novel spaces, and novel objects were significantly reduced.

The loss of visual placing and conditioned avoidance responses were examples of a lesion narrowing the range of adequate stimuli for eliciting a behavioral sequence rather than abolition of the motor response because lesioned rats placed normally to tactile stimuli and escaped normally from foot shock.

Pretreatment with amphetamine or L-DOPA did not restore active avoidance responding. Amphetamine restored visual placing transiently at some time between 5 weeks and 6 months. This prolonged time course for the effect of amphetamine that was mediated by the release of endogenous DA was consistent with the time required for increased DA synaptic homeostasis in the few remaining DA terminals.

The decreased locomotor response of lesioned rats to amphetamine was consistent with the loss of MLC terminals containing DA for release by amphetamine. There appeared to be a quantitative relationship between the number of terminals lost and the response to amphetamine because unilateral loss of MLC fibers produced about half the decrease in locomotion observed after bilateral lesions (Jeste & Smith, 1980).

Pretreatment with low doses of apomorphine reversed the decreased exploration of a novel open field and investigation of a novel object (Fink & Smith, 1980a). The restorative effect of apomorphine was blocked by pimozide. We concluded that MLC DA function was necessary for normal exploration and investigation—two behaviors important for the appetitive phase of finding food.

The fact that lesions of the MLC impaired different motor responses to novel, visual, and aversively conditioned auditory stimuli is consistent with a nonspecific arousal function of DA (Smith, 1976). It is important that these deficits occurred despite normal DA innervation of the dosrsolateral Str and in animals that were eating and drinking spontaneously and maintaining their body weight.

DA neurons apparently had a permissive function; they did not command any specific functional neurological network (Smith, 1973). This permissive function presumably increased the signal-to-noise ratio within a network to facilitate optimal performance. The similarity between the characteristics of the behavioral results of MLC and NStr lesions strengthened Stricker and Zigmond's conclusion that DA neurons produced a widespread arousal function but no specific motivation.

The second important review was by Fibiger and Phillips (1986) and it appeared in the same volume of the *Handbook*. They reviewed the evidence for DA in the reward of intracranial self-stimulation (ICSS), self- administration of psychoactive drugs, and of food. They concluded that the effects of 6-OHDA lesions and DA antagonists were evidence for DA mediation of the rewards of ICSS and self-administration of drugs. This was not true, however, of food reward: "On balance it

appears unlikely that the mesolimbic DA system is involved critically in mediating the primary reinforcing or hedonic properties of food (p.664)." This negative conclusion was based primarily on the reports that 6-OHDA lesions of the Acc and olfactory tubercle increased intake in 30-min tests or did not change food intake in the home cage (Koob, Riley, Smith, & Robbins, 1978). The numerous inhibitory effects of DA antagonists on food intake were attributed to motor deficits despite the extensive experimental results against that interpretation that Wise discussed in two formidable responses to critics of his hypothesis (Wise, 1982, 1985).

The conclusion that DA was important for the rewarding effects of ICSS and of drug self-administration, but not for food, seemed odd because hypothalamic sites for electrical stimulation of eating were invariably positive for ICSS. Thus, in the third review, I surveyed the experimental results concerned with the role of DA in food reward and found considerable evidence for Wise's hypothesis (Smith, 1995). The best evidence was obtained by using specific DA anatagonists, such as SCH 23390 and raclopride. The results were clearest in experiments in which sucrose solutions were sham fed and in operant experiments in which responses to conditioned stimuli (CSs) determined the schedule of food reinforcement. The decreased responding for sucrose and other foods observed after antagonist pretreatment in operant experiments was evidence for DA mediation of the incentive motivating effects of food. These inhibitory effects could be obtained in the absence of detectable sensory or motor deficiencies, or aversive effects of the drugs (see the review for details of the relevant experiments).

The decreased intake of sucrose during sham feeding produced by the DA antagonists was consistent with DA mediation of the orosensory rewarding effect of sucrose that acts to maintain eating when it begins. Potential interactions between the antagonists and postingestive satiating or rewarding effects of ingested sucrose were not necessary for the inhibitory effects of the antagonists because sham feeding eliminated the postingestive effects.

Note that DA antagonists and 6-OHDA lesions blocked transient and chronic effects of central DA neurons. But only transient increases of DA that correlated temporally with the motivational and rewarding effects of food on behavior could be an objective correlate of the motivating and rewarding effects of food. Chronic increases of extracellular DA could not be an objective correlate because they did not change when behavior did. Of course, chronic increases (or decreases) of DA could affect eating and intake, but that is another matter.

There was some evidence for transient increases of DA detected by measuring the ratio of the concentration of DA metabolites and DA, microdialysis, or voltammetry from DA sites prior to, during, or after eating food (Smith, 1995). I concluded that the results obtained with the DA antagonists and the small number of observations of increased DA detected by microdialysis and correlated temporally with ingestion of sucrose or other foods was sufficient to confirm the core of the hypothesis proposed by Wise—that central DA mechanisms were necessary for the normal mediation of the rewarding effect of food.

I thought the case was strong but not ironclad. Others, such as Salamone (Salamone, Cousins, & Snyder, 1997), thought the case was weak or nonexistent.

Review of Microdialysis Experiments

A large amount of new data has appeared since 1995 that are relevant to DA as an objective correlate of the rewarding and motivating effects of food. In reviewing

these new results, I have included only those experiments that used microdialysis to measure changes in extracellular DA in rats that were eating or performing an operant response to obtain food. The relatively small number of experiments that used voltammetry to measure DA have not been included because detection of DA by this technique is usually uncertain (Wightman & Robinson, 2002). New experiments that used DA antagonists or 6-OHDA lesions have not been included because they do not distinguish between transient and chronic changes of DA.

Food Reward and Transient Increases of DA

Eating food is accompanied by transient increases in extracellular DA that last 30–40 min under most, but not all, experimental conditions. The Acc has been investigated more than any other region. DA increased significantly in 44 test meals (Ahn & Phillips, 1999, 2003; Avena, Rada, Moise, Geary, & Hoebel, 2003a; Bassareo, De Luca, & Di Chiara, 2002; Bassareo & Di Chiara, 1997, 1999a, 1999b; Cenci, Kalen, Mandel, & Bjorklund, 1992; Church, Justice, Jr., & Neill, 1987; Cousins, Trevitt, Atherton, & Salamone, 1999; Di Chiara & Tanda, 1997; Feenstra & Botterblom, 1996; Gambarana *et al.*, 2003; Hajnal & Lenard, 1997; Hajnal & Norgren, 2001; Hajnal, Smith, & Norgren, 2004; Hernandez & Hoebel, 1988a, 1988b, 1990; Inoue *et al.*, 1993; Kittner, Krugel, El Ashmawy, & Illes, 2000; Mark, Blander, & Hoebel, 1991; Mark, Smith, Rada, & Hoebel, 1994; Martel & Fantino, 1996a, 1996b; McCullough & Salamone, 1992; Meguid, Yang, & Koseki, 1995; Orosco & Nicolaides, 1992; Pothos, Creese, & Hoebel, 1995; Radhakishun, van Ree, & Westerink, 1988; Salamone, Cousins, McCullough, Carriero, & Berkowitz, 1994; Sokolowski, Conlan, & Salamone, 1998; Taber & Fibiger, 1997; Taber, Zernig, & Fibiger, 1998; Westerink, Kwint, & de Vries, 1997; Westerink, Teisman, & de Vries, 1994; Wilson, Nomikos, Collu, & Fibiger, 1995; Yang, Koseki, Meguid, & Laviano, 1996; Yang & Meguid, 1995; Yang, Meguid, & Oler, 1997; Yoshida *et al.*, 1992). DA increased in the shell region in 10 of 11 test meals and in the core in 5 of 5 test meals (Bassareo & Di Chiara, 1999a, 1999b; Bassareo *et al.*, 2002; Di Chiara & Tanda, 1997; Gambarana *et al.*, 2003; Sokolowski, 1998). DA did not change in nine test meals (Bassareo *et al.*, 2002; Cenci *et al.*, 1992; Datla, Ahier, Young, Gray, & Joseph, 2002; Martel & Fantino, 1996b; McCullough & Salamone, 1992; Salamone *et al.*, 1994).

Other sites were investigated less often. DA increased in the Pfc during all 13 meals in which it was measured (Ahn & Phillips, 1999, 2003; Bassareo & Di Chiara, 1997; Bassareo *et al.*, 2002; Cenci *et al.*, 1992; Di Chiara & Tanda, 1997; Feenstra & Botterblom, 1996; Gambarana *et al.*, 2003; Hernandez & Hoebel, 1990). The Str was investigated in six meals: DA increased in three meals (ventrolateral = 2, central = 1) (Church *et al.*, 1987; Cousins *et al.*, 1999) and did not change in the ventrolateral region in three meals (Hernandez & Hoebel, 1988a; Inoue *et al.*, 1993). The lateral and rostromedial hypothalamus showed a transient increase of DA in all six meals (Meguid *et al.*, 1995; Orosco *et al.*, 1992; Yang, Koseki, Meguid, & Laviano, 1996; Yang & Meguid, 1995) and there was an increase of DA in the amygdala in the only test meal investigated (Hajnal & Lenard, 1997). In contrast to the increase of DA in these sites, DA decreased in the VMH during eating (Yang *et al.*, 1997).

The increases were large (up to 300% above baseline), began within the first 5 min of eating, and usually returned to baseline within 20–40 min after eating ended. The increases of DA occurred when liquid and solid foods were eaten. The foods could be novel or familiar. Increases of DA have been observed after no deprivation and after 1, 16, 20, or 24 hr of food deprivation.

The increase of DA depends on the orosensory stimulation by the food because gastric intubation of liquid food did not increase DA in any of these sites (Wilson *et al.*, 1995; Yang *et al.*, 1997) and sham feeding of sucrose, a procedure that preserves orosensory stimulation by sucrose while eliminating its postingestive effects, increased DA in the Acc (Avena, Rada, Moise, Geary, & Hoebel, 2003a; Hajnal *et al.*, 2004).

The magnitude of the increase of DA in the Acc correlates with the amount or volume of food ingested (Hajnal *et al.*, 2004; Martel & Fantino, 1996a; Meguid *et al.*, 1995; Wilson *et al.*, 1995; Yang & Meguid, 1995). This is true whether the different amounts eaten were determined by the investigator or the rat. The correlation of the magnitude of the increase of DA with the amount ingested means that the DA increase was correlated with the reward value of the food or with the movements of ingestion.

Four experiments have demonstrated that the transient increase of DA in the Acc was correlated with food reward and not with ingestive movements. First, Hajnal *et al.* (2004) allowed rats to sham feed 0.1M and 0.3M sucrose. Rats ingested more 0.3M sucrose than 0.1M and the increase of Acc DA was significantly larger during the of 0.3M sucrose than of 0.1M. In a second experiment they limited the volume of 0.3M sucrose ingested to the volume of 0.1M sucrose ingested. Making the volumes ingested equal presumably made the ingestive movements equal. Despite ingesting equal volumes, Acc DA increased significantly more during sham feeding 0.3M sucrose than during sham feeding 0.1M sucrose. Since the reward value of sucrose solutions is correlated monotonically with concentration (Pfaffmann, 1982), the larger increase of DA during ingestion of 0.3M sucrose correlated with the larger reward value of 0.3M sucrose than of 0.1M sucrose. This is strong evidence that the magnitude of DA release in the Acc was correlated with the reward value of sucrose during sham feeding rather than with the ingestive movements.

Second, eating the same amount of chow produced a significantly larger DA response in the Acc after 20 hr of food deprivation than after no deprivation (Wilson *et al.*, 1995). This extends the well-known synergistic relationship between duration of deprivation and the reward of ingested food from intake to a transient increase of DA in the Acc.

Third, intraoral infusion of saccharin increased DA in the Acc 37%. But after rats learned a conditioned aversion to saccharin, intraoral infusion of the same volume of saccharin decreased Acc DA 40% (Mark *et al.*, 1991).

Fourth, when rats ingested sucrose octoacetate (SOA), a liquid with a mildly bitter taste, Acc DA did not change. When SOA became preferred as the result of associating its taste with postingestive nutrient infusions, ingestion of SOA increased and Acc DA increased (Mark *et al.*, 1994). In contrast to the Acc response, DA in the dorsal Str did not change during ingestion of SOA before or after preference conditioning.

Negative Results

Although an increase of DA in the Acc is correlated with eating under most experimental conditions, DA did not change during 9 of 53 test meals. The explanation of the negative results is not obvious but understanding them is important because they may provide clues to the necessary contingencies for the release of DA in the Acc during eating. The number of negative results is not sufficiently

numerous to reject the conclusion that a transient increase in Acc DA is an objective correlate of food reward.

The positive results in the Pfc, in the lateral and rostromedial hypothalamus, and in the amygdala suggest that transient increases of DA in these regions could also be objective correlates of food reward. Until more experiments are done, however, their reliability is uncertain in comparison to the Acc.

Dopamine and the Motivation to Eat

The transient increase of Acc DA during and after eating in most experimental conditions demonstrates a correlation of Acc DA with the rewarding effect of food. The transient increase of Acc DA may also be a correlate of the motivating effect of food. To attempt to get further evidence on this issue, two kinds of experiments have been used—operant experiments and conditioning experiments in which rats are exposed to stimuli previously associated with a food that increases Acc DA when it is eaten. DA increased in the Acc in six operant tests (Cousins *et al.*, 1999; Hernandez & Hoebel, 1988a, 1988b; Salamone *et al.*, 1994; Sokolowski *et al.*, 1998), but DA did not change in two operant tests (Datla *et al.*, 2002; Salamone *et al.*, 1994).

When CSs were presented alone, Acc DA increased in four tests (Ahn & Phillips, 1999; Bassareo *et al.*, 1999a; Datla *et al.*, 2002; Wilson *et al.*, 1995) and did not change in four (Bassareo & Di Chiara, 1997, 1999a; Hernandez & Hoebel, 1988a; Wilson *et al.*, 1995). One of the four negative results occurred in nondeprived rats; when the same CSs were presented after 20 hr of food deprivation, Acc DA increased (Wilson *et al.*, 1995). In another of the negative results, DA did not increase in the shell of the Acc, but it did increase in the core (Bassareo & Di Chiara, 1999a).

The positive results are encouraging but there are too few of them. More experiments are required to determine how robust the positive results are in the Acc as well as in the smaller number of results in the Pfc (Ahn & Phillips, 1999; Bassareo & Di Chiara, 1997) and Str (Cousins *et al.*, 1999).

The Inhibitory Effect of Diet Preexposure

Recent experience with a test diet can inhibit the increase of Acc DA. For example, Ahn and Phillips (1999) used two foods with which rats had experience eating. One of the two foods was presented in two sequential tests and DA was monitored in Acc and Pfc. Each test consisted of 40 min during which a rat could smell and see the food from behind a screen but could not approach it or eat it; this was the appetitive phase. Forty minutes later the screen was removed and the rat could eat for 40 min; this was the consummatory phase. DA increased in the Acc and Pfc in the appetitive and consummatory phases of the first test. When the same food was presented in the second test, DA did not change in either the appetitive or consummatory phases. When a food was presented in the second test that was different from the food eaten in the first test, however, DA increased in the Pfc during the appetitive and consummatory phases of the test, but the magnitude of the increase was significantly less than the increases in the first test. In contrast to the Pfc, DA did not increase in the Acc during the appetitive phase of the second meal with a different food, but it did increase during the consummatory phase.

The results demonstrated that recent exposure to a diet inhibited the Acc DA response. The inhibition was apparently not the result of recent ingestion of food because the inhibition essentially disappeared when rats ate a different food in the second test. Thus, the DA increase in the Acc correlated with the relative novelty of the ingested food. Furthermore, the Acc DA response during the second meal was dissociated from the amount eaten because rats ate more of both diets in the second meal than in the first.

Di Chiara and his colleagues pursued this effect of relative novelty of food on the increase of Acc DA. In the first experiment, Bassareo and Di Chiara presented a high-carbohydrate, high-fat snack food called Fonzies for 20 min (Bassareo & Di Chiara, 1997). The food was novel in one group of rats and not novel in the other. Eating novel food increased Acc DA significantly more than eating food that was not novel even though rats ate less of the novel food than the food they had eaten before. Unlike the DA responses in the Acc, eating both foods produced equivalent increases of DA in the Pfc.

When Bassareo and Di Chiara presented Fonzies in two 20-min tests separated by 2 hr, the increase of Acc DA was smaller in the second test, but the increase of DA in the Pfc was not. This result replicated an earlier experiment (Di Chiara & Tanda, 1997). They interpreted the decreased response of Acc DA as habituation due to preexposure of the food stimuli rather than sensory-specific satiety because the rats ate more in the second test than in the first.

The characteristics of the inhibitory effect of preexposure were (Bassareo & Di Chiara, 1997, 1999a, 1999b) as follows. First, the inhibitory effect occurred in the shell but not the core of the Acc. Second, the inhibitory effect had to be present for at least 10 min; it lasted at least 24 hr but less than 5 days. Third, the inhibitory effect occurred in non-deprived rats but not in rats deprived of food for 24 hr. Fourth, the inhibitory effect required that rats ate the same food in the first and second tests. When a different food was eaten in the second test, there was no inhibition of DA in the shell. The disinhibition that occurred when a different food was eaten is consistent with their interpretation of the inhibition as habituation. Fifth, the inhibitory effect did not require ingestion of food. When rats could see and smell Fonzies in a box that had been used in a prior feeding test, but were not allowed to eat the food, DA increased in the Pfc but not in the Acc. When the rats were allowed to eat after this test, DA increased further in the Pfc, but there was only a small and brief increase in the Acc. Thus, conditioned olfactory and visual stimuli were sufficient to inhibit the DA response in the Acc but not the Pfc.

Having shown the importance of conditioned olfactory and visual stimuli for affecting the DA response in the shell, Bassareo *et al.* (2002) investigated the effect of taste. They gave intraoral infusions (1–2 ml), measured taste reactivity, and measured the DA response in the shell and core of the Acc and in the Pfc.

Sucrose and chocolate increased DA in all three sites. The DA response in the shell was decreased by prior experience with the stimuli 24 hr before but the responses of the core and Pfc were not. When prior experience decreased the DA response in the shell, taste reactivity and the volume ingested did not change. This lack of change of taste reactivity when DA decreased in the shell is consistent with Berridge's suggestion (Berridge, 1996) that DA in the Acc is not necessary for the hedonic response to taste stimuli. Note that this holds for the shell, but not the core because the DA response of the core did not change.

In contrast to sucrose and chocolate, aversive taste stimuli, such as quinine and concentrated NaCl, produced either no response or a delayed response in the shell.

The Pfc and core responded to these aversive tastes as they had to sucrose and chocolate. Prior experience decreased the DA response in the shell, but not in the core or Pfc.

Gambarana *et al.* (2003) also investigated sequential test meals. They used vanilla sugar pellets to bait one arm of a Y maze and an intraoral infusion of a vanilla sugar solution to evaluate taste reactivity. There were two 5-min tests. In the first test, DA increased significantly in the Acc and Pfc. In the second test, the increase of Acc DA was smaller than in the first test, but the increase of DA in the Pfc was equal to that observed in the first test. Despite the smaller increase of Acc DA in the second test, the latency to eat, the number of pellets eaten, and the taste reactivity to the intraoral solution in the second test were the same as in the first test.

The dissociation in the second test between smaller Acc DA response and unchanged taste reactivity is additional support for Berridge's claim that increases of Acc DA are not determined by the hedonic potency of the stimulus. But the smaller DA response in the Acc was also dissociated from measures of "wanting," such as the latency to eat, the number of pellets eaten, and the volume of the intraoral infusion ingested. This result is not consistent with Berridge's idea that increased Acc DA is associated with incentive salience of food stimuli that occurs when rats "want" food. Note the increase of DA in the Pfc was the same again in both tests.

The inhibition of Acc DA after diet preexposure could be due to inhibition of neurons that project to the Acc and stimulate DA release. For example, inactivation by lidocaine of the central nucleus of the amygdala or the medial Pfc decreased the Acc DA response to eating (Ahn & Phillips, 2003). In contrast, inactivation of the basolateral amygdala had no effect on the DA response in the Acc and it produced long-lasting oscillations of DA in the Pfc that were not related to the ingestion of food.

Characteristics of the Objective Correlate

A transient increase of DA in the Acc is an objective correlate of eating. This correlation has been observed when rats lick liquid foods or chew solids. It occurs after no food deprivation or after short or long periods of deprivation. It is seen with free access to food or when rats obtain food by lever pressing or running a runway. The postingestive effects of food do not contribute to the DA response because it is correlated with sham feeding but not with postingestive loads of liquid food.

The magnitude of the transient increase of DA in the Acc is affected by the relative novelty of the food, the duration of food deprivation, the amount of food ingested, and in the case of sham-feeding sucrose, by the concentration of sucrose solution. Correlation with amount eaten, duration of deprivation, and concentration of sucrose is evidence for the correlation of the DA increase to the rewarding value of the food eaten. This rewarding value of food has sufficient positive reinforcing potency to enable rats to form conditioned associations between eating food and operant responses or arbitrary neutral stimuli. This conditioning with food enables the previously neutral stimulus to acquire incentive motivational effects on eating. Thus, the increase of Acc DA is also correlated with the incentive motivating effect of food. A transient increase of DA has been correlated with the incentive motivating effect of food in fewer experiments than with the rewarding effect of eating. Given the importance of incentive motivation in the control of food intake, more investigation is warranted.

Additional experiments are also required to evaluate the relative importance of increases of DA in the shell and core of the Acc as objective correlates of the rewarding and motivating effects of food. The fact that diet preexposure inhibits the DA response in the shell, but not in the core or Pfc needs to be understood. It is important that it is dependent on rats being non-deprived; 24 hr of food deprivation abolishes the effect (Bassareo & Di Chiara, 1999b). If this site-specific pattern of increases of DA is robust, the transient DA responses in the core or Pfc may be more consistent, and therefore better, correlates than the transient increase of DA in the shell in experiments involving sequential meals or recent dietary experience.

Transient increases of DA in the Str have been investigated relatively little. More data are required to evaluate the suggestion that DA increases in the Str when eating occurs under very predictable conditions (see next section).

The increase of DA in the Acc is not a specific objective correlate of the rewarding and motivating effects of food. It has been observed during ingestion of water (Yoshida *et al.*, 1992), appetitive and consummatory phases of sexual behavior, aversive behaviors, stress, and self-administration of drugs (Fibiger & Phillips, 1986). This nonspecificity was the point Stricker and Zigmond emphasized in 1986 (Stricker & Zigmond, 1986).

The possibility that future research will identify a *specific* objective correlate for the rewarding and motivating effects of food is unlikely. This pessimism is based on the frequent demonstrations that a synaptically active molecule, such as serotonin or Neuropeptide Y, is used in the same or different regions of the brain to integrate different behaviors.

Importance, Function, and Meaning of the Objective Correlate

The availability of an objective correlate brings investigation of the motivating and rewarding effects of food to a new level of analysis—the neurophysiological. This is a major advance. The increase of Acc DA transcends previous work with 6-OHDA lesions and pharmacological agonists and antagonists that demonstrated the importance of DA in mediating the motivating and rewarding effects of food, because these techniques could not decide whether the effect was due to loss of a chronic or transient function of DA. The microdialysis results show that it is a transient increase of DA in the Acc that occurs during and after the motivating and rewarding effects of food under most conditions.

Knowing that it is a transient increase is heuristic for exploring the neural mechanisms underlying the motivating and rewarding effects of food. That the transient increase occurs in the Acc vindicates the suggestion of Mogenson (Mogenson *et al.*, 1980) that the Acc is important for the integration of motivating and rewarding information from the limbic forebrain with brainstem mechanisms that control the sequential movements that occur in the appetitive and consummatory phases of eating.

Having this correlate also facilitates the investigation of the function of Acc DA in the motivating and rewarding effects of food by making contact with other areas of investigation of central DA function. For example, Schultz and his colleagues have shown that DA neurons in the A9 and 10 regions, monitored extracellularly,

are activated by food stimuli in the initial phases of training an association between a tone or light and brief oral infusions of foods (Schultz, 2002). As training proceeds, the activation of the DA unit moves from being correlated with the orosensory food reward to being correlated with the presence of the conditioned stimulus (CS). The migration of the DA response from the orosensory reward to the CS during acquisition of a conditioned response suggests that this dynamic change could be important for the development of secondary positive reinforcement and incentive salience of the CS. This sequential change in the relationship between DA neuronal activation and learning a conditioned response for orosensory reward has catalyzed work on the role of DA in learning (Fiorillo, Tobler, & Schultz, 2003; Suri & Schultz, 2001; Waelti, Dickinson, & Schultz, 2001).

Bassareo and Di Chiara attempted to use this idea to explain the importance of the relative novelty of food stimuli for the increase of DA in the shell and Datla *et al.* (2002) used it to discuss this possibility in their demonstration of the DA increase in the Acc elicited by CSs. But their microdialysis results have not been consistent with the hypothesis of Schultz. Bassareo and Di Chiara discussed two reasons why this might be so. First, there are large differences in the durations of stimulus delivery (200 ms vs 1–2 min) and DA response (seconds of unit activity compared to 10-min samples of dialysate) in the experiments of Schultz and his colleagues, and those of Bassareo and Di Chiara, respectively. Second, Schultz has not determined the effects of the activation of DA units on specific terminal projection sites. Given the numerous local controls of DA release in the various projection sites of DA neurons in the forebrain and the common observation that terminal fields are not always activated simultaneously, the importance of DA unit activity in the midbrain for detection of an increase of DA in a terminal field such as the Acc is an empirical question. Certainly the observation of differential activation of the shell and core by familiar and novel stimuli is not predictable from Schultz's results.

Although these initial attempts to use Schultz's idea have not been positive, any attempt to understand the role of DA in learning and memory is likely to be helpful in understanding the pervasive actions of those processes in the motivating and rewarding effects of food (Sclafani, 2001; Tracy, Jarrard, & Davidson, 2001). For example, it would be very interesting to monitor Acc DA during the acquisition and performance of a conditioned preference produced by the association of taste and postingestive nutrient effects. Mark *et al.* (1994) observed a significant increase in Acc DA during ingestion of SOA *after* preference conditioning but not before. This is the only published result known to me and it does not include information about Acc DA during the acquisition of the preference. Measurement of Acc DA during acquisition of a conditioned flavor preference is likely to be informative because systemic administration of D_1 and D_2 antagonists decrease acquisition and expression of conditioned flavor preferences (Azzara, Bodnar, Delamater, & Sclafani, 2001; Baker, Shah, Sclafani, & Bodnar, 2003; Yu, Silva, Sclafani, Delamater, & Bodnar, 2000a, 2000b).

Finally, increase of Acc DA is relevant to the long search for a common central mechanism for the rewarding and motivating effects of foods and drugs (Di Chiara, Acquas, Tanda, & Cadoni, 1993; Hernandez *et al.*, 1988b; Wise, 2002; Wise *et al.*, 1978). Not only do a number of drugs increase Acc DA, but recent experiments have revealed an intimate relationship between eating sugars, such as glucose and sucrose, and the release of DA in the Acc by amphetamine.

In 1999 Barr and Phillips (1999) showed that the rewarding and motivating effects of 4% sucrose measured by performance on a Progressive Ratio schedule were significantly decreased during withdrawal from amphetamine. Then the relationship between sucrose and amphetamine was pursued in rats that had spontaneous differences in sucrose intake. HIGH-intake rats earned a larger number of reinforcements of 20% sucrose than LOW-intake rats on a Progressive Ratio schedule (Brennan, Roberts, Anisman, & Merali, 2001). HIGH rats also had a larger increase of Acc DA after peripheral administration of amphetamine than LOW rats (Sills & Crawley, 1996). Thus, spontaneous sucrose consumption is correlated with the motivating and rewarding effects of sucrose and both are correlated with the magnitude of the increase of endogenous DA in response to peripheral amphetamine.

Instead of using systemic administration of amphetamine, Wyvell and Berridge (2000) injected amphetamine into the Acc and observed increased incentive salience of a Pavlovian-conditioned cue for sucrose reward. They extended this result by investigating the effect of sensitizing rats to amphetamine on the response to the conditioned cue for sucrose reward. After being drug-free for about 2 weeks, the rats were tested in the same conditioned paradigm. Rats sensitized to amphetamine responded more to the conditioned cue for sucrose than normal rats that had not been sensitized (Wyvell & Berridge, 2001).

Amphetamine-sensitized rats not only responded more to a conditioned cue for sucrose reward, they also locomoted more after eating sucrose for 1 min and they consumed more 10% sucrose in daily 1-hr tests for 5 days than nonsensitized rats (Avena & Hoebel, 2003b). The relationship was reciprocal. When rats were maintained for 21 days on access to 10% sucrose and chow for 12 hr alternating with deprivation for 12 hr, their locomotor response to amphetamine was significantly increased compared to the response of control groups (Avena & Hoebel, 2003c).

This evidence of cross-sensitization between amphetamine and sucrose complements the evidence obtained by Hoebel and his colleagues of signs of dependency to ingested glucose (Colantuoni *et al.*, 2002). Rats were given access to 25% glucose and chow for 12 hr a day for 1–4 weeks. When withdrawal was precipitated by naloxone or food deprivation, rats showed signs of opioid dependency, such as teeth chattering, forepaw tremor, head shakes, and decreased movement in the exposed arms of the elevated plus maze, a measure of anxiety (Pellow, Chopin, File, & Briley, 1985). Naloxone also decreased DA and increased acetylcholine in the Acc, a neurochemical pattern previously seen after withdrawal from morphine induced by naloxone and withdrawal from nicotine produced by mecamylamine. These results suggested that withdrawal from glucose, morphine, and nicotine share an Acc mechanism and they are consistent with endogenous opioid dependence caused by intermittent, excessive sugar intake.

Positive Feedback of Orosensory Sucrose and Accumbens Dopamine

In addition to cross-sensitization to amphetamine and development of opioid dependence, orosensory sucrose stimulation in the mouth and Acc DA have a positive-feedback relationship that is similar to the one described in psychostimulant drug self-administration.

For example, eating sucrose for 7 days increased the Acc DA response to the orosensory stimulation of sucrose more than drinking water did (Hajnal & Norgren, 2002). Similarly, rats that had daily access to 10% sucrose for 12 hr for 21 days had a larger Acc DA response to sham feeding and real feeding of sucrose on day 21 than rats without experience with sucrose (Avena *et al.*, 2003a).

The DA response can also be increased by pharmacological manipulation of the local control of Acc DA. Nomifensine, a blocker of DA uptake by the DA transporter, infused into the Acc by reverse microdialysis increased Acc DA and sucrose intake (Hajnal & Norgren, 2001; Westerink *et al.*, 1994). Low doses of SCH 23390 and sulpiride dialyzed together into the Acc abolished both effects of nomifensine (Hajnal & Norgren, 2001).

Infusion of a large dose of sulpiride alone into the Acc also increased DA in the Acc and increased sucrose intake (Hajnal & Norgren, 2001; Westerink *et al.*, 1994). This effect of sulpiride on Acc Da was presumably due to its blocking D2 receptors on DA terminals and axons that mediate the autoinhibition of extracellular DA on DA release.

Thus, sucrose in the mouth stimulates DA in the Acc, which in turn stimulates sucrose intake. This is a positive-feedback relationship. Under physiological conditions, uptake of DA, autoinhibition of DA release, and postingestive inhibitory stimuli that produce release of acetylcholine in the Acc (Avena *et al.*, 2003a) blunt this positive-feedback effect. Of these three mechanisms, the postingestive inhibitory mechanisms are the most important because when they are eliminated by sham feeding, the positive feedback is obvious in the food-deprived rat: Eating does not end and the rate of intake, the volume ingested, and the magnitude of the Acc DA response are monotonic functions of sucrose concentration (Geary & Smith, 1985; Hajnal *et al.*, 2004) but acetylcholine in the Acc does not increase (Avena *et al.*, 2003a). These concentration-dependent effects of sucrose on sham feeding have been called orosensory self-stimulation (Schneider, 1989).

Human Imaging

The concentration-dependent effects in rodents are similar to the psychophysical measures of intensity and pleasure of sweetness in humans that are a monotonic function of sucrose and glucose throughout most of the range of their concentrations. Thus, sucrose, glucose, and sweet juice have been used in human imaging studies to investigate regions of the human brain involved in the processing of intensity and hedonics of sweet taste. Sweet orosensory stimuli increased activity in primary cortical sensory structures for taste, such as the inferior insula and opercular frontal cortex (Faurion, Cerf, Le Bihan, & Pillias, 1998).

These same structures are also stimulated by pictures and words of sweet taste, and interestingly, by imagined tastes and phantom tastes (Henkin, Levy, & Lin, 2000; Levy, Henkin, Lin, Finley, & Schellinger, 1999). Secondary activation occurs in the orbitofrontal cortex (O'Doherty, Rolls, Francis, Bowtell, & Mc Glone, 2001). There is preliminary evidence that the amygdala responds to the intensity of the gustatory stimulus rather than its hedonic value, while the orbitofrontal cortex is involved in evaluating the reward and the use of the gustatory stimulus in the choice of goal objects (Zald, Hagen, & Pardo, 2002).

Unpredictability affects reward and preference can be dissociated from reward. (Berns, McClure, Pagnoni, & Montague, 2001). Activity in the Acc and medial

orbital frontal cortex produced by drinking sweetened fruit juice was largest when delivery of the juice was unpredictable. Self-reports of preference for juice or water were not correlated with the activity in Acc or orbital frontal cortex, but with activity in the sensorimotor cortex.

There are only three reports relevant to the role of forebrain DA in the mediation of the rewarding, motivating, or pleasurable effects of sweet taste. DA release was measured by the displacement of [(11)C] raclopride-specific binding using positron emission tomography. Drevets *et al.* (2001) showed that peripheral administration of amphetamine in seven people increased DA in the anteroventral striatum (AVS, this included the Acc, ventromedial caudate, and the anteroventral putamen) significantly more than in the dorsal caudate. This result demonstrates the preferential effect of amphetamine on the release of DA in the ventral rather than the dorsal Str; this is well documented in rats and thus this study provides an important link between the microdialysis studies in the rat and the displacement of [(11)C] raclopride in human imaging. There was a correlation between the measure of extracellular DA in the AVS and self-reports of euphoria, tension, and anxiety. Similar correlations were also found in the ventral putamen.

Using the same technique for estimating DA release, Volkow *et al.* (2002) found that the display of food without eating increased the reported desire for food in 10 food-deprived subjects and increased DA in the dorsal but not the ventral Str. Pretreatment with methylphenidate, a drug that blocks DA transporters and thus increases extracellular DA by decreasing reuptake, was required for the detection of significant changes in the displacement of [(11)C] raclopride.

Similar results were obtained by Small, Jones-Gotman, and Dagher (2003) when they measured the effects of eating a favorite meal in seven people. DA increased in the dorsal putamen and caudate nucleus but not in the ventral Str. There was a correlation between the reduction of [(11)C] raclopride binding and ratings of the pleasantness of the meal, but not with the desire to eat or satiety after the meal. The same group has presented evidence for a dissociation between areas activated by sensory intensity and hedonic evaluation of taste stimuli (Small *et al.*, 2003).

Thus, the desire to eat and the pleasantness of a meal have been correlated with estimated DA release in the dorsal but not ventral Str in two human experiments. This is the opposite of the usual effects in rats.

In thinking about the human imaging studies, it is useful to keep the constraints of such experiments in mind. These include technical aspects of imaging, restraint of the subject sufficient to maintain the head in a constant position, significant manipulation of primary data, and individual differences in self-reports (Drevets *et al.*, 2001). To these can be added the validity of the magnitude of the change of the self-reports as measures of the intensity and kind of subjective experience (Bartoshuk, 2000). Finally, there is the formidable problem of translation among self-reports, subjective experience, anatomic regions, and indirect estimates of DA release. The accessibility of language in human imaging studies is often proposed as an advantage in the investigation of the relationships of brain anatomy to subjective experience and behavioral expression. This is to underestimate the difficult problems of meaning and intention that are central to the use and understanding of language. It is unlikely that these translations in humans will be easier than the nonlinguistic translations of the similar relationships in animals.

The long search for an objective correlate of the motivating and rewarding effects of food has been successful. The objective correlate is the transient increase of DA in the Acc that occurs during the ingestion of food and during the presentation of CSs previously associated with the ingestion of food. The magnitude of the increase is correlated with the amount of food eaten and with the concentration of sucrose when the volume of sucrose ingested is held constant. It occurs with liquid and solid foods, after varying durations of food deprivation or after no deprivation, and in free feeding and operant situations. It is reliable—the correlation occurred in 44 of 53 tests. Why it did not occur in nine tests is not clear and should be investigated, particularly in experiments in which the shell and core regions of the Acc are sampled simultaneously (see below).

Orosensory stimuli of food are crucial for the increase of DA. Postingestive food stimuli produced by gastric loads in the absence of eating do not increase DA in the Acc under conditions in which the ingestion of the same food did. Furthermore, sham feeding of sucrose, a procedure that preserves the orosensory stimuli of sucrose and eliminates its postingestive effects, also increases Acc DA. Among the orosensory effects, retronasal stimulation of the olfactory receptors is not necessary because the DA increase did not change after olfactory bulbectomy (Meguid *et al.*, 1995). At least in the case of sucrose, the major oral stimulus is gustatory.

There is growing evidence that DA responses to eating and CSs are different in the shell and core regions of the Acc. The reliable increase of DA in the shell (10 of 11 tests) is inhibited by preexposure to the diet either by ingestion of food or by presentation of CSs previously associated with the food within the 24 hr preceding the test. This inhibition does not occur in the core of the Acc. The inhibition in the shell depends on the same diet being used for preexposure and testing. If different diets are used, the inhibition does not occur. The block of inhibition by a relatively novel food in the second meal is evidence that the inhibition is an example of habituation. The inhibition occurs in the non-deprived state; deprivation of food for 24 hr abolishes it.

Thus, the increase of DA in the shell of the Acc is sensitive to recent diet ingestion or CSs exposure, and to the state of food deprivation. These are the characteristics of a central mechanism related to eating and metabolism. The fact that the shell of the Acc, but not the core, has significant projections to the LH also favors this interpretation because the LH is a nodal point in the central network for the control of eating and food intake.

Despite these characteristics, the DA response in the Acc shell is not necessary for intake. When the shell is inhibited, intake and taste reactivity do not change. This raises the fundamental question of the functional relationship between the DA increase in the shell and the rewarding and motivating effects of food measured by intake. It is possible that these effects of ingesting food are decreased when the Acc response is inhibited—the food is devalued. The effects of different food and long deprivation are consistent with such an interpretation. This is an important question that deserves experimental attention because it probes the function of the objective correlate in the shell.

Taste and food stimuli produce differential responses of the Acc shell and core. The possibility of these differential responses has not been part of most experimental designs in the past. This could affect the results. For example, if the microdialysis

probe sampled the extracellular DA of the shell of the Acc, this could account for the failure to detect a DA response to eating in a protocol where preexposure occurred.

The Acc is not the only site of DA release that correlates with the ingestion of food. The Pfc, lateral and rostromedial hypothalamus, and the amygdala have also shown increases of DA during the ingestion of food. Of these, the Pfc has been investigated the most and it is quite a reliable correlate—it increased in all 13 tests. Its response is not affected by diet or stimulus preexposure. The apparent high fidelity of the Pfc needs to be investigated more.

The differential DA responses in the shell and core of the Acc raise problems for the heuristic ideas of Berridge and Schultz. The problems may be clarified by further experiments using similar protocols.

The identification of a transient increase of Acc DA as an objective correlate of the motivating and rewarding effects of food has two important consequences. First, it is heuristic because it enables the investigation of the function of Acc DA in the shell and in the core in the central network for the control of eating. Second, it facilitates the comparison of the function of Acc DA in the rewarding and motivating effects of self-administration of psychostimulants and eating food.

The past decade of research in this subject has made significant progress. The concept of the rewarding effect of food can now be differentiated into explicit questions of its roles in associative learning about food in the development of preferences or aversions, in incentive motivation, and in the control of meal size. The imaging studies of humans, so intrinsically interesting, have begun to deal with the experience of food, particularly its taste and the regional release of DA.

The next decade should be even better. New techniques, such as fast-scan voltammetry (Wightman & Robinson, 2002) and genetic manipulations (Benoit *et al.*, 2003; Cannon & Palmiter, 2003; Pecina, Cagniard, Berridge, Aldridge, & Zhuang, 2003) will have an impact. And new ideas, such as the interaction between Acc DA and adiposity signals implicated in the control of food intake and body weight (Figlewicz, 2003), can be tested rigorously. Thus, in these and other unforeseen ways the identification of an objective correlate has brought the investigation of neurophysiological mechanisms of the motivating and rewarding effects of food within experimental reach.

Acknowledgments

I thank Drs. Anthony Azzara and Roy Wise for giving me detailed and constructive criticism on preliminary versions of this chapter. I am also grateful to Dr. Bartley Hoebel for copies of many of his papers and Ms. Marcia Miller, Librarian of the Westchester Division of the New York-Presbyterian Hospital, for her many helpful bibliographic services. Dr. Edward Stricker gave me good editorial advice and was patient beyond the call of duty with the glacial pace of preparation of this chapter.

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3

The CNS Physiology of Food Reward

Current Insights and Future Directions

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In 2003, we are confronted with the incontrovertible fact that the United States and similar societies across the globe are experiencing a virtual epidemic of obesity. Rigorous and systematic documentation of the increased prevalence and severity of obesity has been presented repeatedly over the past decade, in the form of website "slide shows" that include data from the Centers for Disease Control and Prevention, publications in medical journals (Mokdad *et al.*, 2001), and publications in high-visibility basic research journals, most recently a special focus issue of *Science* (Hill, Wyatt, Reed, & Peters, 2003; Pi-Sunyer, 2003). Obesity is recognized as a significant risk factor for diabetes, cardiovascular disease, and several cancers, as well as shortened life span. Medical and public health acknowledgment of the seriousness of this problem should lead to more opportunities for basic scientists to study its etiology. As such, we are offered challenges as well as opportunities. Clearly we have learned a lot but still have a long way to go in terms of understanding the basic physiology of food intake regulation by the central nervous system (CNS) and the physiological or pathophysiological mechanisms that underlie overeating.

Several chapters in this *Handbook* review the extensive and elegant work that has been done to delineate the role of specific CNS neuronal pathways, and their neurotransmitters, in the regulation of individual meal size and the defense of body weight and body adiposity; that is, the maintenance of caloric intake for the physiological needs of an animal. The original model proposed by Woods, Porte, and colleagues

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Neurobiology of Food and Fluid Intake, 2nd Ed., Volume 14 of *Handbook of Behavioral Neurobiology,* edited by Edward Stricker and Stephen Woods, Kluwer Academic / Plenum Publishers, New York, 2004.

(Woods, Lotter, McKay, & Porte, Jr., 1979) of a negative feedback loop between the brain and adiposity signals (circulating factors whose concentrations reflect the size of adipose stores, and which signal this information to the CNS) has received substantial experimental support (Baskin *et al.*, 1999). While there also is support for the concept that food intake can be modified by the rewarding aspect(s) of food, the concept that the rewarding aspect(s) of food in turn may be regulated is still somewhat novel. The purposes of this chapter are to summarize the current knowledge regarding CNS mediation of food reward and the evidence in support of its potential regulation; and to offer suggestions for new lines of investigation to further validate or refute the concept of food reward regulation. As there are numerous excellent and recent reviews that cover specific aspects of this information, this chapter is a selective summary, with the intent to guide the reader to the primary studies rather than to provide either an exhaustive or a balanced review of any of the concepts or topics mentioned.

"Reward" is a construct that has served as a rich source for debate and study by psychologists as they have attempted to define and differentiate between "motivation," "reinforcement," and "reward" (Berridge & Robinson, 1998; Robbins & Everitt, 1996; Wise, 2002; Wise & Hoffman, 1992). For the purposes of this chapter I define "reward" functionally: the "rewarding" aspect of food is gauged by the function of its being sought out and consumed, be it in an animal experiment or a free-choice setting for humans. This psychologically loose definition thus collapses both "motivation" and "reinforcement," and as I am considering it here, it takes into account caloric need, palatability of food as a primary experience, and emotional/learned aspects of feeding experience. Whereas elaborate psychological studies have deconstructed these components of reward, I propose that physiologists should begin to synthesize these components. It is clear that in real life they are inextricably connected. An illustration of this connection comes from religious traditions across cultures and centuries, in which fasting is followed by feasting. Post-fasting and somewhat calorically deprived, an individual's motivation to eat is enhanced (see discussion below) and a larger than normal meal may be consumed. However, palatability and experience play a critical role in the selection of food types and quantities. The result is that a group of people who spend a comparable amount of time at a meal in fact consume a vastly different range of macronutrients and calories. One physiologic endpoint, of course, is the number of calories consumed, and evidence suggests that the basic macronutrient proportion (fat vs carbohydrate calories) is also physiologically critical for the regulation of energy balance and body weight. How this "bottom line" of food intake is arrived at in a free choice situation is the complex result of numerous factors, only one of which is true caloric need. Thus, as physiologists we need to study, at both anatomical and functional levels, how caloric need, food palatability, and food experience are interwoven, and how they might be manipulated either behaviorally or through the use of pharmacotherapeutics to impact caloric intake in a meaningful way.

Evidence available to date supports the concept that both the nutritional status of an animal and its environment—type of food, obstacles to obtain food—modulate the rewarding or motivational value of food (Levine & Billington, 1997; Salamone, Correa, Mingote, & Weber, 2003; Wilson, Nomikos, Collu, & Fibiger, 1995). How or whether this modified value of the food impacts on the energy balance regulatory circuitry of the CNS remains essentially unknown. One reason, perhaps, that there are so few data currently available to address this issue is that,

historically, studies of CNS reward function and studies of regulation of food intake have for the most part focused on anatomically distinct circuitry and have used behavioral paradigms that might not be appropriate for asking or answering questions about food reward.

Studies evaluating the physiological defense of caloric intake by the CNS have focused upon the medial hypothalamus as a major anatomical target (Saper, Chou, & Elmquist, 2002; Williams *et al.*, 2001) and have evaluated the actions of hormones and neurotransmitters experimentally using highly controlled and stimulus-deprived environments for these tests. While this experimental approach has been necessary and is correct for these sorts of studies, the applicability of the findings to eating by humans has been challenged. Hill *et al.* (2003) have pointed out that the current obesity epidemic may be ascribed to an environment of convenient and economically affordable food that is both highly palatable and high in caloric density and fat content. If, in fact, the medial hypothalamic circuitry acts as the final common arbiter of caloric intake, then why doesn't caloric intake (and in adults, body adiposity) remain constant and appropriate in the face of whatever foodstuffs are available? One response to this query is that the data we have gleaned regarding the calorie-regulatory circuitry of the hypothalamus have been obtained in circumstances where there are no environmental challenges and probably limited activation of the CNS reward circuitry. Thus, the majority of the data on food intake have been collected from animals feeding in their home cages on commercial rodent chow, a bland, monotonous, and relatively low-fat diet which is presented in abundance; thus the animal needs minimal engagement of motor systems in order to eat and can generally expect that there will be as much to eat as it wants. As suggested above, this situation offers an almost-perfect model for studying the regulation of caloric need by neural and endocrine factors. However, in 1988 a key study from the lab of Bray made it clear that the function of the CNS–adiposity signal feedback loop can be altered by an environmental intervention; that is, changing the fat content of the diet. In their study, they demonstrated that rats fed a high-fat diet lost less body weight in response to a direct CNS infusion of insulin than rats maintained on standard lab chow (Arase, Fisler, Shargill, York, & Bray, 1988). This finding was replicated subsequently by Chavez, Woods, and colleagues who demonstrated that the effect was "dose dependent" on the concentration of fat in the diet (Chavez, Riedy, Van Dijk, & Woods, 1996). While the precise mechanisms of this effect remain unclear, these studies made the point that diet composition has a major impact on the function of the calorie-regulatory CNS circuitry. Interestingly, in the Chavez study the ability of insulin to decrease food intake was lost when diet fat concentration reached about 30%, the value that represents the maximum proportion of fat recommended by nutritionists for the daily American diet (i.e., any proportion under 30% is considered "lower fat" or "low fat"); thus, the typical American adult probably consumes a higher proportion of fat. If the same effect of dietary fat to suppress adiposity signaling occurs in humans as in rats, then one might postulate that most Americans are eating a diet composition that would make them resistant to the action of one of their own endogenous adiposity signals, essentially inactivating the calorie-regulating negative feedback loop. This consideration highlights the need for understanding the contribution of food reward to caloric consumption: in an environment of abundant food choice, individuals select food based on what they like or want. This choice drives the actual intake, and when the caloric balance tips in favor of high percentage of fat in the diet, the CNS calorie-regulatory circuitry is silenced.

Historically, study of the CNS and reward has focused anatomically on the lateral hypothalamic area (LHA), and functionally on paradigms such as brain self-stimulation or self-administration of various neurally active substances. Following the work of Olds (1962), half a century of research has pursued specific neuroanatomical substrates that mediate reward. Thus in addition to the mapping of specific areas in the LHA, the role of the median forebrain bundle (a large collection of fibers passing through the area) and midbrain dopaminergic cell bodies and their projection sites have been the target of intensive study (Wise, 1988). Not surprisingly, it has become appreciated that additional CNS sites have a role in mediating the rewarding aspects of stimuli. Figure 1 is a schematic diagram of the relevant anatomy (all connectivity is not shown). As an anatomical basis for potential cross-talk between calorie-regulatory circuitry and reward circuitry, it must be appreciated that the medial hypothalamic nuclei are extensively connected with the CNS regions that mediate reward and motivation. For example, the LHA is a major relay area for projections from the mediobasal hypothalamus and thus could serve as a critical integrator for signals from both reward circuitry and calorie-regulatory circuitry.

The limbic reward system can be functionally defined as those CNS structures that mediate the rewarding, reinforcing, and emotional aspects of stimuli. From an anatomical perspective, there is a general consensus that the LHA, amygdala, select regions of the cerebral cortex, the ventral tegmental area (VTA), and the ventral striatum or nucleus accumbens (NAc) are components of this circuitry (DeOlmos & Heimer, 1999; Everitt *et al.*, 1999). Reciprocal synaptic connections exist between the amygdala and cortex, and between the NAc and cortex, and there are substantial efferent projections from the amygdala to the hypothalamus and the VTA/substantia nigra pars compacta (SNc). There appear to be limited forebrain inputs directly to the paraventricular nucleus of the hypothalamus (PVN) although PVN

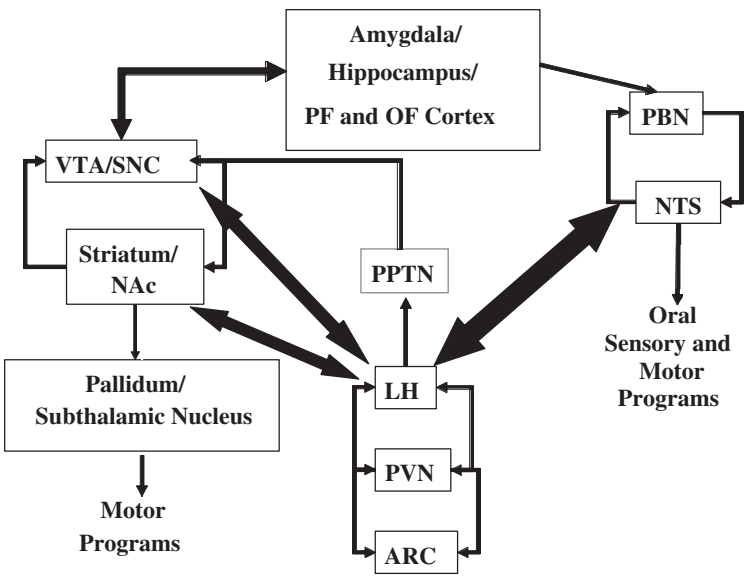

Figure 1. CNS reward circuitry. Hypothalamic subareas have reciprocal interconnections as well as extensive connections with various components of the reward circuitry. For clarity, not all interconnections are shown; see text for discussion of specific synaptic interconnections.

efferent projections to the LHA are abundant. Rather the arcuate nucleus of the mediobasal hypothalamus appears to receive critical limbic inputs projections from the LHA containing the feeding-stimulatory peptide orexin, as well as input from the central nucleus of the amygdala (CeA). In turn, there are direct projections from the arcuate nucleus to the LHA. The CeA also projects to the LHA, and the LHA and amygdala receive direct taste inputs from the nucleus of the solitary tract (NTS, the critical primary-to-secondary relay site for the taste pathway). Other relevant synaptic connections include reciprocal projections from the NAc to the VTA, and projections from the NAc to the LHA. For more detailed anatomical discussion, the reader is referred to Chapter 9 in this *Handbook* by Berthoud that reviews brainstem contributions to the control of feeding, and to the recent reviews by Berthoud and Kelley (Berthoud, 2002; Kelley *et al.*, 2002).

The collection of mesocorticolimbic (VTA) dopamine neurons, which project to the ventral striatum or NAc, and to the prefrontal cortex, has been viewed as a central neuroanatomical substrate for reward and motivation (Ikemoto & Panksepp, 1996; McBride, Murphy, & Ikemoto, 1999). Activation of VTA dopamine neurons, and release of dopamine within the NAc, have long been viewed as indicative of reward enhancement. What does activation of the VTA/NAc pathway reflect in terms of food reward? Although this remains a topic of lively debate (Hoebel, Hernandez, Schwartz, Mark, & Hunter, 1989; Salamone, Correa, Mingote, & Weber, 2003; Schultz, 2002; and see Chapter 2 by G. P. Smith in this *Handbook*), Berridge and colleagues have demonstrated that this activity is not correlated with enhanced hedonic value of food as evaluated in the "taste reactivity" paradigm, and thus dopaminergic activation does not reflect an increase in the animal's "liking" of the food (Berridge, 1996). Rather those authors have proposed that mesocorticolimbic dopamine activity reflects an increase in the "incentive salience" of a stimulus, including food. This property can be modulated by the nutritional status of an animal. In the schema of Berridge, the food stimulus would be more relevant and more motivating. It has been documented that with repeated training to gain access to a diet in a defined physical environment, initial exposure leads to increased release of dopamine in the NAc shell whereas subsequent exposure leads to either no increase of dopamine (Richardson & Gratton, 1996); an increase of dopamine prior to the actual presentation of the food (Kiyatkin, 1995); increased release of dopamine within a different part of the NAc (Bassareo & DiChiara, 1997); or sustained release of dopamine in the prefrontal cortex (Bassareo & DiChiara, 1997). Although interpretation of these findings remains controversial, the concept that the contextual stimuli themselves (odor or visual cues) become salient, and can elicit dopamine release with repeated exposure to food in the same context, seems experimentally validated. However, if dopamine release in the NAc shell reflects "reward," the habitual presentation of the same food could be predicted to result in a loss of its primary reward value.

Most current studies of candidate endocrine or neural factors for the regulation of food intake include an evaluation of these factors in association with chronic consumption of a high-fat diet and perhaps diet-induced obesity. While this experimental approach may be valuable for understanding the impact of diet composition on the efficacy of putative calorie-regulatory factors, it may not be a meaningful diet manipulation for evaluating the effect of diet composition on reward circuitry function. As summarized above, one might speculate that there is very limited NAc dopamine release when rats eat commercial rat chow in the habitual home cage environment, and, after the initial exposures, there probably would be limited

dopamine release to a high-fat diet if that was the only food available. Experiments testing this hypothesis have not yet been conducted. In addition to the possibility that chronic, noncontingent exposure to any diet might neutralize its primary rewarding properties, studies from the scientific literature on drug abuse have highlighted the point that there are differences in CNS dopamine release, the neuroendocrine response, and drug-seeking behavior between rats that passively (noncontingently) receive a drug such as cocaine, and rats that have the opportunity to self-administer it (Gallici, Pechnick, Poland, & France, 2000; Markou, Arroyo, & Everitt, 1999; Wilson, Nobrega, Corrigal, Coen, & Kish, 1994). This effect also might hold true for diets: that is, the consequences of diet choice on VTA/NAc activity (or any other component of the reward circuitry) may be different from those that occur when there is no choice, regardless of the diet composition. This point may seem obvious when one considers human eating experiences, but it has never been evaluated systematically in laboratory experiments.

Experiments in which the nutritional status of the animal and the diet (whether offered as a treat or as the maintenance diet) are systematically manipulated, while the components of CNS reward circuitry are evaluated, are a feasible initial approach to determining the involvement of these specific components of the reward circuitry in realistic feeding simulations. The studies of Bassareo and DiChiara (1997, 1999) provide an example of such experiments. They demonstrated that the initial presentation of a food treat to rats resulted in increased dopamine release in both the NAc shell and prefrontal cortex. With a second presentation of the treat within 24 hr, the NAc shell response habituated but the prefrontal cortex response remained unchanged. A stimulus that signaled a food treat (i.e., the food container with an associated olfactory cue) also could block the subsequent food-induced rise of dopamine in the NAc. Waiting 5 days between presentations of the treat prevented the initial habituation in the NAc shell, and the habituation also could be partially reversed by food deprivation. These studies make the point that mesolimbic dopamine release is not just a simple response to a feeding situation, and anatomically specific dopamine outflow is contingent on experience, timing of palatable food access, and the nutritional status of the animal. Compared with other studies in which animals are maintained on a strict training paradigm for weeks to months at a time, this more acute experimental paradigm is perhaps more relevant for discerning the role of mesocorticolimbic dopamine in food reward for humans, since humans generally do not eat exactly the same food in the identical environment every day.

Perhaps the strongest clue to common *functional* links between the reward circuitry and calorie-regulatory circuitry is the observation that fasting or food restriction have marked behavioral and neurochemical effects on both. The reader is referred to other chapters in this book as well as a recent review (Schwartz & Seeley, 1997) that discusses the function of medial hypothalamic calorie-regulatory circuitry in the context of starvation. Fasting or food restriction activates, or enhances the activation of, reward circuitry as evaluated in several different behavioral paradigms (Carr, 2002; Shizgal, Fulton, & Woodside, 2001). In one of the most striking illustrations of this effect, Carroll and colleagues studied rats allowed to self-administer a threshold dose of cocaine (Carroll & Meisch, 1984). Cocaine and other drugs of abuse cause release of dopamine in the NAc (although by different mechanisms). In Carroll's study, rats were fasted or fed on an alternating-day schedule prior to having access to the cocaine. When tested on days after they were fed overnight, they self-administered almost no cocaine. When tested on days after they

had been fasted overnight, rats robustly self-administered cocaine. This result demonstrates that reward circuitry in the CNS is markedly responsive to changes in metabolic status. The finding has been replicated with food restriction rather than fasting and has been observed in a somewhat different self-administration paradigm: food restriction will enhance the propensity to drug-taking relapse in rats that have extinguished drug self-administration (Shalev, Grimm, & Shaham, 2002).

A second behavioral task, the conditioned place preference (CPP) paradigm, assesses the strength of a learned association between the perceived reward value of a stimulus (such as a drug treatment or food) and the location in which the animal received the stimulus (Bardo & Bevins, 2000). The strength of the conditioning can be sensitive to the nutritional status of the animal. Conditioning of a place preference by cocaine is enhanced by food restriction (Bell, Stewart, Thompson, & Meisch, 1997). Place preference also can be conditioned by food and, perhaps not surprisingly, the strength of the CPP is enhanced by food restriction (Agmo, Galvan, & Talamantes, 1995; Figlewicz, Higgins, Ng-Evans, & Havel, 2001; Lepore, Vorel, Lowinson, & Gardner, 1995; Papp, 1988; Swerdlow, Vander Kooy, Koob, & Wenger, 1983). Place preference conditioning by food is dependent on intact dopaminergic activation, as development of the CPP is blocked by administration of dopamine receptor antagonists during training sessions (Agmo *et al.*, 1995; Figlewicz *et al.*, 2001).

Given the central role of the VTA dopamine neurons in reward circuitry, it has been hypothesized that the effect of food restriction is due to enhanced activation of these neurons. Indeed, Wilson and colleagues demonstrated that food-restricted rats trained to drink a palatable liquid food had greater dopamine release in the NAc than free-feeding rats (Wilson, Nomikos, Collu, & Fibiger, 1995). One question, then, is whether these dopamine neurons are a target for neural or endocrine factors that change in association with fasting and food restriction. The Carroll study suggests that neural or humoral factors modulating these phenomena must be able to change with a time course of several hours. Adrenal glucocorticoid levels are elevated with fasting, and Piazza and colleagues have provided evidence that glucocorticoids can facilitate dopamine release and dopamine-mediated behaviors (Marinelli & Piazza, 2002). Additionally, both insulin and leptin levels rapidly decrease in association with food restriction or fasting (Havel, 2000). As summarized in a recent review (Figlewicz, 2003), there is evidence for effects of insulin at the level of dopamine re-uptake as well as behaviorally: *in vitro* insulin administration (at a physiological concentration) facilitates striatal dopamine re-uptake, which should in turn curtail dopaminergic signaling. This observation suggests that, by decreasing dopamine signaling, insulin should decrease the rewarding aspect of stimuli. Consistent with this hypothesis, both insulin and leptin inhibit performance in food reward behavioral tasks that are dopamine dependent. Receptors for both insulin and leptin have been identified on the VTA dopamine cell bodies (Figlewicz, Evans, Murphy, Hoen, & Baskin, 2003). These results suggest the possibility that insulin and leptin may act directly at the VTA and/or on NAc nerve terminals to blunt dopaminergic activity and its contribution to food reward. Collectively, studies of the effects of glucocorticoids, insulin, and leptin support the conclusion that a neuroendocrine milieu exists in fasted animals that would bias them toward enhanced dopaminergic function.

As described above, enhanced performance may be observed when either drugs or food are used as stimuli in paradigms where behavioral performance is sensitive to food restriction. This leads to the question of whether or not the reward circuitry

functions in a uniform or nonspecific way such that all types of stimuli are essentially interchangeable. Two observations suggest that this is not the case. First, Shalev and colleagues have reported that the enhanced relapse to heroin self-administration in response to acute food deprivation could be reversed by intraventricular administration of leptin, but leptin was ineffective at preventing the enhanced relapse caused by the nonnutritional stressor of footshock (Shalev, Yap, & Shaham, 2001). Second, the possibility that there may be specificity in activation of the NAc in response to a food stimulus is suggested by the electrophysiological studies of Carelli (2002) which have identified unique classes of NAc neurons that respond differentially depending upon whether the stimulus is a natural reward (water, sucrose solution, food pellets) or a drug (cocaine). The implication of these findings is that NAc-neuronal activation is not nonspecific in response to every stimulus, and that parallel inputs (such as from the cortex) in addition to NAc dopamine release (which would be released nonspecifically by cocaine) may become entrained to food and water stimuli leading to unique patterns of NAc activation. If, in fact, there are NAc neurons that respond specifically to food, then the possibility might be explored that their recruitment, or their responsivity to afferent inputs, could be modulated by food stimuli or the nutritional status of the animal.

Collective evidence demonstrates that activity of the mesocorticolimbic dopamine neurons is modified by the nutritional status of the animal such that there may be enhanced behavioral responding in association with food deprivation. In addition to the mesocorticolimbic dopaminergic system, other neurotransmitter systems (e.g., GABAergic, cholinergic) have a role in the VTA/NAc reward circuitry. Brain opioid systems have served as a focus for investigation as endogenous opioid neural networks appear to play a role in the regulation of food intake, food hedonics, and food choice (Glass, Billington, & Levine, 1999; Levine, Kotz, & Gosnell, 2003). Mu, kappa, and delta opioids and opiatergic agonists stimulate feeding independent of the nutritional status of the animal, and this robust effect has been documented when the drugs are administered into multiple CNS sites. Conversely, opiate antagonists decrease feeding. Although experimental evidence demonstrates that DA and the opioids play different roles in the mediation of food reward, the neuroanatomical circuitry that is implicated in opioid effects overlaps significantly with the VTA/NAc reward circuitry. At an intuitive level, the potential interaction of opioidergic and dopaminergic systems seems obvious: one would predict that a "more pleasing" food would be more rewarding. Thus, opioidergic activation may mediate the hedonic valuation of foods (which the VTA dopamine neurons do not) whereas activation of the VTA/NAc mediates the reward (motivating, reinforcing, incentive salient) properties of food. A compelling reason for targeting the opioids for continued investigation by basic scientists is the observation that endogenous opioids may play a role in hedonic valuation of foods in human subjects. In one report, the opiate antagonist nalmefene decreased fat and protein intake from a standardized buffet meal in nonobese subjects (Yeomans, Wright, Macleod, & Critchley, 1990). Drewnowksi and colleagues reported that both taste preferences for, and intake of, sweet high-fat foods (such as cookies or chocolate) were decreased by treatment with the opioid antagonist naloxone in binge eaters but not in nonbinge eaters (Drewnowski, Krahn, Demitrack, Nairn, & Gosnell, 1992, 1995). This finding suggests that some endogenous opioid systems may be (more) active in association with food binges.

For extensive historical summaries of the work on the role of CNS opioid systems in mediating food intake, the reader is referred to the numerous reviews of

Levine, Billington, and colleagues, who have made substantial contributions to this field (Gosnell & Levine, 1996; Levine & Billington, 1990; Morley, Levine, Yim, & Lowy, 1983). Together, their work and that of others (e.g., Bodnar, Glass, Ragnauth, & Cooper, 1995; Cooper & Kirkham, 1993) suggest that the opioids act to sustain, rather than initiate, feeding; that stimulation of all major subclasses of opioid receptor (mu, delta, kappa) can result in enhanced food intake; and that activation of these receptor populations can stimulate intake of preferred food. Opioids do not appear to act by enhancing the actual sensory properties of palatable foods. Rather, as shown by Berridge and colleagues in their taste reactivity paradigm, exogenous opioids appear to enhance the hedonic value or perceived palatability of food (Pecina & Berridge, 2000), and hence its motivational valence. Thus, mu opioid activation within the VTA can stimulate short-term intake of palatable food in non-deprived rats (Figlewicz, 2003) and can enhance motivated work (i.e., running) to obtain food (Noel & Wise, 1995). In another study, the effect of the non-selective opiate agonist butorphanol was evaluated in the "progressive ratios" paradigm (Rudski, Billington, & Levine, 1994). In this paradigm, rats receive a food reward after pressing a lever for a successively increasing number of times within a session. Thus, initially one lever press may result in a food reward, but the rat then has to press more often (i.e., work harder) for each successive food reward. Butorphanol enhanced responding for food pellets, and, conversely, the opiate antagonist naloxone decreased this responding. The effect of naloxone could be partially reversed by food restriction, demonstrating that—similar to the VTA dopaminergic system—there is an interaction between nutritional status and this component of reward circuitry.

The influence of opioids to stimulate intake of preferred foods has been demonstrated in several studies; some examples follow. Naloxone can specifically inhibit intake of preferred sweetened chow versus regular chow (Levine, Weldon, Grace, Cleary, & Billington, 1995); and naloxone also decreases the intake of the individually preferred diet when rats are offered high-fat and high-carbohydrate diets (Glass, Grace, Cleary, Billington, & Levine, 1996). The longer-acting opiate antagonist naltrexone blocks the reinstatement of sucrose-diet feeding in rats that had prior access to a sucrose diet and then were returned to a less preferred diet (Levine, Grace, Cleary, & Billington, 2002). Endogenous opioid-mediated preference for high-fat diets may have a genetic basis, as studies from the lab of Bray, York, and colleagues have shown that administration of the dynorphin antagonist, norbinaltorphimine, preferentially decreases intake of high-fat diet in Osborne Mendel rats, but does not do so in non-fat-preferring Sprague Dawley rats, when the rats are given a choice between a high-fat and a high-carbohydrate diet (Ookuma, Barton, York, & Bray, 1998). Thus, the CNS opioids play a potentially unique role in feeding behavior, mediating the expression of food preferences when food choices are available.

Discrete opioid receptor populations within several CNS areas mediate the feeding effects of endogenous or exogenous opiate peptides. Mapping of c-fos in response to peripheral administration of butorphanol has revealed neuronal activation in the PVN, the NTS, and the CeA (Kim *et al.*, 2001). Studies from Billington and colleagues have led to the current conclusion that "opioid receptors in the NTS and PVN appear to be important in the total amount of energy content consumed, while opioid receptors in the CeA may be more important in affecting consumption influenced by the sensory or hedonic value of ingestates" (Glass *et al.*, 1999, p. 364). Thus, administration of naltrexone into the PVN suppresses both

preferred and non-preferred diets, but administration of naltrexone into the CeA decreases intake of only the preferred diet (Glass, Billington, & Levine, 2000). Naltrexone administration into the NTS decreases deprivation-induced feeding and can decrease body weight (Kotz, Billington, & Levine, 1997). Functional links appear to exist between the opioid receptor populations of the NTS and CeA, since naltrexone administration into the NTS also increases dynorphin mRNA expression in the amygdala (Glass, Briggs, Billington, Kotz, & Levine, 2002). Although these observations have not yet been tied to behavior, the effect of an opioid antagonist, in this case at the molecular level, reveals neural connections that must be intrinsically active. One speculative scenario would be that NTS opioidergic neurons facilitate deprivation-induced feeding, and the connection with the amygdala would enhance the rewarding aspect of the food in this condition. Further, this finding is consistent with other studies implicating the amygdala in feeding (Petrovich, Setlow, Holland, & Gallagher, 2002). Opiate administration directly into the CeA stimulates feeding (Gosnell, Morley, & Levine, 1986). The NAc is another direct limbic target for opioids: opiates stimulate feeding when administered directly into the NAc. Further study of this phenomenon by Kelley and colleagues has demonstrated that mu opiates can preferentially stimulate intake of fat (Zhang, Gosnell, & Kelley, 1998). Conversely, only high-fat diet intake is blocked by naltrexone administration into the NAc. This phenomenon is independent of baseline macronutrient preference of the animals, and it can be reversed by concomitant inactivation of either the dorsomedial nucleus of the hypothalamus, the LHA, the VTA, or the NTS (Will, Franzblau, & Kelley, 2003). These latter findings again emphasize the potential role of CNS opioid systems to connect reward circuitry with other feeding-relevant CNS sites such as those in the mediobasal hypothalamus and the brainstem.

As discussed above, a central neuroanatomical substrate for coordinating both reward inputs and energy circuitry inputs may be the LHA. Studies from both relevant scientific literatures support this possibility. The anatomical basis for this concept also is well established, as the LHA receives direct and indirect limbic inputs and direct projections from the arcuate nucleus of the hypothalamus (which is a major target for candidate adiposity signals) as well as numerous intrahypothalamic and neuroendocrine inputs (Elias *et al.*, 1998). Studies dating from the 1960s have demonstrated the capacity of animals to electrically self-stimulate their brains when electrodes are placed within specific sites of the LHA. This behavioral paradigm has been interpreted as representing "activation of reward circuitry within the CNS" (Wise, 1996). While the physiological meaning of this behavioral paradigm may be open to question, it is clear that the choice to electrically self-stimulate the brain in lieu of pursuing other activities or other stimuli reflects access to some powerfully motivating neural circuitry. Thus, studies of LHA self-stimulation, interpreted cautiously, may provide insights into the modulation of CNS reward circuitry. Relevant for the present discussion are the facts that the self-stimulation activity can be enhanced by complete or partial food deprivation, and that the threshold current necessary to sustain this behavior is decreased in association with food deprivation (Carr, 1996). The question of which neurochemical or neuroendocrine substrate(s) mediate(s) this phenomenon has been pursued with some interesting results. Enhanced opioidergic activity may be the intrinsic neurochemical change that mediates the shift in current threshold since administration of naltrexone into the lateral ventricles can reverse the effect of fasting on threshold current shift within individual rats (Carr & Wolinsky, 1993).

Shizgal has localized some of these food restriction-sensitive sites to the perifornical area of the LHA (Shizgal *et al.*, 2001), a region that contains an abundance of neurons that synthesize melanin-concentrating hormone (MCH), an orexigenic neuropeptide (Broberger, DeLecea, Sutcliffe, & Hokfelt, 1998). The LHA also contains neurons that co-synthesize dynorphin and orexin, the latter of which has been implicated in both feeding and arousal (Chou *et al.*, 2001). Both of these neuronal phenotypes might be altered in their activity in response to food restriction, as they receive projections from arcuate nucleus neurons that are a critical component of the hypothalamic calorie-regulatory circuitry. Recent studies by Berthoud and colleagues demonstrate that in association with NAc-induced feeding responses, neuronal activation (enhanced Fos expression) has been observed in some LHA neurons expressing orexin but not MCH (Zheng *et al.*, 2003). Finally, we have recently localized receptors for both insulin and leptin within the LHA (Figlewicz, 2003), and have postulated that the LHA may be a direct target for these peptides after they are transported into the CNS. Collectively, this evidence suggests that the LHA is potentially responsive to both signals of nutritional status and CNS inputs reflecting reward.

Other forebrain structures are implicated in both limbic function and food reward/motivation. Studies from Baunez and colleagues have demonstrated that the subthalamic nucleus plays a critical role in modulating food-related motivation (Baunez, Amalric, & Robbins, 2002). This nucleus is a component of the basal ganglia circuitry, within a functional loop that includes the NAc and ventral pallidum. The subthalamic nucleus is connected to the prefrontal cortex through a two-synapse pathway (subthalamic nucleus–substantia nigra–cortex) as well as through the pallidal loop (Maurice, Deniau, Glowinski, & Thierry, 1999). Baunez and colleagues observed that bilateral lesion of the subthalamic nucleus results in an increased rate of eating food pellets, an increase in performance in the "progressive ratios" paradigm, and increased reinforcing properties of food-associated stimuli. These effects were situation dependent and therefore probably were not due to a nonspecific enhancement of motor responding. In fact, the investigators have observed that the use of cocaine or amphetamine as reinforcers in their behavioral paradigms results in opposite effects to those obtained with a food stimulus in rats with lesions of the subthalamic nucleus, arguing for both behavioral specificity and stimulus specificity of the lesion effects (Baunez, Cador, Dias, Robbins, & Amalric, 2002).

Specific subcomponents of the cerebral cortex are integrally involved in taste recognition, taste memory and valuation, and executive function in initiating ingestive decisions based on visual and olfactory cues. Primary taste cortex (i.e., agranular insular cortex) has efferent connections to the orbitofrontal cortex (OFC) and the other major limbic areas, including NAc, LH, and amygdala. Additionally, there are direct projections to the NTS and autonomic motor CNS structures. The OFC receives multimodal inputs including gustatory, olfactory, visual, and somatosensory information. For example, some OFC neurons respond to the oral texture of fat (Rolls, Critchley, Browning, Hernadi, & Lenard, 1999). Outputs from this region of the cortex project to the striatum, the ventral midbrain, and the sympathetic nervous system (Berthoud, 2002). In rats, electrical stimulation of the OFC initiates feeding (Bielajew & Trzcinska, 1994), and infusion of various neuropeptides or neurotransmitters into the OFC can alter respiratory quotient and energy expenditure as thermogenesis (McGregor, Menendez, & Atrens, 1990a, 1990b; Westerhaus & Loewy, 2001). OFC activation has been observed to be low in obese (relative to lean) men in response to a satiating meal (Gautier *et al.*, 2000). The basis of this effect remains to be determined and might be evaluated in animal

models. Given the "executive function" of the cerebral cortex, and the anatomical connections that would allow direct information flow to and from taste input/feeding motoric structures, some bypassing the hypothalamus, it would seem that systematic study of cortical function and activity in feeding paradigms that include choice, associative learning, and motivation components would be valuable in enlarging our understanding of links between reward circuitry and circuitry that regulates energy balance.

Consideration of the reward circuitry as a whole can offer a framework for hypotheses and experiments evaluating the interaction between "food reward" and caloric regulation. For example, it might be hypothesized that when food increases the activation of reward circuitry, for whatever reason, the efficacy of adiposity signals at the medial hypothalamus is decreased. One might ask whether there are impairments in the action of the hormonal adiposity signals, insulin or leptin, at the medial hypothalamus following activation of one of the CNS sites for reward. Additionally, or alternatively, one could test for the altered efficacy of intrinsic CNS neurotransmitters that have well-characterized effects on feeding, such as melanocortin agonists. Another conceptual framework for pursuing food reward physiology is to pose the question: how do the calorie-regulatory circuitry and the reward/motivational circuitry communicate with each other? The adage "there's always room for dessert" highlights the fact that at a point in a meal where neurohumoral satiety signals should be providing physiological input to the CNS for meal termination, the recalled experience with, and hedonic valuation of, the offered dessert frequently guides the individual to make a food choice independent of any short-term or chronic caloric need. What are the mechanistic bases for this phenomenon? It should be abundantly clear from the summary above that a large amount of detailed information already is available about the anatomical and neurochemical characteristics of both sets of "circuitry," and that both can impact directly or indirectly on the motor output of feeding. Further, excellent technical approaches are available for asking questions about activation or inactivation of putative neuronal pathways, for evaluating the functionality of specific neuropeptides or transmitters, and for more sophisticated behavioral measurements than the amount of chow that experimental animals are consuming. Recent work from Tecott, for example, takes advantage of simultaneous activity measurements in a slightly more complex environment in which feeding is only one of several behavioral options (Nonogaki, Abdallah, Goulding, Bonasera, & Tecott, 2003).

How does calorie regulatory circuitry communicate with reward circuitry? A large cast of neuropeptide and neuroendocrine players has been identified in roles contributing to the regulation of food intake and body weight (Ahima & Osei, 2001; Beck, 2000; see also Chapters 6 and 10 by Seeley and Woods in this *Handbook*). For many of these molecules, the locations of either neuronal cells of origin, or receptor-containing neuronal populations, have begun to be identified. Having this knowledge, it should be possible to test the effects of these signaling molecules in behavioral paradigms of reward and motivation. One might begin with the admittedly simple hypothesis that calorie-regulatory signals that are known to decrease feeding may decrease reward values of foods (or, conversely, orexigenic signals might enhance reward values of foods). As mentioned above, such studies as these are beginning to evaluate the role of insulin and leptin in food reward. One approach that has been taken by Shizgal and colleagues is to determine whether leptin, the orexigenic transmitter, Neuropeptide Y (NPY), or the anorexic peptide corticotropin-releasing factor (CRF), can specifically modulate food

restriction-sensitive LHA sites of self-stimulation (Fulton, Woodside, & Shizgal, 2000; Shizgal *et al.*, 2001). Likewise, Carr and colleagues have demonstrated that the food restriction-induced changes in LHA self-stimulation can be reversed by intraventricular insulin administration (Carr, Kim, & Cabeza de Vaca, 2000). These findings argue for the usefulness of this experimental approach. However, the focus of much of the work to date has been on evaluating the effects of food deprivation or food restriction. Given the reasonable perspective that, in fact, the calorie-regulatory circuitry has evolved to defend caloric intake rather than to inhibit it (Pi-Sunyer, 2003; Schwartz *et al.*, 2003), one may not be able to make inferences for overeating based on differences observed with feeding versus fasting. Not only is this issue crucial for those who study the calorie-regulatory circuitry, it also is probably an important issue for the study of mechanisms underlying the overingestion of calories. The point here is that the delineation of mechanisms underlying food reward that is responsive to food restriction may not be useful for understanding motivation that is enhanced purely in response to palatable food. Thus, while food restriction is a relatively simple experimental model that has been studied extensively, its relevance for questions of overfeeding may be limited.

How does reward circuitry talk to calorie-regulatory circuitry? Given the central anatomical placement in reward circuitry of the medial hypothalamus (and the "calorie regulatory-circuitry" as I am defining it here), the possibilities for direct and indirect inputs to the medial hypothalamus are numerous. One obvious interface is the LHA, with its abundant primary and secondary projections from the medial hypothalamus and its many links to other limbic structures and taste inputs. Thus, inputs from components of the reward circuitry such as the NAc or amygdala might synergize with orexigenic inputs from the medial hypothalamus when a palatable food is offered. These inputs (such as the NAc/LHA neuronal activation described above) might be substantial enough to result in feeding even when medial hypothalamic orexigenic input is decreased (e.g., dessert at the end of a meal).

With few exceptions to date, food reward studies have originated from the drug abuse field, and therefore minimal consideration has been given either to the physiological state of the animal or to the physiological consequences of the food reward as a *caloric* entity. It would seem that studies of the regulation of food intake and drug abuse, that have been traditionally two separate lines of investigation, might be creatively merged to take advantage of the technologies and experimental design strengths of both. Concepts that are explored by drug abuse researchers may have direct applicability to the issue of eating not based on caloric need, including the ingestion of foods simply because they are preferred. Translational work in the drug abuse field, moving from basic research toward clinical practice, is already taking advantage of these parallels, for example, by testing the use of a food substance as a substitute to compete with drug-taking (Campbell & Carroll, 2000; Foltin, 1999). Given that animals appear to have innate taste preferences (e.g., for sweet taste [Shide & Blass, 1991]), the analogy of "food addiction" may not be perfectly appropriate; for example, humans who go on diets and stop eating candy, desserts, or potato chips do not overtly go through classic signs of drug withdrawal. However, a more meaningful application of findings from the drug abuse literature might be in consideration of learned habits and the contribution of specific contextual or environmental cues to the eating of certain foods. Thus the concepts of sensitization and tolerance might be examined in a model where the rewarding substance is a food that is highly preferred by the animal. Sensitization reflects increased activation of reward circuitry and some aspects of behavior in

response to the repeated access to a stimulus in an identical environment (e.g., level pressing to administer a drug; Robinson & Berridge, 2003; Self & Nestler, 1995). Tolerance reflects the development of opponent physiological responses to repeated drug administration (both within-session and between-session), and includes the possibility that the "reward value" of the stimulus might decrease (Siegel & Ramos, 2002; Woods & Ramsay, 2000). The molecular bases for these phenomena are being actively investigated for drugs of abuse, but palatable food has not been used as the rewarding stimulus for such studies. This line of research may provide a scientific basis for asking questions such as whether repeated ingestion of an initially preferred food confers higher or lower reward value to that food? Expressed in lay terms, does one eat the whole bag of snack food because it becomes progressively more rewarding, or less rewarding?

In closing, I would like to return to the point made early in the chapter: that calorie-regulatory circuitry is intricately linked with reward circuitry. As such, it would seem to be a logical, rather than a radical, proposition that calorie-regulatory signals communicate directly with reward circuitry and *vice versa*. The concept has been put forth that calorie regulatory circuitry is part of a negative feedback loop which includes the generation of peripheral signals that reflect body adipose stores, and these signals act primarily at the medial hypothalamus to regulate the efferent components of this feedback loop, specifically food intake and energy balance (see Chapter 7 by Porte in this *Handbook*). However, the CNS anatomy suggests that reward circuitry ultimately should not be viewed as functionally separate from calorie-regulatory circuitry but as part of the loop. Inputs from reward circuitry may not just be "modulatory input" but are undoubtedly one critical component of the total CNS network that regulates food intake. The currently available data that suggest that food reward is regulated are limited but sufficiently substantive to justify future investigation.

Acknowledgments

Dianne Figlewicz Lattemann is supported by the Merit Review Program of the Department of Veterans Affairs and NIH Grant RO1-DK40963. The assistance of Drs. Ed Stricker and Steve Woods in the preparation of this chapter is gratefully acknowledged.

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Taste

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4

Neurobehavioral Analysis of Taste Function

Alan C. Spector and James C. Smith

Introduction

It is common for the sense of taste to be regarded as an expendable luxury and not as critical to survival as other sensory modalities such as vision or audition. Of course, the relative functional significance of a sensory system depends heavily on the environment in which an animal dwells. The fact that most species possess some type of contact chemoreceptor that has access to external chemical compounds (i.e., taste receptors) demonstrates the phylogenetic breadth of the sense of taste and suggests that the gustatory system provides a fundamental service in promoting fecundity. In many mammals, including rats and humans, input arising from taste receptors is one of many types of signals that contribute to the regulation of the internal milieu. The contribution of taste to regulatory processes should not be trivialized nor overstated. It is, however, a fact that in the complex chain of signals controlling the ingestion of foods and fluids, neural input arising from taste receptors has a high degree of temporal precedence. After all, the animal must first decide whether to swallow or expel and this decision occurs before the stimulus reaches the receptors of the gastrointestinal tract. Our agenda in this essay is to summarize some of the ways in which this neural input impacts upon the behavior of the laboratory rat—a commonly used animal model for feeding and drinking research. More to the point, we attempt to provide examples of the interpretive power of careful and rigorous measurement of taste-related behavior.

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Neurobiology of Food and Fluid Intake, 2nd Ed., Volume 14 of *Handbook of Behavioral Neurobiology,* edited by Edward Stricker and Stephen Woods, Kluwer Academic / Plenum Publishers, New York, 2004.

Taste represents just one of several cephalic receptor systems that provide information regarding the chemical properties of potentially ingestible substances. The olfactory and trigeminal systems also contribute relevant input. The neural integration of these various sensory signals gives rise to the perception of *flavor*. Strictly speaking, a *taste* stimulus is a relatively nonvolatile chemical compound that interacts with taste receptor cells influencing the excitation of afferent fibers destined for the brain. It would therefore be useful to start by reviewing the basic anatomy and physiology of the gustatory system.

Basic Anatomy and Physiology of the Gustatory System

In vertebrates, taste receptor cells are grouped within structures that each contain about 50 epithelial cells called taste buds (see Miller, 1995). In rats, taste buds are distributed in several fields in the oral cavity (see Bradley, 1971; Miller, Jr., 1977; Miller, Jr. 1995; Miller, Jr. & Spangler, 1982; Travers & Nicklas, 1990). The anterior tongue contains ~13% of the total taste buds, and these are innervated by the chorda tympani (CT) branch of the seventh cranial or facial nerve. A second sensory branch of the facial nerve, the greater superficial petrosal (GSP), innervates the taste buds on the palate (~16% of total), including the nasoincisor ducts, the geschmacksstreifen, and the posterior palatine field. The posterior tongue and nearby regions are innervated by the lingual-tonsilar branch of the glossopharyngeal (ninth cranial) nerve accounting for ~56% of the total. Finally, <10% of the taste buds are found in and around the laryngeal epithelium and these are innervated by the superior laryngeal branch of the vagus (tenth cranial) nerve (SLN). The small number of taste buds found on the buccal wall and sublingual organ comprise the remainder.

All of the gustatory nerves respond more or less to most taste stimuli placed on their receptor fields, but there is some variation in the degree of responsiveness to certain classes of compounds. For example, the CT responds best of all the nerves to sodium salts, whereas the glossopharyngeal nerve (GL) responds best to quinine and the GSP responds best to sucrose (e.g., Boudreau, Do, Sivakumar, Oravec, & Rodriguez, 1985; Boudreau, Hoang, Oravec, & Do, 1983; Frank, 1991; Lundy & Contreras, 1999; Nejad, 1986; Ogawa, Sato, & Yamashita, 1968; Travers & Norgren, 1991). Consistent with its suggested role of protecting the airways, in rodents the SLN responds well to water, acid, milk, and hypertonic NaCl (Andrew, 1956; Shingai, 1980; see also Dickman & Smith, 1988; Smith & Hanamori, 1991). All of these nerves project in an overlapping orotopic pattern to the rostral nucleus of the solitary tract (NST; Hamilton & Norgren, 1984). Although the relative responsiveness of these nerves suggests some degree of regional chemospecificity in the oral cavity, the outcomes of studies in which nerves were transected suggest that the functional organization of the peripheral gustatory system is not as simple as that (see Spector, 2003b).

In recent years there has been significant progress in understanding signal transduction mechanisms in taste receptor cells. Salts and acids use ion channels as their receptor elements (Heck, Mierson, & DeSimone, 1984; Herness & Gilbertson, 1999; Lindemann, Gilbertson, & Kinnamon, 1999). Sugars, artificial sweeteners, alkaloids, and amino acids bind with G-protein coupled receptors (GPCR; Margolskee, 2002). Two families of taste GPCRs have been identified. The T1R family has three subtypes, T1R1, T1R2, and T1R3, which are thought to form heterodimers that determine the

ligands with which they interact (Bachmanov *et al.*, 2001; Hoon *et al.*, 1999; Kitagawa, Kusakabe, Miura, Ninomiya, & Hino, 2001; Max *et al.*, 2001; Montmayeur, Liberles, Matsunami, & Buck, 2001; Nelson *et al.*, 2001). L-amino acids are thought to bind with the T1R1 + R3 heterodimer, and some D-amino acids as well as some, but not all, sugars and artificial sweeteners are thought to bind with the T1R2 + R3 heterodimer (Li *et al.*, 2002; Nelson *et al.*, 2001, 2002). The second family is referred to as T2R and consists of ~30 receptors that bind with ligands that are bitter tasting to humans and avoided by animals (Adler *et al.*, 2000; Chandrashekar *et al.*, 2000; Matsunami, Montmayeur, & Buck, 2000). Finally, a splice variant of the metabotropic glutamate receptor mGluR4 has also been found in taste receptor cells (Chaudhari, Landin, & Roper, 2000).

There are several excellent reviews of taste transduction pathways and we will not cover those details here (Gilbertson, Damak, & Margolskee, 2000; Herness & Gilbertson, 1999; Margolskee, 2002). However, it is worth noting that there is currently a debate regarding whether the output of a given taste receptor cell represents the first stages of a labeled line, the activity in which eventually gives rise to a single qualitative perception. On the one hand, Gilbertson, Boughter, Jr., Zhang, and Smith (2001) reported that single taste receptor cells can display changes in K^+ currents in response to compounds that are prototypically associated with different taste qualities, replicating the findings of an earlier group of researchers but with more modern techniques (Kimura & Beidler, 1961; Ozeki, M., & Sato, 1972; Sato & Beidler, 1997; Tonosaki & Funakoshi, 1984). For example, some cells displayed responses to both quinine ("bitter") and sucrose ("sweet"). Indeed, calcium imaging data also support this view (Caicedo, Kim, & Roper, 2002, see also Zhao & Herness, 2002). These observations challenge the qualitative specificity of taste receptor cells. On the other hand, recent gene deletion and rescue experiments in mice suggest that taste receptor cells that respond to bitter-tasting compounds are independent of those that respond to sweet-tasting compounds (Zhang *et al.*, 2003). In that experiment, deletion of the PLCβ2 gene (gene for a phospholipase C enzyme that is expressed in taste receptor cells important in signal transduction) rendered mice completely unresponsive to several bitter- and sweet-tasting compounds as assessed both electrophysiologically from nerve recordings and behaviorally with brief-access taste tests and two-bottle intake tests. In a manipulation that highlights the analytical power of molecular procedures, the PLCβ2 gene was reinserted only into taste receptor cells that expressed T2R receptors (i.e., those that bind with bitter-tasting ligands). Responsiveness to bitter-tasting compounds was completely restored in these genetically "rescued" mice, but responsiveness to sweet-tasting compounds was absent. These findings suggest that compounds that taste sweet stimulate a different population of taste receptor cells than compounds that taste bitter. The latter hypothesis is buttressed by the fact that very few afferent fibers in the peripheral gustatory system are appreciably responsive to both sucrose and quinine (although some can be found that respond to both compounds; Frank, 1991; Frank, Contreras, & Hettinger, 1983; Lundy & Contreras, 1999; Ogawa *et al.*, 1968). There are, however, some broadly tuned fibers that respond to salts, acids, and alkaloids, and their existence suggests that the receptor elements and transduction pathways for these particular classes of compounds coexist in single taste receptor cells (Frank *et al.*, 1983; Lundy & Contreras, 1999; Ogawa *et al.*, 1968). Alternatively, input from cells that have a specific transduction pathway may converge given that a single taste afferent fiber synapses with several taste receptor cells not necessarily in the same taste bud (see Miller, Jr. 1995). One caveat to consider is

that the presence of labeled-line neural representations of certain stimuli in the periphery does not necessitate that a similar coding organization exists in central gustatory circuits (see Smith & St. John, 1999).

The second order taste-responsive neurons of the NST project to the parabrachial nucleus (PBN; Norgren, 1995; Travers, 1993). Taste-responsive neurons in the PBN are found primarily within the "waist" area which includes the central medial and ventral medial subdivisions as well as the cellular bands traversing the brachium conjunctivum (Halsell & Travers, 1997; Norgren & Pfaffmann, 1975). The external medial and external lateral subdivisions, which cap the lateral margins of the brachium, also contain taste responsive neurons (Halsell & Travers, 1997). The PBN represents an interesting hub in the ascending gustatory system. It is believed that this area represents an obligate relay for all taste input destined for the forebrain. The PBN projections to the forebrain form two basic pathways. One pathway represents the classic thalamocortical route of the lemniscal system. The parvicellular subdivision of ventroposterior nucleus of the thalamus is the target and these neurons, in turn, project to agranular insular cortex (e.g., Kosar, Grill, & Norgren, 1986a, 1986b). It has been suspected for some time that this pathway is important in sensory-discriminative function (see below). The other pathway projects to ventral forebrain structures known to be important in autonomic regulation, feeding and drinking, and reward and aversion. These structures include the lateral hypothalamus, the central nucleus of the amygdala, the bed nucleus of the stria terminalis, and the substantia innominata. A third pathway arising from the waist area projects to premotor neurons of the medullary reticular formation that in turn project to the oromotor nuclei (Karimnamazi & Travers, 1998).

It has been a long-standing goal of researchers studying the central gustatory system to unravel the mysteries of its functional organization. Although some features of the basic anatomy described above provide clues as to what that organization might be, the relationship between structure and function has been somewhat elusive. On the other hand, progress has certainly been made, especially in terms of the identification of which portions of the gustatory system are necessary or sufficient for particular taste functions to be maintained. Accordingly, it is worthwhile discussing what those functions are.

The Domains of Taste Function

There is substantial evidence suggesting that taste signals contribute to at least three functions (Norgren, 1985; Pfaffmann, Frank, & Norgren, 1979; Scott & Mark, 1986; Spector, 2000). The first is stimulus identification. This function involves the sensory-discriminative processes that allow animals to qualitatively distinguish among taste compounds and identify them. The second is ingestive motivation. This function involves processes that promote or discourage the ingestion of a taste stimulus. Finally, taste signals are capable of triggering physiological reflexes (e.g., salivation) that aid in the digestion and assimilation of ingested foods and fluids. A given taste stimulus can either innately engage these three functions or do so through learning. The interpretation of the effectiveness of an anatomical, physiological, pharmacological, or genetic manipulation to influence a taste-guided behavior should be considered with this functional framework in mind. For example, if the injection of a drug (e.g., a dopamine antagonist) affects an animal's ingestion of sucrose solutions, dismissing direct oromotor impairments, this effect

could potentially occur because the drug interfered with sensory-discriminative processes, motivational processes, or both. The dissociation of these possibilities requires a combination of methods that are designed to focus on the respective functional domains.

Sensory-Discriminative Function

The sensory-discriminative domain of taste function is most commonly studied by training the animal to selectively respond to a specific taste stimulus. This can be accomplished through operant or classical conditioning procedures or in some cases measurement of innate responses to a specific stimulus. In the former, an animal is trained to perform a specific response (e.g., press a lever) upon presentation of a particular taste stimulus. For example, St. John and Spector (1998) trained thirsty rats to sample from a centered drinking spout in a specially designed gustometer similar to the one depicted in Figure 1. If the rats pressed one lever (e.g., to the right of the sample spout) when the fluid presented was quinine hydrochloride, they received brief access to water. When the test fluid was KCl, the rats were required to press the other lever (e.g., to the left) to obtain water. The lever assignments were counterbalanced across animals and incorrect responses were punished with a time-out, further delaying the opportunity to receive water. Concentration was varied so that intensity could not serve as a reliable cue. Interestingly, performance in the discrimination task remained entirely normal after transection of the GL, which innervates close to 60% of the taste buds in the rat and contains fibers that respond differentially to these two compounds (Frank, 1991; Miller, Jr., 1977). In contrast, performance was significantly disrupted by transection of the CT, which innervates only ~13% of the taste buds and has not been reported to contain fibers that differentially respond to quinine and KCl (Frank *et al.*, 1983; Lundy & Contreras, 1999). When the CT was transected in combination with the GSP nerve depriving the brain of the combined gustatory input from the seventh cranial nerve, performance dropped to close to chance levels (Figure 2). These and other observations suggest that GL transection is without effect on a variety of tasks that require the rat to identify a taste stimulus, whereas transection of one or both of the gustatory branches of the facial nerve always leads to significant impairments. These findings led St. John and Spector (1998) to hypothesize that the taste input provided by the facial nerve is necessary and likely sufficient for normal sensory-discriminative function to be maintained. In this type of procedure, the taste stimulus serves as a signal for reinforcement irrespective of its motivational characteristics. Rats generally avoid quinine and KCl, yet they sampled these stimuli and performed the discrimination with accuracy when their taste nerves were intact (Contreras & Studley, 1994; St. John, Garcea, & Spector, 1994). Because of their features, these types of procedures can be used to examine sensory-discriminative taste function relatively uncontaminated from affect.

One caveat to consider in the interpretation of the experiment just described (St. John & Spector, 1998) is the possibility that the effects of CT transection on discrimination performance were attributable to the partial denervation of the sublingual and submaxillary salivary glands because the parasympathetic supply of these glands is provided in large part by the CT. By making detailed measurements of feeding (more on this below), Smith *et al.* (1988) found that CT transection emulated the effect of extirpation of the sublingual and submaxillary glands in that meals of powdered chow were much less efficient (normal meal sizes but longer

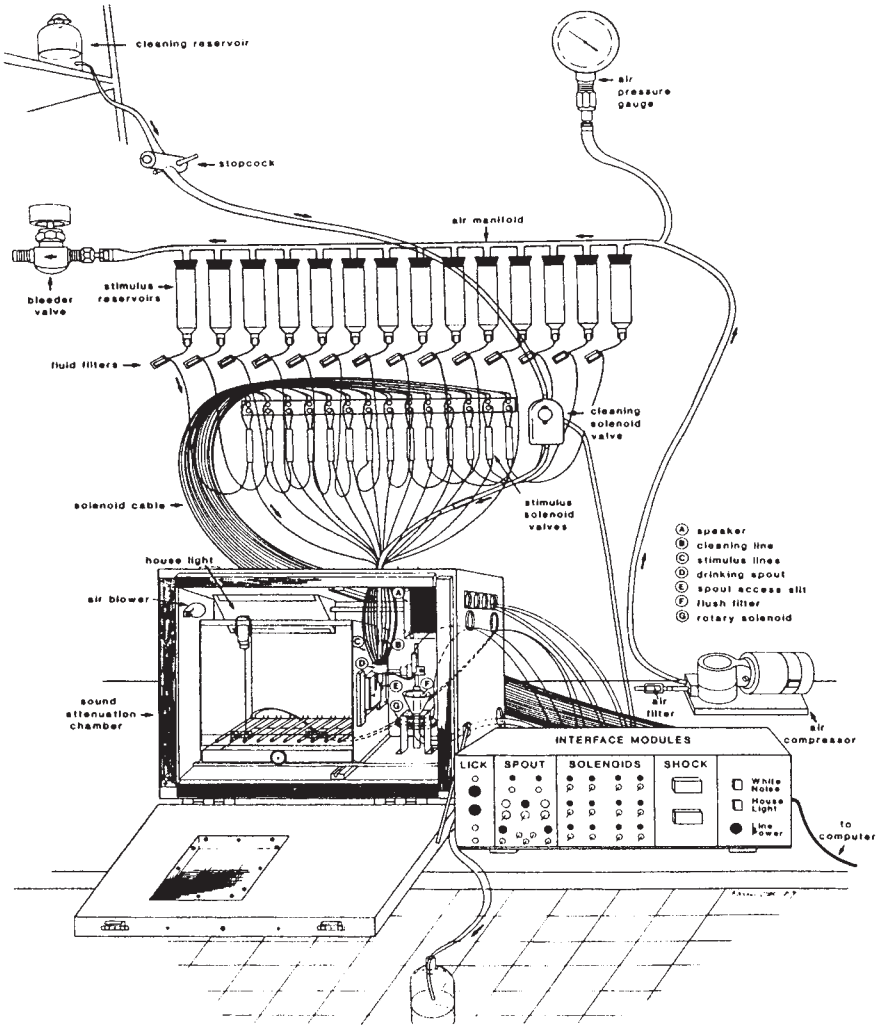

Figure 1. A prototype of a gustometer used in several of the experiments described in this chapter. Newer versions have two levers installed on either side of the stimulus access slot centered in the sidewall of the inner chamber. The manipulanda and stimulus delivery are controlled and monitored through a custom-designed electronic interface, which, in turn, is controlled by computer programs. This apparatus allows for the delivery of small volumes of up to 12 stimuli that can be intermittently delivered in brief trials throughout a session during which immediate responses can be measured. As discussed in the text, these features increase the confidence that the animal's behavior is guided by orosensory cues. (Reprinted from Spector, Andrews-Labenski, and Letterio [1990] with permission.)

meal durations). To address this concern, St. John and Spector (1998) included a group of rats that had their sublingual and submaxillary glands surgically removed, and found that the discrimination performance of these animals was unaffected by the manipulation in this particular task.

Classical conditioning techniques (sometimes referred to as Pavlovian or respondent conditioning) also can be used to train animals to respond in specific ways to taste stimuli, thereby allowing for sensory-discriminative function to be

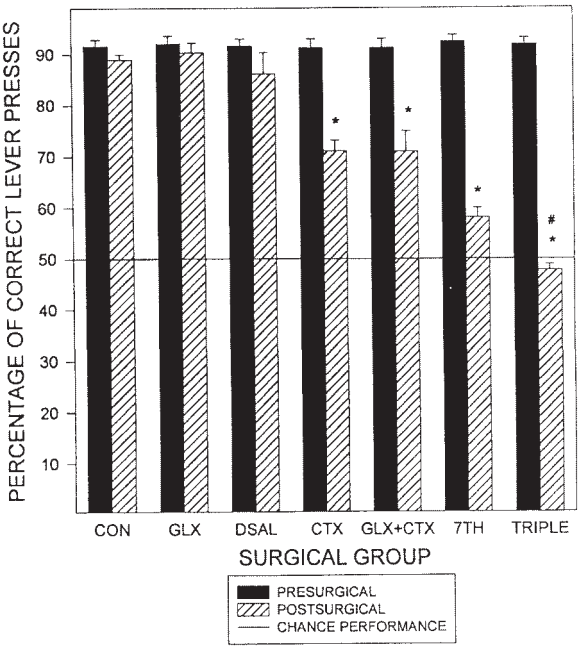

Figure 2. Mean (± SE) percentage correct on all trials for rats trained to press one lever in response to quinine hydrochloride and another lever in response to KCl. Concentration was varied to render intensity an irrelevant cue. Filled bars represent performance before surgery and hatched bars represent performance after surgery. Unexpectedly, GLX had no effect on the discrimination whereas transection of the gustatory branches of the facial nerve dropped performance to near chance levels. The combined transection of the CT, GSP, and GL resulted in unequivocal chance performance indicating that the input from the SLN or the olfactory and trigeminal systems was not sufficient to maintain any degree of competence in the task. CT: chorda tympani nerve; GL: glossopharyngeal nerve; GSP: greater superficial petrosal nerve; CON: sham-operated controls; GLX: bilateral transection of the GL; CTX: bilateral transection of the CT; GLX + CTX: combined bilateral transection of the GL and CT; 7TH: combined bilateral transection of the CT and GSP; TRIPLE: combined transection of the CT, GL, and GSP. Asterisks indicate significant difference before versus after surgery ($p \leq .05$); Pound sign indicates failure to find a significant difference from chance. (Reprinted from St. John and Spector (1998) with permission.)

assessed. The most widely used procedure of this type is the conditioned taste aversion (CTA) paradigm. When animals ingest a novel tasting substance (conditioned stimulus, CS) followed by visceral malaise (unconditioned stimulus, US), they subsequently avoid ingesting that specific stimulus on future occasions (unconditioned response, UR; e.g., Garcia, Kimeldorf, & Koelling, 1955). Once a CTA has been established to a particular CS, the degree of avoidance expressed to other taste compounds relative to unconditioned control animals is taken as a measure of qualitative similarity between the CS and the test stimuli (TSs; Nachman, 1963; Nowlis, Frank, & Pfaffmann, 1980; Smith, Travers, & VanBuskirk, 1979; Tapper & Halpern, 1968). Because such generalization can occur along both intensity and quality dimensions, the concentrations used as CSs and TSs must be considered carefully (Spector & Grill, 1988). Moreover, if intake tests are used in which single TSs are tested on separate days, the possibility that the CTA is extinguishing must be watched closely. The latter concern can be overcome by presenting all of the TSs in very-brief-access trials in a gustometer and measuring immediate lick avoidance

in thirsty rats in a single session. In any event, the CTA paradigm is a relatively efficient way to behaviorally map taste stimulus similarity, caveats notwithstanding.

Indeed the CTA paradigm has been applied to the evolving issue of fat taste. Traditionally, fats were not thought to be capable of stimulating taste receptor cells. In the last several years, however, Gilbertson and his colleagues (Gilbertson, 1998; Gilbertson, Fontenot, Liu, Zhang, & Monroe, 1997) have been able to measure changes in K^+ currents of a subset of taste receptor cells in response to *cis*-polyunsaturated fatty acids, such as linoleic acid, in very weak concentrations (as low as 10 μM) delivered to taste bud cells. Although free fatty acids are relatively rare in foods, it is postulated that triacylglycerides can be cleaved by lingual lipase. Smith, Fisher, Maleszewski, and McClain (2000) were able to show that rats could form CTAs to corn-oil, which is composed of about 60% linoleic acid. Smith *et al.* (2000) tested whether corn-oil aversions would generalize to 22 μM linolelic acid, which does not have the viscosity and textural components associated with corn-oil. They found very robust cross-generalization. In further work, Smith and his colleagues have found that the conditioning of an aversion to linoleic acid in rats does not require intact olfactory bulbs, but is dependent on an intact CT (Pittman *et al.*, 2002, & unpublished observations). Thus, the CTA paradigm has been useful in illuminating some of the chemosensory parameters of fat perception.

As a procedural postscript, it is worth noting that the linoleic acid must be dissolved in ethanol, and when added to water the final ethanol concentration is 0.1%. Smith *et al.* (2000) included the ethanol solvent as the comparison solution in the two-bottle tests of the CTA. This point is important because rats can detect the presence of this very weak ethanol solution as indicated by the fact that an aversion can easily be conditioned to it (Smith, Denblecker, & Ferrence, 2003). Therefore, one should always take into account the solvent when conducting taste experiments no matter how insignificant its concentration might appear.

Interestingly, CTAs engage neural processes involved with ingestive motivation in addition to those involved with sensory-discriminative function. The affective valence of the CS changes from positive to negative as a result of conditioning. Presumably once the stimulus is identified as CS-like (sensory-discriminative function), neural circuits that promote ingestion are inhibited (alternatively, neural circuits that discourage ingestion may be activated). The forebrain is necessary for this process. Chronic supracollicular decerebrate rats, which have the forebrain neurally isolated from the rest of the nervous system, do not express a presurgically conditioned taste aversion nor can they acquire one postsurgically as assessed by oromotor consummatory behavior (Grill & Norgren, 1978a). Moreover, an intact PBN is necessary for a CTA to be acquired, but not for retention or expression of a presurgically conditioned aversion (DiLorenzo, 1988; Flynn, Grill, Schulkin, & Norgren, 1991; Grigson, Shimura, & Norgren, 1997; Reilly, Grigson, & Norgren, 1993; Spector, Norgren, & Grill, 1992). The fact that rats with PBN lesions can form a conditioned aversion to capsaicin solution, an oral trigeminal stimulus, demonstrates that these animals are capable of learning, of processing aversive visceral signals, and of expressing stimulus-induced suppression of ingestive behavior (Grigson, Reilly, Shimura, & Norgren, 1998). These data collectively suggest that the PBN or the forebrain nuclei to which it is anatomically connected are critical in the integration of taste and visceral signals required for CTA formation.

Sensory-discriminative taste function also can be assessed through the measurement of innate responses to specific taste stimuli. The prototypical example is the phenomenon of sodium appetite in herbivores and omnivores. If rats are fed

a sodium-deficient diet for a prolonged period or treated with a natriuretic drug promoting rapid sodium loss, they will display an enhanced ingestive response to sodium-containing salt solutions even at concentrations that are normally avoided (e.g., Denton, 1982; Richter, 1956; Schulkin, 1991; Stricker & Verbalis, 1990). The response is observed immediately upon stimulus presentation and is specific for sodium (lithium is also effective; Wolf, 1969). In brief-access taste tests in which a variety of salts are offered, the licking of those solutions containing the sodium cation is specifically potentiated (Breslin, Spector, & Grill, 1993, 1995; Geran & Spector, 2001; Markison, St. John, & Spector, 1994). Like a CTA, this sodium appetite engenders both sensory-discriminative function and ingestive motivation. The former is engaged because of the stimulus specificity of the behavior, and the latter is involved because of the potentiation of both appetitive and consummatory responses to sodium salts.

The specificity of the innate responses of rats to sodium-containing salt solutions under conditions of sodium deficiency has been exploited to help relate the biophysics of suspected salt taste transduction pathways to perception. Salts are transduced through at least two pathways in taste receptor cells (see Heck *et al.*, 1984; Lindemann *et al.*, 1999; Simon, Holland, Benos, & Zampighi, 1993). One pathway is suppressed by oral treatment with the epithelial sodium channel (ENaC) blocker amiloride (e.g., Brand, Teeter, & Silver, 1985; Doolin & Gilbertson, 1996; Hettinger & Frank, 1990; Ye, Heck, & DeSimone, 1993). The other(s) is unaffected by amiloride (e.g., Elliott & Simon, 1990; Simon, 1992; Ye *et al.*, 1993). Sodium salts with large organic anions, such as sodium acetate and sodium gluconate, appear to only stimulate the ENaC pathway as indicated by the fact that treatment with amiloride virtually eliminates the responses of the CT to these stimuli (Elliott & Simon, 1990; Formaker & Hill, 1988; Simon, 1992; Ye *et al.*, 1993). Amiloride treatment only partially suppresses CT responses to NaCl indicating that in addition to the passage of sodium through apically located ENaCs in taste receptor cells, NaCl stimulates amiloride-insensitive transduction pathways as well. The stimulus responsiveness of single fibers in the CT that are narrowly tuned to respond to sodium chloride relative to other nonsodium chloride salts is suppressed by lingual application of amiloride (Hettinger & Frank, 1990; Lundy, Jr. & Contreras, 1999; Ninomiya & Funakoshi, 1988). In contrast, CT fibers that are broadly tuned to respond to sodium and nonsodium chloride salts are unaffected by amiloride. Apparently, the presence of ENaCs that show relatively selective permeability for sodium in the apical membranes of taste receptor cells, coupled with the pattern of connectivity between these cells and CT fibers, suggests that the peripheral gustatory system has a labeled line, the activity in which provides a reliable signal that the taste stimulus contains sodium. If true, then sodium gluconate, which purportedly stimulates only the ENaC-related pathway, should be sufficient to generate potentiated licking responses in sodium-depleted rats. Moreover, treatment with amiloride should entirely eliminate that potentiation, as has been shown to occur when NaCl is the stimulus (Bernstein & Hennessy, 1987; Brot, Watson, & Bernstein, 2000; McCutcheon, 1991). Geran and Spector (2001) tested this hypothesis and confirmed the predicted outcome demonstrating that the amiloride-sensitive transduction pathway and its associated afferent fibers are both *necessary* and *sufficient* for the taste-guided recognition of sodium salts.

The work just described represents a very satisfying correspondence between the neurobiology of the peripheral gustatory system and the psychophysical characteristics of the animal. However, sensory-discriminative taste function as assessed

behaviorally does not always conform to predictions based on molecular or biophysical data alone. For example, as noted above, bitter-tasting ligands bind with GPCRs from the T2R family (Adler *et al.*, 2000; Chandrashekar *et al.*, 2000; Matsunami *et al.*, 2000). Although each of the 30 or so T2R receptors is thought to be relatively selective for a given stimulus, members of this family appear to be co-expressed in subsets of taste receptor cells. Thus, if a taste receptor has one type of T2R it is highly likely that it will have others. From these findings, researchers predicted that rats and humans would be unable to qualitatively discriminate among bitter-tasting compounds (Chandrashekar *et al.*, 2000). However, a subsequent study of intracellular calcium responses to an array of commonly used bitter-tasting compounds measured *in situ* in rat taste bud slices suggested that taste receptor cells were more narrowly tuned than was suggested by the T2R expression pattern (Caicedo & Roper, 2001). Out of 374 taste bud cells tested from the foliate papillae, 69 responded to at least one of the five bitter-tasting compounds tested. Of these, 93% responded to only one or two of the ligands. Accordingly, these

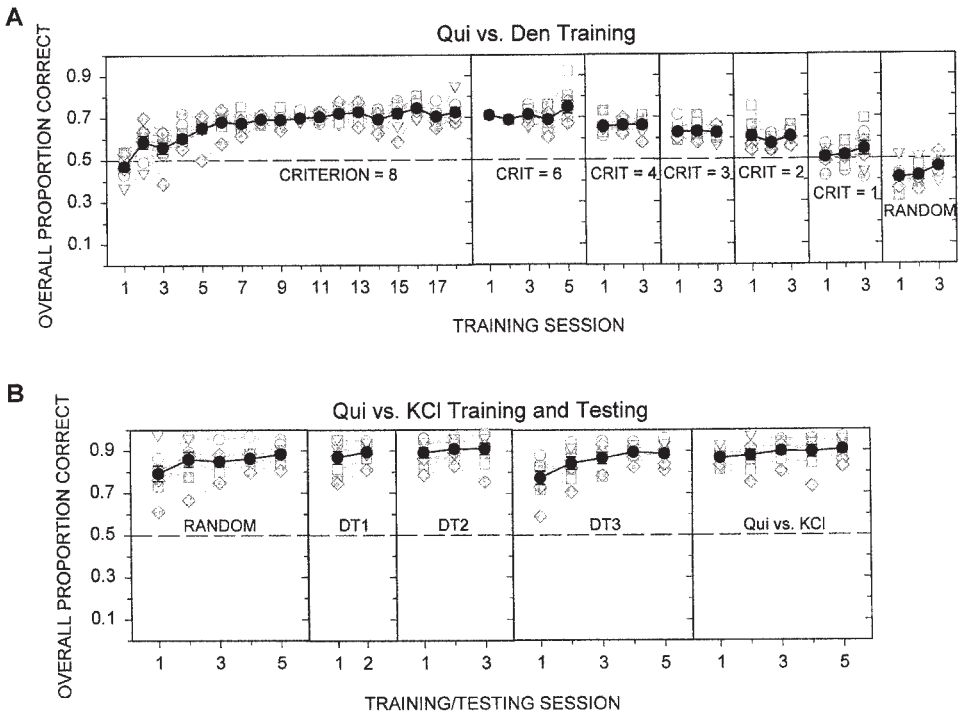

Figure 3. Mean (± SE; dark filled circles and error bars) percentage correct on all trials for rats trained to press one lever in response to quinine hydrochloride and another lever in response to (A) denatonium benzoate or (B) KCl. A mid-range concentration for each stimulus was chosen for training sessions. (A) Rats could not be trained to discriminate between quinine and denatonium. The apparent learning curve early in training was due to the use of a correction procedure in which the stimulus was not switched until a criterion number of correct presses (CRIT) were made. Once this correction procedure was eliminated (RANDOM), performance dropped to chance levels (0.5). (B) These same rats were later trained to discriminate quinine and KCl. The data presented are from the late phases of training and the final testing phase. It is clear that the rats performed well on this task demonstrating that they could competently perform another taste discrimination involving quinine and a non-alkaloid stimulus. Lightly shaded symbols represent the performance of individual animals. (Reprinted from Spector and Kopka (2002) with permission.)

researchers predicted that humans and animals could discriminate among bitter-tasting compounds. In the taste bud calcium-imaging work, quinine hydrochloride and denatonium benzoate appeared to be highly discriminable as indicated by the fact that only 2 of the 29 cells that were responsive to either compound were responsive to both. Spector and Kopka (2002), however, were unable to successfully train rats to discriminate quinine from denatonium (Figures 3 and 4). These same rats were able to learn to discriminate quinine from KCl, showing that they were capable of performing competently in the task. These data strongly suggest that quinine and denatonium give rise to a unitary qualitative taste sensation leaving open the possibility that other compounds fall into this perceptual class.

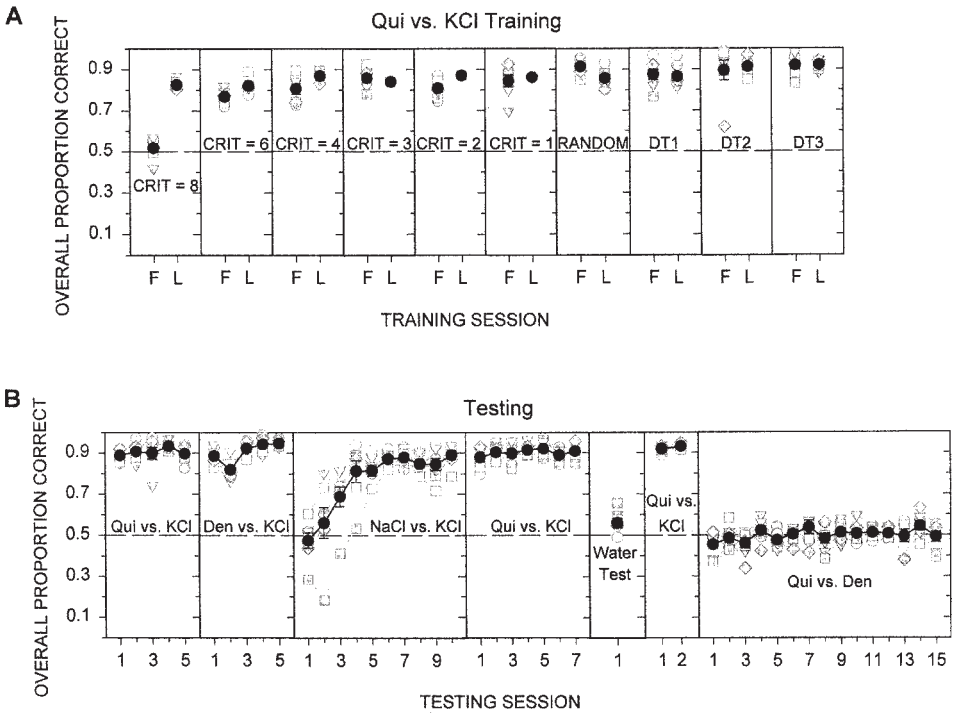

Figure 4. Mean (± SE; dark filled circles and error bars) percentage correct on all trials for naive rats trained to press one lever in response to quinine hydrochloride and another lever in response to KCl (A). A mid-range concentration for each stimulus was chosen for training sessions. (A) Rats without prior experience could be trained to discriminate between quinine and KCl. Only the first (F) and last (L) days of each training phase are shown. (B) The first panel displays the final test performance of these rats on the quinine versus KCl task. The next panel shows that the performance of the rats was maintained at very high levels when denatonium was substituted for quinine, even on the first session of the switch. This finding suggests a very high degree of similarity between the two stimuli. When denatonium was switched with NaCl, however, performance dropped to chance and then progressively improved over sessions. This finding demonstrates that simply substituting any stimulus for quinine will not necessarily result in undisrupted performance on the first few sessions. When water was placed in all of the stimulus reservoirs and some were assigned as "quinine" and others as "KCl," performance was severely disrupted demonstrating that the animals needed the chemical cue in the solution to perform competently. Finally these rats were tested on a quinine versus denatonium discrimination task. Over 15 sessions, these animals displayed absolutely no evidence of discriminating between these 2 compounds suggesting that these alkaloid stimuli were giving rise to a unitary qualitative perception. Lightly shaded symbols represent the performance of individual animals. (Reprinted from Spector and Kopka (2002) with permission.)

The more important point of this story with regard to the current discussion, however, is that predictions based on results from reduced levels of analysis may not be validated by more molar tests of function. In the above example, the molecular biology predicted something about the response properties of taste receptor cells that was not confirmed by calcium imaging measures. The latter findings, in turn, predicted that rats could discriminate between two prototypical bitter-tasting ligands and this was not confirmed by a behavioral test. The failure of rats to behaviorally distinguish these compounds is most likely due to the potentially discriminable signals originating from taste receptor cells converging somewhere downstream of information flow. Nevertheless, the point is that it is the behavior (or autonomic physiology) of the animal that is the ultimate output of the nervous system and that therefore must guide the interpretation of neurobiological findings in regard to taste function.

Ingestive Motivation

Ingestive motivation refers to those taste processes that promote or discourage eating and drinking. It is commonly viewed as the hedonic or affective characteristics of a taste stimulus. Behavior associated with this functional domain can be analytically subdivided into two classes (see Craig, 1918). The first is the appetitive class referring to the behavior that brings the animal to the stimulus. The second class is consummatory behavior representing the final act(s) of an appetitive sequence. Consummatory behavior is triggered by the contact of the stimulus with cephalic receptors, most notably, in the case of ingestion, those of gustatory origin (see Grill & Berridge, 1985; Grill, Spector, Schwartz, Kaplan, & Flynn, 1987).

Purely appetitive responses are best measured when the stimulus is not in contact with taste receptors. Accordingly, a rat performing an operant behavior, such as lever pressing, to gain access to a taste reinforcer, such as sucrose, would represent an example of appetitive behavior unadulterated by consummatory responses (e.g., Guttman, 1954). In this case, the rate and pattern at which the operant was performed could be analyzed as the concentration of sucrose changed or the animal's physiological state was altered. In the context of free feeding, the approach to the food trough or drinking bottle can be considered appetitive (see Sederholm, Ammar, & Sodersten, 2002). Likewise, avoidance of a food or fluid source falls into the same category. In the case of research involving feeding by rats, the stimulus often is presented in liquid form. Thus, each lick could potentially be considered an appetitive or consummatory response because it fits the definition of both. Rats, however, generally lick in bursts that appear to be governed by a central pattern generator (Corbit & Luschei, 1969; Travers, DiNardo, & Karimnamazi, 1997; Wiesenfeld, Halpern, & Tapper, 1977). It is not unreasonable to assume that the number of bursts within a meal is a measure of the output of appetitive processes, whereas the burst size (or duration) is a measure of consummatory responsiveness (see Hulse, 1966, 1967). Consequently, the size of a meal, and therefore total daily intake, is the product of both appetitive and consummatory processes.

The quantification of licking patterns within a circumscribed episode of ingestion (i.e., meal, short-term intake test) is referred to as microstructural analysis (see Davis, 1989). Although the pause length that constitutes the termination of a burst of licking is subject to different definitions, it is clear that the taste properties of a stimulus influence licking microstructure (see Allison & Castellan, Jr., 1970; Slater, 1982; Spector, Klumpp, & Kaplan, 1998). For example, as the concentration of a sugar

solution increases in a short-term drinking test (~30 min), the rate of drinking in the initial minute of exposure (i.e., total licks in the first minute) increases monotonically. Similarly, the average burst size (or duration) from the session increases monotonically as a function of concentration (e.g., Davis & Perez, 1993; Spector *et al.*, 1998). In contrast, burst number shows an inverted-U-shaped function, the rising limb of which likely reflects the motivational properties of the taste solution; the descending limb likely reflects the growing competition between the inhibitory strength of postingestive signals and the affective value of the sugar as concentration is raised. It would appear, then, that postingestive inhibition has a much greater effect on appetitive behavior, as reflected in burst number, than on consummatory behavior, as reflected in burst size. One caveat regarding such a conclusion is that the two measures are not completely independent when taken during a fixed time period. Obviously, if an animal had one long burst, it would decrease the opportunity for other bursts to occur. However, when long pauses occur there is potential for additional bursts to be initiated. It is noteworthy that burst size during meals of sucrose does not increase when postingestive inhibition is precluded by allowing the ingested contents to drain out of an open gastric cannula (i.e., sham drinking), but burst number markedly increases (Davis & Smith, 1992; Davis, Smith, Singh, & McCann, 2001).

As might be expected, the microstructure of drinking chemical solutions can be affected by manipulations of the gustatory system. For example, water-restricted rats given access to water for daily 45-min sessions initiated about 35 bursts (defined as runs of licks with pauses ≤1 s) of approximately 90 licks each. When these rats were presented with a novel 0.2 mM solution of quinine hydrochloride, burst size dropped precipitously to about 10 licks and burst number strikingly increased to close to 150. In other words, it appeared as though the aversive taste of quinine was suppressing the expression of the consummatory behavior, but the physiological state caused by the water restriction schedule (i.e., thirst), coupled with delayed satiation because of the low burst size, was potentiating appetitive behavior. This observation provides further evidence that consummatory and appetitive behavior can operate simultaneously in different directions. Rats that had the CT and GL transected in combination displayed little change in the number and the size of bursts as compared with water, demonstrating that the altered microstructure observed with quinine was dependent on input from those gustatory nerves (Spector & St. John, 1998).

The difference between a microstructural analysis and a meal pattern analysis is simply one of time scale. Daily intake is a direct function of the number and size of meals distributed over a 24-hr period and which can be measured in a specially equipped cage (Figure 5). In turn, meal intake is a direct function of the number and size of licking bursts (again assuming a liquid diet). In this sense, the number of meals represents the action of feeding-related variables, including taste, on appetitive processes. As noted above, the meal size is a reflection of both appetitive and consummatory processes. Just as was the case for the microstructural features of feeding and drinking, taste also influences the pattern and macrostructural features of meals. For example, Spector and Smith (1984) documented orderly changes in the size, rate, number, and 22-hr distribution of sucrose drinking bouts as a function of concentration of the sucrose solution. Bout size and bout number initially increased as concentration was raised but then decreased at higher concentrations. The average rate of licking within drinking bouts, however, increased as a direct monotonic function of sucrose concentration. This latter finding suggests that the descending limb of the concentration–response function describing bout number and bout size was influenced by the postingestive factors associated

with the caloric density of the high sucrose concentrations. It also is noteworthy that as the sucrose concentration increased, drinking bouts became distributed more uniformly throughout the 22-hr observation period. Apparently, the combination of the postingestive inhibition related to the caloric and colligative properties of the more highly concentrated sucrose solutions coupled with the potent affect originating from gustatory input was sufficient to substantially disrupt the normal nocturnal bias of ingestive behavior.

Another example of the power of detailed analyses of ingestive behavior is the remarkable ingestion of glucose and saccharin solutions. Rats given a mixture of 3% glucose and 0.125% saccharin will ingest amounts sometimes exceeding their body weight in a 24-hr period (Valenstein, Cox, & Kakolewski, 1967). Interestingly, when one bottle of 6% glucose and one bottle of 0.25% saccharin are presented simultaneously, rats also will drink enormous amounts. By monitoring licking behavior over a 24-hr period, Smith, Williams, and Jue (1976) were able to demonstrate that during drinking episodes rats were rapidly switching between licking from the glucose and saccharin bottles and thus were essentially mixing a "cocktail." It is worth noting that this kind of behavioral insight would have been lost if these researchers had simply measured total intake. The fact that the animals repeatedly switched from one bottle to the other within the span of a drinking bout strongly implies that taste was driving the excessive intake of the solutions.

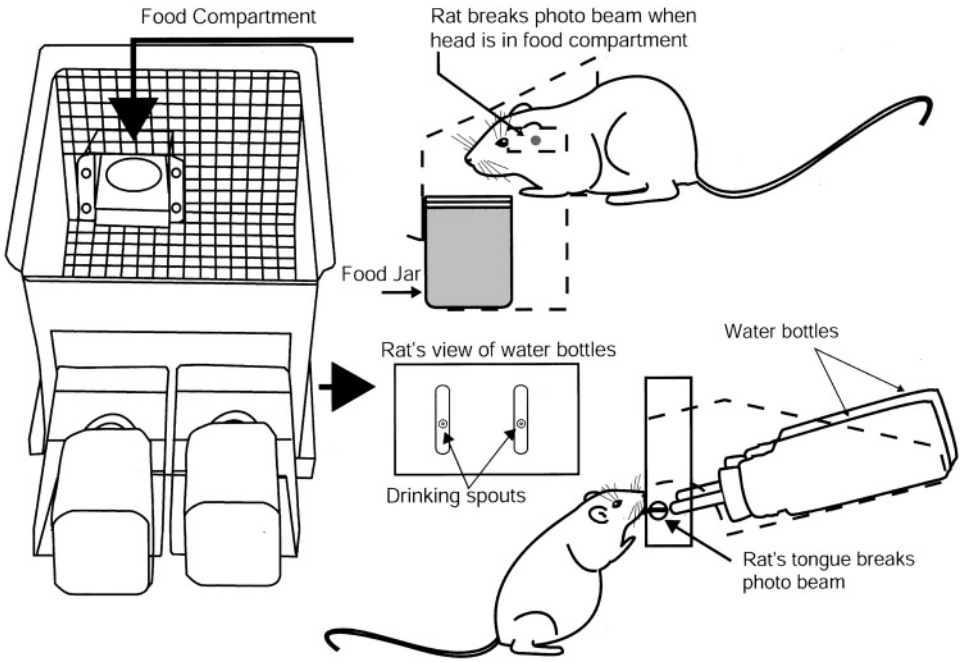

Figure 5. A specially equipped cage used by the laboratory of J. C. Smith to monitor feeding and drinking over a 24-hr period. A food compartment is attached to the front of a cage. When the rat enters this compartment to eat the powdered food, the animal's head breaks an infrared beam, allowing for measurement of food bout duration. In some variations of this apparatus, a load beam is placed under the food jar to record amount of food consumed during a bout. Smith has found a very high correlation between bout duration and amount consumed. On the rear of the cage, two bottles are mounted. The sipper tubes are recessed so either a break in the beam or a contact circuit records licks during drinking bouts. See Smith (2000) for more detail.

Purely consummatory responses can be measured by infusing taste stimuli directly through a chronically implanted intraoral cannula. Because the rate and pattern of stimulus delivery is under the control of the experimenter and the responses are triggered by stimulus contact with the receptors, the observed behavior represents consummatory action. Rats, as well as other mammals, display two categories of stereotypical oromotor and somatic responses to taste compounds. Collectively these responses are referred to as *taste reactivity* (e.g., Grill & Berridge, 1985; Grill & Norgren, 1978b; Grill *et al.*, 1987; Ossenkopp & Eckel, 1995; Parker, 1991). The *ingestive* category of responses includes tongue protrusions, lateral tongue protrusions, and mouth movements. These responses are generally elicited in a concentration-dependent manner by taste stimuli that normally are preferred such as sucrose. The *aversive* category of responses includes gapes, chin rubs, forelimb flails, and head shakes. These responses generally are accompanied by expulsion of the fluid and are elicited in a concentration-dependent manner by taste stimuli that normally are avoided such as quinine.

Ingestive stimuli can be conditioned to elicit aversive responses. When sucrose is paired with a systemic injection of LiCl (a salt which causes visceral malaise), as noted above, animals will form a CTA to the sucrose. The expression of this CTA can be seen not only in decreased sucrose ingestion in subsequent intake tests, but also in marked alterations in the sucrose-elicited profile of taste reactivity, changing from ingestive to aversive. Interestingly, when conditioning takes place over several trials, the first ingestive response type to decrease most markedly is the tongue protrusion followed by a decrease in mouth movements (Breslin, Spector, & Grill, 1992). Likewise, gapes are the first aversive response to appear after the first trial followed by an increase in chin rubs on subsequent trials. In other words, gapes depicted less aversion than chin rubs. These findings suggest an ordering of the individual taste reactivity responses along what might be viewed as an affective dimension with tongue protrusions representing the extreme of the positive pole and chin rubs near the extreme of the negative pole (forelimb flails and head shakes were relatively infrequent but there were hints that they might represent greater degree of rejection than chin rubs). Interestingly, quinine and HCl can be conditioned to be less aversive by immediately following their intraoral delivery with a sucrose infusion. When this is done over several trials, the number of aversive oromotor responses decreases and the number of ingestive responses increases to the CS taste paired with the sucrose (Breslin, Davidson, & Grill, 1990).

Appetitive and consummatory behavior can be dissociated neurally. The best example of this dissociation comes from the behavior of chronic supracollicular decerebrate rats. These rats do not spontaneously eat and drink and must be fed by gavage. Thus, they do not demonstrate appetitive behavior associated with stimuli relevant to energy and fluid homeostasis. Nevertheless, these rats do display concentration-dependent taste reactivity to various chemical compounds (Flynn & Grill, 1988; Grill & Norgren, 1978c). These findings show that the caudal brainstem contains sufficient circuitry to support oromotor consummatory and rejection responses, but the forebrain is necessary for the expression of appetitive behavior. Interestingly, decerebrate rats do not display changes in their taste reactivity following taste aversion conditioning, nor do they express a presurgically conditioned aversion (Grill & Norgen, 1978a). These findings imply that conditioned changes in oromotor responses to tastes depend on descending input from forebrain.

Although transection of the GL, a gustatory nerve that is especially responsive to quinine, results in relatively minor effects on performance in a variety of

taste-related tasks including some that employ quinine as a stimulus, it does cause a striking decrease in the occurrence of unconditioned gaping to the "bitter" alkaloid (Grill, Schwartz, & Travers, 1992; King, Garcea, & Spector, 2000; Markison, St. John, & Spector, 1995; Spector & Grill, 1992; Spector, Markison, St. John, & Garcea, 1997; St. John *et al.*, 1994; St. John & Spector, 1996; Travers, Grill, & Norgren, 1987). Quinine-elicited gaping returns to normal as the GL regenerates (King *et al.*, 2000). However, rats with GL transections not only are able to acquire a CTA to sucrose, they also express the aversion by gaping just as frequently as sham-operated conditioned controls (Eylam, Garcea, & Spector, 2000). Thus, it appears that while the GL is necessary for the expression of normal unconditioned gaping to naturally aversive taste compounds, the absence of input from the GL does not preclude signals in the remaining nerves from gaining conditioned control over oromotor rejection circuits (i.e., circuits that generate gapes) presumably through forebrain processing.

The so-called brief-access taste test is an effective and increasingly popular technique for quantitatively assessing the ingestive motivation associated with taste stimuli (Contreras, Carson, & Pierce, 1995; Contreras & Studley, 1994; Davis, 1973; Glendinning, Gresack, & Spector, 2002; Krimm, Nejad, Smith, Miller, & Beidler, 1987; Smith, Davis, & O'Keefe, 1992; Spector, Redman, & Garcea, 1996; St. John *et al.*, 1994). In this procedure animals are presented with very brief trials (several seconds in duration) of taste stimuli delivered in a gustometer and licking responses are measured. For normally preferred stimuli, the animals can be tested in a nondeprived state. For normally avoided stimuli, animals are placed on a water restriction schedule and their responses to the taste stimuli are compared to their water licking. Using this technique, researchers have shown that licking responses to a variety of compounds including sucrose, maltose, NaCl, quinine, denatonium, and citric acid vary in a monotonic concentration-dependent manner. Disruption of peripheral afferent input or lesions in the gustatory zones of the NST or PBN can severely blunt taste responsiveness measured in this fashion (Krimm *et al.*, 1987; Shimura, Grigson, & Norgren, 1997; Spector, 1995; Spector, Grill, & Norgren, 1993; Spector *et al.*, 1996; Spector, Travers, & Norgren, 1993; St. John *et al.*, 1994). Moreover, this procedure has been very effective at revealing robust alterations of responsiveness to "sweeteners" and "bitter" taste stimuli following specific molecular modifications of taste receptor cells (see above) in mice (Zhang *et al.*, 2003). The small volumes of stimuli ingested on each trial coupled with the measurement of immediate responses raises the confidence that the responsiveness measured with the brief-access taste test is based on oral stimulation, an inference supported by the collective findings of experiments demonstrating that manipulations of the gustatory system can have profound and specific effects on this behavior (Spector, 2003a).

In the case of a ubiquitously preferred and reinforcing taste stimulus such as sucrose, some of the appetitive and consummatory responses can be influenced by postingestive factors in addition to taste. However, the fact that responses of peripheral gustatory nerves and single units in the central gustatory system display a similar monotonic increase as a function of sucrose concentration compared with bout drinking rate, brief-access licking rate, operant response rate (e.g., lever pressing), oromotor ingestive responses, and especially sham drinking, strongly implies that taste signals are significantly promoting ingestion (see Smith, 2000; and Figure 6).

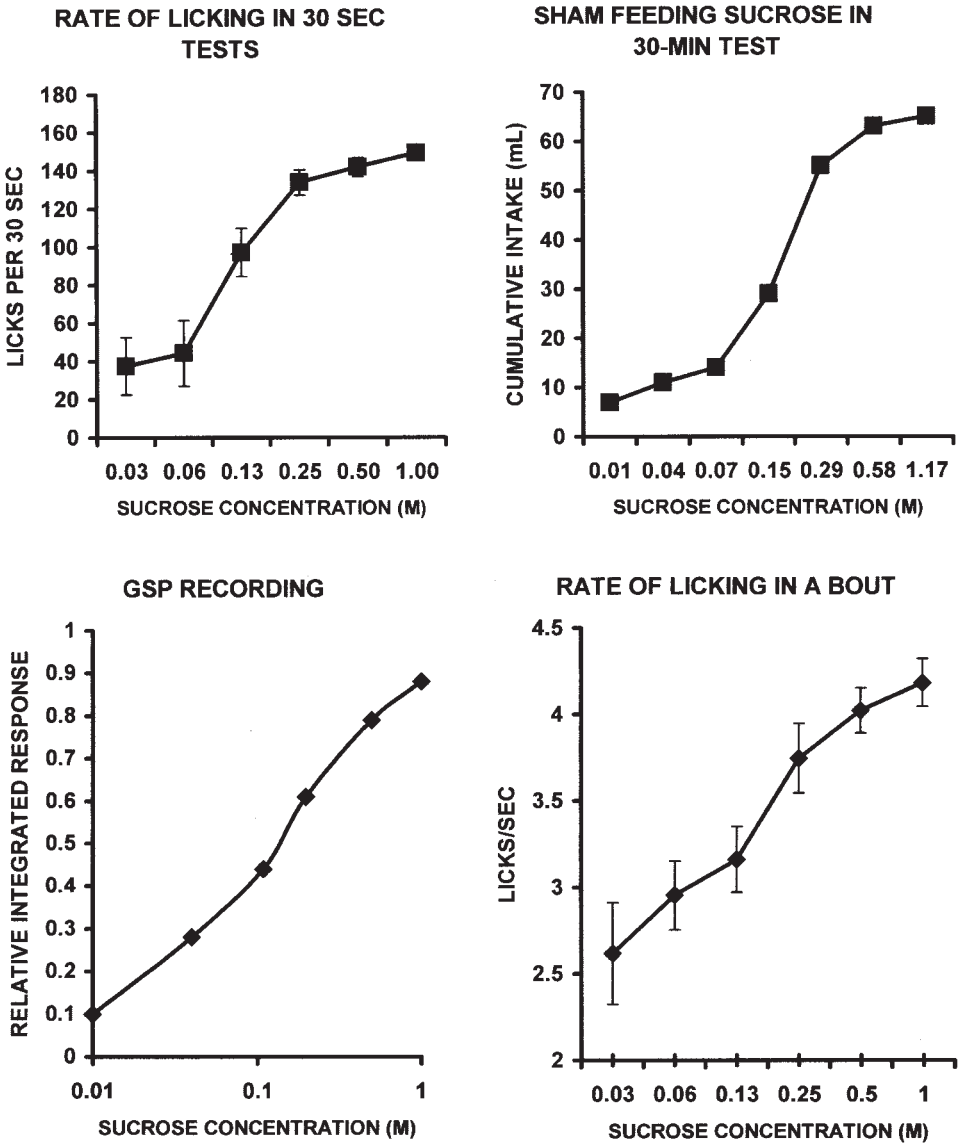

Figure 6. Upper left panel: Mean (± SE) rate of licking by rats in single-bottle 30-s tests as a function of varying sucrose concentrations. Upper right panel: Mean cumulative intake of sucrose in 30-min tests by sham-fed rats plotted as a function of sucrose concentration. Lower left panel: The magnitude of the integrated electrophysiological response from the greater superficial petrosal nerve to stimulation with varying concentrations of sucrose solution (replotted from Nejad, 1986). Lower right panel: Mean (± SE) rate of licking during bouts of sucrose drinking as a function of concentration during 24-hr two-bottle preference tests (water was in the other bottle). Note that all four panels depict functions that increase monotonically with sucrose concentration.

Cephalic Phase Reflexes

Taste stimuli can trigger physiological responses in addition to behavioral ones. This general phenomenon is referred to as the cephalic phase reflex, a term derived from the notion that stimulation of receptors in the head during the beginning

stages of a feeding episode can lead to reflexive visceral events (see Nicolaidis, 1969; Pavlov, 1902; Powley, 1977; Teff, 1999). The clearest example of a cephalic phase reflex is the salivation that accompanies oral stimulation with particular taste compounds (see Bradley, 1991). There also is evidence that oral ingestion of certain sugars and nonnutritive sweeteners can lead to preabsorptive increases in serum insulin levels (Berthoud & Jeanrenaud, 1982; Berthoud, Trimble, Siegel, Bereiter, & Jeanrenaud, 1980; Flynn, Berridge, & Grill, 1986; Grill, Berridge, & Ganster, 1984). Cephalic phase reflexes are thought to facilitate digestion and assimilation of ingested substances. The fact that these responses display some degree of chemospecificity strongly suggests the functional involvement of the gustatory system.

In fact, conditioned changes in the affective valence of a gustatory stimulus as assessed by appetitive or consummatory behavior can modulate the effectiveness of the taste compound to trigger certain cephalic phase responses. A striking example of this phenomenon can be found in the work of Berridge, Grill, and Norgren (1981) who conditioned an aversion to glucose by pairing its intraoral infusion with an injection of LiCl in rats. The glucose-elicited preabsorptive insulin release (PIR), which was observed prior to the first LiCl injection, was abolished after a single conditioning trial. After a second conditioning trial the PIR to glucose remained absent. Concomitantly over the course of conditioning, these rats decreased their ingestive taste reactivity responses and increased their oromotor and somatic rejection responses to the glucose CS and displayed a robust avoidance of glucose in a 24-hr two-bottle preference test relative to the unequivocal preference exhibited by unconditioned control rats. It is noteworthy that Flynn *et al.* (1986), using the chronic supracollicular decerebrate rat preparation, have demonstrated that the caudal brainstem is sufficient for the generation of a glucose-elicited PIR. It would be instructive to examine whether glucose–LiCl pairings are capable of eliminating this cephalic phase response in decerebrate rats as it does in intact rats. Based on the necessity of an intact connection with the forebrain for CTAs to be expressed in consummatory behavior, it would be expected that the caudal brainstem would not be sufficient to support conditioned changes in taste-elicited PIR, but this remains to be explicitly tested.

Final Remarks

The sense of taste is functionally complex. Because taste function is multidimensional, there is no one behavioral procedure that can serve as a definitive assay for gustatory competence following a given neural manipulation. For many years, researchers were unable to disrupt two-bottle preference behavior after various types of gustatory nerve transections. These findings were generally interpreted as demonstrating the redundancy of the gustatory system. That is, the signals in the remaining nerves were considered sufficient to maintain taste function. Although this conclusion may have been true with reference to the domain of affect (caveats regarding the potential contribution of postingestive receptor systems aside), severe deficits were revealed as a consequence of gustatory neurotomy once researchers started assessing other aspects of taste function with appropriately designed behavioral tasks.

The behavior of the animal provides the functional context in which to understand the underlying neurobiology. In this sense, behavioral analysis is an indispensable component in the overall effort to elucidate the functional organization of the gustatory system. It is our hope that in the coming years there will be just as much emphasis placed on rigorous behavioral experimentation as there is on more

molecular levels of analysis, and that all of these approaches will be better coordinated conceptually, if not methodologically (see Spector, 2000).

Acknowledgments

We would like to thank Connie Colbert, Cedrick D. Dotson, and Shachar Eylam for helpful comments on an earlier draft of this essay. We would also like to acknowledge the support from the following grants: R01-DC01628 (ACS), R01-DC04475 (ACS), R01-DC-03198 (JCS).

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Psychophysical Measurement of Human Taste Experience

Linda M. Bartoshuk and Derek J. Snyder

Measure what is measurable, and make measurable what is not so.
Galileo Galilei (1564–1642)

In his *Elemente der Psychophysik* (1860), Fechner described psychophysics as "an exact theory of the functionally dependent relations … of the physical and psychological worlds." He noted that "since the measure of physical magnitudes is already known, the first and main task of this work will be to establish the as yet nonexistent measure of psychic magnitudes …" (Fechner, 1966/1860).

Many texts restrict psychophysics to "psychic magnitudes" evoked by stimuli from the traditional sensory modalities (i.e., vision, audition, touch, pain, temperature, taste, smell). Actually, psychophysics has a much broader scope, embracing all internal sensations (e.g., thirst, hunger) as well as affective and emotional experiences (e.g., food preferences, the satisfaction of satisfying a craving). In short, psychophysics seeks to measure anything that can be experienced.

Assessing this experience, however, is an extremely challenging task. By its very nature, individual experience is subjective: We can *describe* our experiences, but we cannot directly *share* the experiences of another person. With this understanding, how can we develop measures that will permit us to compare our experiences with those of others? By extension, can we use these measures to compare experiences between individuals we observe, or between groups of people? Strictly speaking, we cannot make any of these comparisons with certainty, yet these comparisons have enormous significance; they must be measured well. We need to know how the

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Neurobiology of Food and Fluid Intake, 2nd Ed., Volume 14 of *Handbook of Behavioral Neurobiology,* edited by Edward Stricker and Stephen Woods, Kluwer Academic / Plenum Publishers, New York, 2004.

hunger of bulimics compares to that of controls, how the craving of addicts compares to that of the unaddicted, how the sweet taste of sucrose differs between women and men, and so on. The purpose of this chapter is to examine the history of efforts to solve this problem, to assess where we are today (paying special attention to flawed comparisons), and to highlight recent findings that might lead to future progress.

Measuring Individual Differences: A History of Magnitude Matching

Classical Psychophysics: Fechner

Fechner was not the first to set forth to measure subjective experience (Boring, 1942), but his *Elemente der Psychophysik* placed psychophysics on the map. His delineation of the three threshold methods (i.e., the method of limits, the method of adjustment, and the method of constant stimuli) is still considered classic today (see Engen, 1972 for a superb description of these methods). Thresholds present some technical difficulties, but they are conceptually easy to understand: The *absolute threshold* for a stimulus is the smallest amount of the stimulus that can be detected, while the *difference threshold* is the smallest increment from one amount of stimulus to a higher amount that can be detected (i.e., the "just noticeable difference" or jnd). Threshold methods continue to be developed; it is a tribute to Fechner that these "new" methods are usually variants of his original techniques.

Weber had discovered earlier that over a wide range of stimulus intensities, the amount of stimulus required to produce a jnd is a constant proportion of the initial stimulus intensity. Fechner assumed, therefore, that the jnd was the basic unit of perception. Thus, one could scale perceived intensity by simply counting the number of jnds from absolute threshold to the stimulus value of interest. But if the jnd is the basic unit of sensory scales, it should behave like a unit. For example, if one stimulus is X jnds above threshold and a second stimulus is $2X$ jnds above threshold, then the second stimulus should be twice as intense as the first. However, S. S. Stevens (1957) showed that this second stimulus is often perceived as more intense than that; as he noted, "the subjective size of the jnd grows rapidly as we go up the scale."

S. S. Stevens and Direct Scaling

S. S. Stevens introduced direct scaling methods with ratio properties, the most popular of which was magnitude estimation: The subject is asked to provide a number reflecting the perceived intensity of the stimulus; he/she is then instructed to give a number twice as large to a stimulus that is twice as intense, a number half as large to a stimulus half as intense, etc. The size of the numbers is irrelevant, since only the ratios among the numbers carry meaning.

Comparisons Using Magnitude Estimation. S. S. Stevens's work culminated in the Power Law, expressed as $\psi = \phi^{\beta}$, in which perceived intensity (ψ) plotted against stimulus intensity (ϕ) produces a psychophysical function (i.e., power function) for a specific sensory domain. The term β is a constant that varies across sensory domains; Stevens and his colleagues measured β for many stimulus modalities.

Of critical importance, power functions can describe only how perceived intensity varies with stimulus intensity *within* an individual. As we will show, power functions may be averaged and their shapes may be compared, but they do not reflect meaningful differences of absolute perceived intensity *between* individuals or groups. Among researchers, this limitation has not always been appreciated. One reason for this oversight is that magnitude estimate data are commonly averaged across subjects to produce group functions. This procedure is acceptable because the ratios between ratings remain constant, but the logic behind it deserves further explanation.

To average magnitude estimates while preserving the ratios among them, the experimenter must bring the numbers into register with one another so that one subject's data are not weighted more heavily just because that subject used larger numbers. For example, when asked to rate the saltiness of three NaCl concentrations, subject A might respond, "10, 20, 30"; subject B, "100, 200, 300"; and subject C, "0.1, 0.2, 0.3." These ratings have identical meaning in magnitude estimation because the ratios among them are equal. If we multiply any subject's data by a constant, we do not change these ratios, so we can "normalize" ratings prior to averaging by multiplying each subject's data by a constant. If we set each subject's response to the first solution as 10, we would multiply subject A's data by 1, subject B's data by 0.1, and subject C's data by 100, producing average normalized ratings of 10, 20, 30. (Alternatively, to allow averaging across subjects without normalization, the experimenter can instruct subjects to assign a number to the first stimulus, which then becomes a standard. In the example above, the experimenter might have assigned the number 10 to the first of the NaCl solutions.)

As we have noted, the absolute values of numbers have no meaning in magnitude estimation. A study on the perception of sourness across three age groups provides an example of how this logic has been misunderstood (Chauhan & Hawrysh, 1988). The experimenters assigned the number 10 to a given concentration of acid and instructed subjects to rate the sourness of a series of concentrations relative to that standard. Not surprisingly, the average ratings for the standard concentration were very close to 10 for all three groups. The authors concluded that all three groups perceived the same sourness at that concentration. In fact, the results simply show that all three groups could follow directions.

Stevens considered individual differences less interesting than the functions produced by his power law. Nevertheless, comparisons across individuals or groups have proven fundamental to many important research questions, including our work on genetic variation in taste perception.

Genetic Variation in Taste: Magnitude Matching. "Taste blindness" was discovered in 1931 when Fox, an industrial chemist, was synthesizing phenylthiocarbamide (PTC) in his laboratory. Some of the PTC flew into the air, but when a colleague tasted the airborne chemical and noted its intense bitterness, Fox tasted nothing. Fox and his colleague tested PTC on coworkers, observing that 60% were tasters and 40% were nontasters (Fox, 1931). Subsequently, two family studies (Blakeslee & Salmon, 1931; L. H. Snyder, 1931) suggested that nontasting was a simple Mendelian recessive trait. Blakeslee and Fox (1932) took PTC crystals to a meeting of the American Association for the Advancement of Science and tested 2,550 attendees; 28% found the crystals tasteless, 65.5% found them bitter, and 6.5% reported other taste sensations. When this study was published in *The Journal of Heredity*, the editor had small pieces of paper impregnated with PTC inserted into

the journal; the journal later sold these papers for a nominal fee, generating a series of experiments.

Work in this early era focused on measuring percentages of tasters by race and sex, and on associations between PTC tasting and disease. Since methods varied considerably across these studies, Harris and Kalmus (1949) developed a threshold technique intended to standardize PTC research; this technique actually was a variant of Fechner's method of limits. Threshold studies using the Harris–Kalmus method dominated research on taste blindness for more than 25 years.

In the 1960s, Fischer took these studies in new directions. He substituted PROP (6-*n*-propylthiouracil) for PTC because PROP lacks the sulfurous odor of PTC (e.g., Fischer, 1967); also, since PROP is used as a medication, safety information is available (Lawless, 1980). Fischer discovered associations between PROP tasting and food preferences, alcohol abuse, smoking, and body weight; this insightful work foreshadowed issues about PROP tasting that concern us today.

All of Fischer's experiments were performed using thresholds. Given the success of Stevens's direct scaling methods, we wanted to scale the *suprathreshold* bitterness of PTC/PROP using magnitude estimation, but in our previous work we always used each subject as his/her own control. For example, we looked at the effects of adaptation on taste (e.g., McBurney & Bartoshuk, 1973), but each subject experienced all testing conditions and could make direct comparisons. We were suddenly faced with a problem: Magnitude estimates have *relative* meaning, but we needed to compare the *absolute* bitterness of PTC across nontasters and tasters. Our answer came from early PTC studies, which suggested that the bimodal distribution associated with PTC was limited to compounds containing the N − C = S chemical group. Based on this idea, we asked subjects to rate PTC relative to NaCl, a compound lacking the N − C = S group. Using this ratio technique, we found that caffeine, a bitter compound also lacking the N − C = S group, is less bitter to nontasters than to tasters (Hall, Bartoshuk, Cain, & Stevens, 1975).

Expressing the bitterness of caffeine in terms of the saltiness of NaCl required essentially the same normalization procedure that we discussed above when averaging magnitude estimate data: In both cases, we multiplied each subject's data by a constant chosen to make their ratings of NaCl equal. However, our purpose here was quite different. We wanted to compare ratings across different groups, so we made the assumption that the saltiness of NaCl is not related to the bitterness of caffeine. If this assumption is correct, then we can use the ratio between caffeine and NaCl to make meaningful comparisons of caffeine ratings between nontasters and tasters.

Following Fischer, we switched from PTC to PROP and expanded the group of compounds tasting more intense to tasters; sweet as well as bitter compounds fell into this group (Bartoshuk, 1979; Bartoshuk, Rifkin, Marks, & Hooper, 1988; Gent & Bartoshuk, 1983). In addition, we began to test associations between PROP tasting and the tastes of foods; for example, some milk products tasted more intensely bitter to tasters than to nontasters (Marino *et al.*, 1991). Even some nontaste sensations were related to PROP status; capsaicin (the compound producing burn in chili peppers) produced a stronger oral burn to tasters (Karrer & Bartoshuk, 1991). At first, these experiments showed us simply that differences between nontasters and tasters extend beyond the perception of compounds carrying the N − C = S group, but mounting data made a broader point: These groups inhabit entirely different oral sensory worlds.

As studies accumulated, we realized that among tasters were a distinct group of supertasters (Bartoshuk, 1991), who perceive the most intense sensations not only from taste stimuli but also from oral irritants, oral thickeners, and fats (Bartoshuk, 1993a, 1993b, 2000; Bartoshuk *et al.*, 1992; Duffy, Bartoshuk, Lucchina, Snyder, & Tym, 1996; Phillips, Bartoshuk, Peterson, & Duffy, 2001; Prescott, Johnstone, & Munro, 2001; Prescott & Swain-Campbell, 2000; Prutkin, Fast, Lucchina, & Bartoshuk, 1999; Snyder, Lucchina, Duffy, & Bartoshuk, 1996; Tepper & Nurse, 1997). The discovery of supertasters added to our growing concerns about the use of NaCl as a standard: What if NaCl tasted saltier to supertasters? Fortunately, J. C. Stevens and Marks had expanded their work on cross-modality matching (J. C. Stevens, 1959; Stevens & Marks, 1965) to develop the method of "magnitude matching" (Marks & Stevens, 1980; Stevens & Marks, 1980). Magnitude matching uses the same logic we used in selecting NaCl as a standard, but it extends the range of standards to nontaste modalities. We began to use sound as our standard, assuming (as we did with NaCl) that nontasters, medium tasters, and supertasters have equal average auditory ability. Doing so, we learned that the saltiness of NaCl varies with taster status: Nontasters perceive the least saltiness and supertasters perceive the most (Bartoshuk, Duffy, Lucchina, Prutkin, & Fast, 1998). As a result, all of our PROP effects obtained with an NaCl standard are conservative estimates; the actual differences are larger than we originally reported.

J. C. Stevens's pioneering work on cross-modality matching also had an early impact on the study of pain. Peck, a physician in private practice, asked subjects to turn the knob of an audiometer until the sound intensity matched that of the pain (Peck, 1967) or anxiety (Peck, 1966) they were experiencing. Earlier still, Kast (1962) used one painful stimulus to assess another by applying mechanical pressure to a patient's leg. This pressure could be varied in intensity, so it could be matched to a patient's pathologic pain. Studies like these presaged the formal development of magnitude matching by many years.

In summary, we have "solved" the problem of group comparisons by changing the task. We cannot compare sensory intensities directly across nontasters, medium tasters, and supertasters, but we can ask each subject to rate stimuli of interest as well as a sensory standard. By assuming that any variability in the standard is unrelated to genetic variability in oral sensation, we can compare stimuli of interest across groups as a function of PROP taster status. If our assumption is valid, then we have effectively derived an across-group comparison from within-subject comparisons.

Category Scales: What Do Labels Mean?

Galileo's admonition to "Measure what is measurable, and make measurable what is not so" expresses our need to quantify in order to study. This need spurred the development of numerous methods to assess the perceived intensity of experience. Most of these methods involve the use of scales labeled with intensity descriptors. This is certainly a reasonable idea, as we compare sensations throughout everyday life using intensity adjectives (e.g., "This solution tastes *strong* to me. Does it taste *strong* to you?"), and we use adverbs to modify those adjectives (e.g., "This solution tastes *very strong* to me. Does it taste *very strong* to you?").

Types of Labeled Scales

Category scales date back at least to the astronomer Hipparchus (190–120 BC), who classified stars into six categories by their brightness. More recently, Likert (1932) introduced a category scale for the study of attitudes; this scale has become the basis for a variety of so-called "Likert scales" used today across many disciplines. (Category scales are sometimes called Likert scales, but technically this name applies only to Likert's original attitude scale.) Another well-known category scale is the Natick nine-point scale, which was developed in 1949 for military studies on food preference (e.g., Jones, Peryam, & Thurstone, 1955; Peryam & Girardot, 1952).

The visual analogue scale (VAS), which came into widespread use during the 1960s (e.g., Aitken, 1969; Aitken, Ferres, & Gedye, 1963; Clarke & Spear, 1964; Silverstone & Stunkard, 1968), is essentially a category scale with all labels removed except for those at the extreme ends. It is typically described as a line labeled at its ends with the "minimum" and "maximum" ratings for a particular attribute (e.g., Hetherington & Rolls, 1987).

Invalid Comparisons: The Use (and Misuse) of Labeled Scales

S. S. Stevens (1958) noted: "Mice may be called large or small, and so may elephants, and it is quite understandable when someone says it was a large mouse that ran up the trunk of the small elephant." As this observation clearly shows, "large" and "small" can denote very different absolute sizes, depending on whether we are talking about mice or elephants.

Just as the range in size among mice is much smaller than it is among elephants, the range of perceived intensity varies in size among different sensory domains. We demonstrated this empirically (Bartoshuk *et al.*, 2002) by asking subjects to rate the perceived intensity of the strongest stimuli they had ever experienced in a variety of domains (e.g., strongest odor of a flower, bitter taste, oral pain, light, sound, etc.) using the general Labeled Magnitude Scale (LMS; described below). Some of these data are shown in Figure 1. Not surprisingly, the strongest sensations from some domains (e.g., sound) were much stronger than those from others (e.g., flower odor).

Similarly, ranges of perceived intensity within a given domain vary in size across individuals based on their experiences with that domain. We have shown that differences in individual taste experience can lead to systematic differences in the absolute intensities denoted by scale labels that refer to taste. As described earlier, supertasters of PROP live in a neon taste world, while nontasters live in a relatively pastel taste world. This suggests that a label like "very strong taste" would denote a greater perceived intensity to a supertaster than it would to a nontaster. Figure 2 shows the results of an experiment in which subjects provided magnitude estimates for tastes, tones, and adjectives (Bartoshuk, Duffy, Fast, & Snyder, 2004). Using the tones as a standard, we compared the perceived intensities denoted by the adjectives, just as we compared taste intensities across PROP groups. As the figure illustrates, the label "very strong" describes a significantly different intensity experience for nontasters and supertasters.

To summarize, labeled scales are elastic in terms of both the domain to be measured and the individual's experience with that domain. Labeled scales can be used for within-subject comparisons; they also can be used for across-group comparisons when subjects are assigned to groups randomly. However, across-group comparisons are invalid whenever subject classification (e.g., sex, age, weight,

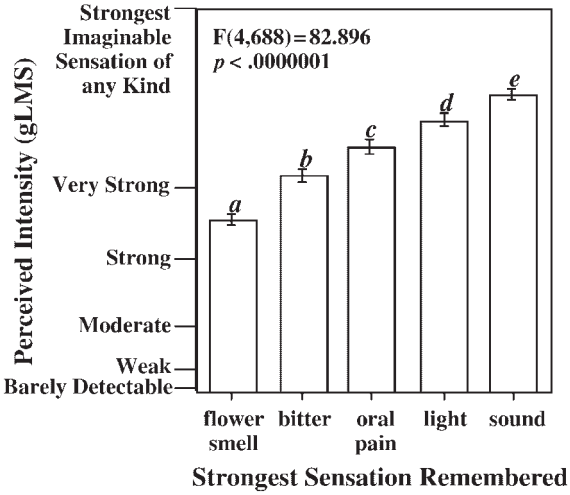

Figure 1. Perceived intensity (±SE) of the strongest remembered sensations from several sensory modalities. For each sensation, the range from zero to maximal has a different absolute size. Letters in italics indicate significantly different effects ($p < .01$) based on Tukey HSD tests following ANOVA (Bartoshuk *et al.*, 2002).

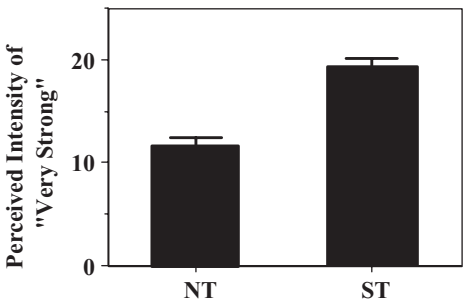

Figure 2. The perceived intensity (±SE) of the descriptor "very strong" differs for nontasters (NT) and supertasters (ST). Subjects were classified by magnitude estimates for 0.0032M PROP; the top 25% of respondents were assigned as ST ($N = 66$), while the bottom 25% were assigned as NT ($N = 65$). Data were normalized to the geometric mean of ratings for 50–98 dB tones at 1,000 Hz (Bartoshuk *et al.*, 2004).

clinical status, etc.) produces groups for whom scale labels may denote different absolute intensities.

Consequences of Invalid Comparisons. Figure 3 shows errors that can result from the false assumption that intensity descriptors denote the same absolute intensity to all. (This figure is idealized and reflects our view of the PTC/PROP taste literature.) On the left side of the figure, we have depicted a series of stimuli that produce equal perceived intensities to nontasters. The diverging lines connecting nontaster ratings to supertaster ratings show PROP effects of differing sizes: Line A indicates a stimulus that is much more intense to supertasters than to nontasters (e.g., bitterness of quinine); lines B and C indicate successively smaller PROP effects; and dashed line D indicates a stimulus that is slightly more intense to supertasters than to nontasters (e.g., NaCl). The intensity difference between

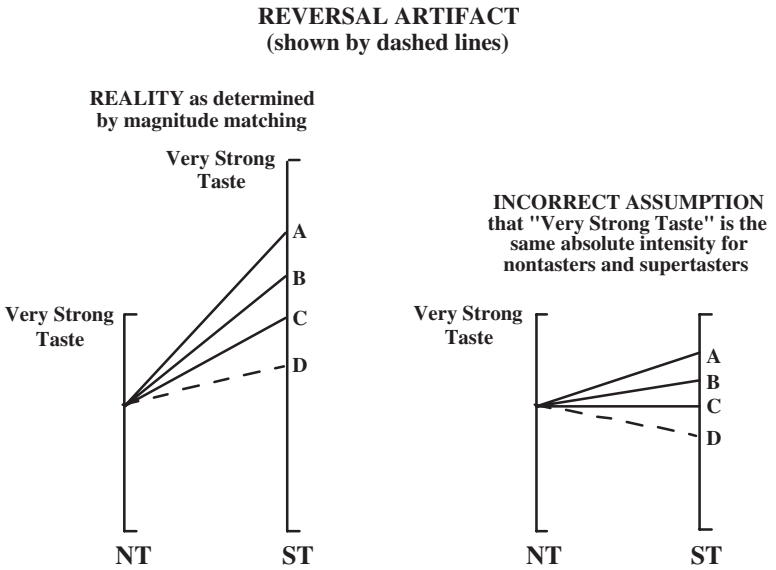

Figure 3. The left panel shows taste functions reflecting real differences between nontasters (NT) and supertasters (ST) measured with magnitude matching (see Figure 2 for details). The right panel shows the consequences of incorrectly assuming that "very strong taste" indicates the same absolute perceived intensity to NT and ST: Valid effects appear truncated and may reverse direction inaccurately (Bartoshuk *et al.*, 2004).

groups for the label "very strong taste" is the same difference shown in Figure 2 (Bartoshuk *et al.*, 2004).

When the label "very strong taste" is treated as if it denotes the same average intensity to nontasters and supertasters, reality is distorted as shown on the right side of Figure 3. The stimulus effect indicated by line A shows a larger difference across nontasters and supertasters than does the label "very strong taste." When equal intensity is attributed to that label for both groups, stimulus A still appears more intense to supertasters than to nontasters, but the effect is blunted compared to the actual difference. Meanwhile, consider stimulus C: The actual difference between nontasters and supertasters is equal to the difference between the labels, so this difference disappears when "very strong taste" is assumed equal to both groups.

Perhaps the most bizarre effect is illustrated by stimulus D, where the actual difference between nontasters and tasters is smaller than that for "very strong taste." In this case, group differences actually appear to go in the wrong direction when "very strong taste" is assumed equal to everyone. We call this phenomenon a *reversal artifact* (see Prutkin *et al.*, 2000 for an example from our earlier work; see Drewnowski, Henderson, & Shore, 1997; Drewnowski, Henderson, Shore, & Barratt-Fornell, 1997; Schifferstein & Frijters, 1991; Yackinous & Guinard, 2001 for additional examples).

Most investigators initiate a study on group differences because they suspect that such differences exist. But if these effects do exist, then so do the very circumstances that could make scale labels mean different things to different groups. We now understand the extent of this problem in studies on genetic variation in taste (e.g., Bartoshuk *et al.*, 2004). Other research areas are likely to have similar problems, as across-group comparisons are common, and such comparisons can be

considered valid only to the extent that no systematic differences exist in scale usage. Nevertheless, this issue is manageable, since the development of more precise scaling methods allows us to verify such differences (see below).

Distorted Scaling is Widespread. Aitken (1969) was aware of the limitations of the VAS, noting that "The same word used by different people need not convey that they experience the same feeling, neither does comparable positioning of marks on lines." Nevertheless, within a short time papers appeared using the VAS to make across-group comparisons.

The limitations of labeled scales also have been observed by other researchers. Among sensory psychophysicists, it is clear that intensity ratings can be altered both by context and experience (e.g., Gescheider, 1988; Helson, 1964; Parducci, 1965; Zoeke & Sarris, 1983). Although these effects generally are demonstrated using subjects as their own controls (i.e., context or experience causes the subject's ratings to change), the results extend to across-group comparisons as well: Whenever context or experience varies systematically across subject groups, comparisons between those groups are invalid.

Experts in a variety of fields have noted the consequences of invalid comparisons across subjects or groups. Narens and Luce (1983) disputed the economic principle of "intercomparability of utility," which states that value can be compared across individuals. In a delightful example, they noted that our confidence in assessing others' preferences may lead us to introduce two people whom we predict will be compatible, when in fact they are not. Biernat and Manis (1994) noted the importance of the target to which a rating is applied: "Very tall" suggests a different height when describing a woman versus describing a man. Birnbaum (1999) showed that one can actually create a false comparison between groups by manipulating the contexts used by those groups to make judgments. He asked different groups to judge the size of the numbers 9 or 221 on a 10-point scale ranging from "very very small" to "very very large." The number 9 suggests a context of relatively small numbers (e.g., Parducci, 1965), so "9" received a relatively high average rating from the group rating only that number. On the other hand, the number 221 suggests a context of larger numbers, so "221" received a *lower* average rating from the group rating only that number. The average scale values for 9 and 221 were 5.13 and 3.10, respectively, leading to the absurd conclusion that 9 is greater than 221.

Insights like these led us to reexamine some of the standards commonly used in magnitude matching. Standards for magnitude matching must be sensory or hedonic experiences with absolute intensity attributes. We suspect that line length and grayness do not function appropriately as standards, as they seem to behave more like labeled scales that can shrink or expand to cover any domain.

The perils of across-group comparisons extend beyond intensity judgments, as frequency and probability descriptors can denote different absolute values to different individuals or groups (e.g., Hakel, 1968, Kong, Barnett, Mosteller, & Youtz, 1986; Mapes, 1979; Simpson, 1944; Wallsten & Budescu, 1995; Wallsten, Budescu, Rapoport, Zwick, & Forsyth, 1986). This scenario was used to humorous effect in a scene from the 1977 Woody Allen film "Annie Hall" (Rollins, Joffe, & Allen, 1977). Alvy (Allen) and Annie (Diane Keaton) visit their respective psychiatrists and describe the frequency with which they have sex. Alvy says, "Hardly ever." Annie says, "Constantly." Both continue, "Three times a week."

Given the significant theoretical and practical concerns we have described in measuring subjective human experience, can we construct scales that allow valid across-group comparisons? We believe that useful options do exist. In fact, our growing understanding of the properties of labeled scales has suggested how we might produce a labeled scale suited for this purpose.

Adding Labels to Ratio Scales

As an early assessment of the spacing of intensity labels, Lasagna (1960) asked patients to place pain descriptors (e.g., slight, moderate, severe, very severe) at the appropriate places on a "pain thermometer." The resulting data showed that these descriptors are not equally spaced across the intensity range.

Students and colleagues of S. S. Stevens embedded intensity labels in ratio scales produced by magnitude estimation. Borg (1961, 1982, 1990) selected a range of descriptors and tested various label positions that seemed reasonable to him, producing a scale anchored from zero to "maximal" sensation. Range theory, proposed by Borg (1982, 1990) and Teghtsoonian (1973), proposed that sensory maxima for all domains are equal (with the possible exception of pain). If range theory had been true (see Figure 1 for evidence that it is not), then Borg's scale would have been a universal sensory ruler.

Moskowitz took a more empirical approach (Moskowitz, 1977; Moskowitz & Chandler, 1977). At the end of a typical magnitude estimation experiment, he asked subjects to provide ratings for the intensities of descriptors; in a study on sweeteners, for example, he would ask, "What rating would you have given if you had tasted a weak sweetener?" "… a strong sweetener," "… a very strong sweetener," and so on. Moskowitz assessed a comprehensive array of food attributes (including hedonic ratings) in this way, but the spacing of the adjectives turned out to be essentially the same every time. Moreover, it matched the spacing Borg had derived in his experiments (Borg, 1961, 1982, 1990). Other investigators were embedding intensity descriptors in ratio scales during this period, and they too replicated this characteristic spacing (e.g., Gracely, McGrath, & Dubner, 1978; Heft & Parker, 1984; Karrer & Bartoshuk, 1991; Schutz & Cardello, 2001).

The similarity of intensity descriptor usage across widespread modalities suggests a cognitive model for intensity perception. We learn the relative spacing among intensity descriptors early in life; these ratios remain constant. However, the absolute sensory range covered by intensity descriptors is variable, so we stretch or compress our descriptive vocabulary to fit the extremes of whatever we wish to describe. As a result, we may use the same words to describe just about anything, but our experience with the object at hand is critical in guiding our frame of reference. Based on this model, a person describing roses can easily identify a "weak" or a "strong" rose odor, and they can identify a "weak" or a "strong" headache equally well; but in absolute terms, the "strong" headache is probably much more intense than the "strong" rose odor.

Evolution of the General Labeled Magnitude Scale (gLMS)

One of the projects to embed intensity descriptors in a ratio space deserves special mention. Green, Shaffer, and Gilmore (1993) devised the Labeled Magnitude

Scale (LMS) to measure oral sensations. Building on the pioneering efforts of Borg and Moskowitz, the LMS was the first scale to place ratings empirically (i.e., using magnitude estimation) *and* include a sensory maximum (i.e., "strongest imaginable [oral sensation]").

Snyder was the first researcher to use the LMS in an across-group comparison (Snyder, 1995; Snyder *et al.*, 1996). He scaled the oral burn produced by capsaicin, the saltiness of NaCl, and the bitterness of PROP, and his data showed a bizarre result: Supertasters appeared to perceive less saltiness from NaCl than did nontasters. This reversal artifact (see Figure 3) revealed to us that the labels on the LMS denote different absolute intensities to nontasters, medium tasters, and supertasters.

We altered our instructions, asking subjects to treat the top of the LMS as the "strongest imaginable sensation of any kind." With this change, differences between nontasters, medium tasters, and supertasters became the same size as those produced by magnitude matching with an auditory standard (Bartoshuk *et al.*, 2000). The revised scale also produced the predicted relationship between saltiness and PROP bitterness: Supertasters perceived the most intense salty taste sensations (Prutkin, 1997).

In collaboration with Green, we have continued to explore descriptors embedded in a ratio scale. Fast (e.g., Bartoshuk *et al.*, 2002; Fast, 2004) replicated the study that produced the LMS (Green *et al.*, 1993), only she instructed subjects to make their ratings in the context of all sensations rather than just oral sensations. As expected, the relative spacing between descriptors was essentially the same as in previous studies, and it matched the scale we had constructed simply by changing the top label of the LMS (Bartoshuk *et al.*, 2000). We call this new scale the *general LMS* (gLMS).

We can think of the gLMS as the elastic intensity scale we all learn through experience, stretched here to its maximum. So, is the gLMS a universal sensory ruler? If it were, then the label "strongest imaginable sensation of any kind" would have to represent an equally intense experience for everyone. We are unlikely to ever know with certainty if this is true, but we need not meet such a stringent criterion for the gLMS to allow valid comparisons across nontasters, medium tasters, and supertasters. Instead, we must assume only that any variation across individuals in the absolute intensity denoted by our top label is not systematically related to variation in taste. Under these conditions, the top label functions as a standard, indicating the same average intensity for all three groups. The gLMS is, in fact, quite similar to magnitude matching, only it uses the top label as the standard instead of separate stimuli (e.g., tones); this similarity explains why the two methods produce similar psychophysical functions (Bartoshuk *et al.*, 2000). As such, the gLMS performs similarly to magnitude matching for any sensation of interest: It is a valid sensory ruler as long as the strongest imaginable sensation of any kind is unrelated to that sensation.

The gLMS as a Hedonic Scale. Moskowitz and Chandler (1977) found that the spacing of hedonic intensity labels is similar to the spacing of sensory intensity labels, suggesting that sensory and hedonic experiences have the same intensity properties. Based on this finding, we created a bipolar hedonic scale by extending two gLMSs in opposite directions from a common midpoint; "neutral" is in the center, "strongest imaginable disliking" anchors the left end, and "strongest imaginable liking" anchors the right end (Bartoshuk *et al.*, 1999; Bartoshuk, Duffy, Fast, Green, & Snyder, 2002; Chapo, Bartoshuk, Peterson, Phillips, & Duffy, 2001; Chapo,

Phillips, Ilich, & Duffy, 2001; Duffy, Fast, Cohen, Chodos, & Bartoshuk, 1999; Duffy, Lucchina, & Bartoshuk, 2004; Duffy & Peterson, 2000; Duffy, Phillips, Peterson, & Bartoshuk, 2001; Fast, Green, & Bartoshuk, 2002; Peterson, Bartoshuk, & Duffy, 1999; Peterson & Duffy, 2000; Phillips *et al.*, 2001; Reed *et al.*, 2002; Snyder, Duffy, Fast, Hoffman *et al.*, 2001; Snyder, Duffy, Fast, Weiffenbach *et al.*, 2001). This scale has proven quite useful in our continuing examination of food preferences. In particular, we have shown that preferences for high-fat foods increase with advancing age in women (Snyder, Duffy, Fast, Hoffman *et al.*, 2001). This finding is especially interesting because it suggests that the intensities described by scale labels may change across the lifespan. Age effects may therefore be subject to distortions like those illustrated in Figure 3.

Schutz and Cardello (2001) also constructed a hedonic scale for food preferences using the methods of Green *et al.* (1993). The fact that they found similar spacing among scale descriptors further supports the idea that hedonic and sensory ratings show similar intensity properties. Consequently, the logic we have discussed with respect to sensory scaling can be extended: When hedonic scales are anchored in terms of all possible hedonic experience, comparisons are permitted across groups that may inhabit very different hedonic food worlds.

Oral Anatomy: A Tool for Comparing Scales. Taste intensity is associated with the density of fungiform papillae (i.e., structures containing taste buds) on the anterior tongue (Bartoshuk, Duffy, & Miller, 1994). This association allows us to evaluate the ability of various scales to provide valid comparisons across nontasters, medium tasters, and supertasters. Gardiner (Prutkin *et al.*, 2000) did a pilot study measuring the association between fungiform papillae density and taste ratings obtained using several scales. The gLMS and magnitude matching both produced a significant correlation between fungiform papillae density and the bitterness of quinine, while two visual analogue scales did not. This finding further demonstrates that conventional labeled scales can distort a valid and meaningful difference, in this case masking it completely.

Corrected Scaling Can Salvage Invalid Comparisons. We have shown that magnitude matching (with appropriate standards) and the gLMS will permit valid across-group comparisons, and we have shown why other existing scales will not. That said, magnitude matching and the gLMS are hardly the only solutions to this problem. In fact, the logic we have used to highlight the flaws of certain scales allows us to modify those scales so that they enable across-group comparisons. For example, to correct the VAS, we simply label a line "zero" on one end and "strongest imaginable sensation of any kind" on the other. By now this line should look familiar, as the "modified VAS" is identical to the gLMS with its inside labels removed. For all scales, corrections for across-group comparisons converge to a common but critical theme: Scale labels must refer to experiences unrelated to the domain of interest.

Our logic allows us to do more than just correct inappropriate scales; it also permits us to verify the conclusions of earlier studies conducted with those scales. The core question remains: Do scale labels from the original study have the same absolute meaning across experimental groups? Using a valid scaling method like the gLMS or magnitude matching, we can answer this question. Unfortunately, this essential control is possible only in studies where group assignments did not depend on scaling data. As such, we are unable to verify several PROP tasting effects because the classification of subjects as nontasters, medium tasters, or supertasters was itself determined by invalid scaling.

Magnitude matching was devised using sensory standards that were actually presented to subjects, but standards that require special equipment (e.g., tones, lights) can be cumbersome. Fortunately, subjects can rate the perceived intensities of remembered sensations instead (Fast, Green, Snyder, & Bartoshuk, 2001), and these remembered sensations may be used as standards. In fact, commonly used labels like "strongest pain ever experienced" are themselves remembered sensations. Among these sensations, "brightest light ever seen" (usually the sun) has proven especially useful as a standard, perhaps because it is experienced universally by sighted subjects.

Chapo *et al.* (2001) used this method to compare the sensations produced by NaCl in young and aged females; older subjects rated NaCl as more intense. (Future studies will determine whether the salty taste or the trigeminal "sting" associated with NaCl were responsible.) This effect was observed using raw gLMS ratings, but it was also present when gLMS ratings were normalized to remembered non-oral sensations. The use of remembered sensations as standards with the gLMS shows great promise, since normalizing gLMS ratings to standards is magnitude matching.

By using the gLMS with a variety of standards (i.e., real stimuli and remembered sensations), researchers make certain that their conclusions will hold over a variety of assumptions. In the case above, Chapo *et al.* (2001) reached the same result twice using methods that assume different things. With raw gLMS ratings, we assume that the *top label* (i.e., "strongest imaginable sensation of any kind") is unrelated to taste, so any variation in the absolute intensity denoted by that label is assumed to occur equally among nontasters, medium tasters, and supertasters. When data are normalized to ratings for a real stimulus (e.g., loudness of a tone), the study becomes a magnitude matching task, where we assume that the *standard domain* is unrelated to taste. So, as long as we can reasonably assume that a remembered sensation (e.g., brightest light ever seen) is unrelated to taste, that remembered sensation may be used as a standard for magnitude matching.

Ungrouped Data: Comparisons Among Individuals. By necessity and design, most research on individual differences uses across-group comparisons to demonstrate variation across a population. But, by their very nature, across-group comparisons are based on *group* averages and related measures of central tendency. Although many such measures convey a sense of the variability across subjects (e.g., standard error), they tend to obscure specific *individual* effects. However, there are occasions when it is especially important to measure differences across individual subjects.

With remembered sensations showing such powerful utility as standards, we wondered if they could be used to produce a new kind of scale altogether, one anchored by stable real world experiences instead of elastic intensity descriptors. This new scale still would require that labels denote the same absolute intensity for everyone, but as we will explain, it could accommodate individual as well as group comparisons.

To determine if remembered sensations could function reliably as labels in such a scale, Fast *et al.* (2002; Fast, 2004) asked subjects to rate remembered sensations and PROP bitterness. When each subject's ratings were expressed relative to

the rating for "brightest light ever seen," most non-oral sensations showed no correlation with PROP bitterness, but taste and oral burn sensations did. In other words, the expected PROP-related differences were observed when an independent remembered sensation was used as a standard, confirming our belief that remembered sensations, if appropriately chosen, may be used as valid standards in taste studies.

More importantly, Fast's finding shows us how we might create the scale we have in mind. If we can identify an array of everyday sensations that covers a sufficiently large sensory range, shows stability across subjects, and avoids specific domains of interest, then that array can serve as an intensity ruler. Then, a sensation showing significant variation across subjects (e.g., taste, pain) could be quantified on an individual basis, as individuals would rate it at different locations within the array. Since the scale is linked to memories of actual experience chosen for their invariance with the sensation of interest, we need not worry about fluctuations in sensory range due to context (as with adjective or adverb labels). In effect, this new method is equivalent to using many standards simultaneously (see Bartoshuk *et al.*, 2004 for further details).

Summary and Conclusions

Psychophysics attempts to measure that which defies measurement: We cannot share subjective experiences directly, but we strive to compare them objectively. Early psychophysicists approached this issue by emphasizing thresholds and the jnd, but an interest in measuring perceptually relevant stimuli eventually led to the suprathreshold scaling techniques of S. S. Stevens. These techniques, however, posed a problem: While they had useful ratio properties, they could be used only for within-subject comparisons, not for comparisons across individuals or groups. With the discovery that people can easily match intensities across different sensory domains, the answer became clear: We can select one domain as a standard and express sensations of interest relative to it. As long as the standard does not vary systematically with the sensation of interest, across-group comparisons are valid. We first used the salty taste of NaCl as a standard for our experiments on PROP taste genetics, but we soon learned that these two stimuli are not independent. We have used sound as our standard ever since, but recent data suggest that sound and taste may be related as well; ear disease can damage taste and hearing because the chorda tympani taste nerve traverses the middle ear. If we find that taste and audition are related, all of our findings to date would be conservative estimates (as with the NaCl standard), and actual effects would be larger than we have reported.

Meanwhile, labeled scales (e.g., Likert, category, VAS) were developed to assess a wide range of sensory, hedonic, and affective qualities. We became interested in the properties of labeled scales when they failed to replicate robust orosensory effects obtained with magnitude matching. Although the creators of these scales recognized that they could not be used to assess across-group comparisons, the scales have been misappropriated over the years for just this purpose, and their limitations have been widely ignored in practice. Essentially, intensity labels have invariant relative spacing, but the absolute intensity range is elastic, stretching and compressing to fit both the domain of interest and the individual differences among those perceiving it. So, when labeled scales like the VAS are used to make comparisons between groups with markedly different experiences in the domain of interest (e.g., nontasters, medium tasters, and supertasters), those comparisons are

distorted because group members interpret the scale labels differently. In fact, we have shown that inappropriate scale use can make real effects appear truncated, absent, or even reversed.

As with ratio scales, category scales require the use of standards for across-group comparisons. The gLMS, one empirical answer to this problem, stretches the intensity range to its maximum with its top anchor of "strongest imaginable sensation of any kind." With this scale, we assume that the top anchor serves as a standard, since it is all-encompassing and thus removed from any particular stimulus of interest. Note that if this assumption is false for a particular domain, the gLMS will not provide valid across-group comparisons for that domain. For example, if pain were always the most intense sensation imaginable, the gLMS could not provide valid pain comparisons across groups (unless a standard unrelated to pain was also used). Our use of appropriate standards has been central to our use of magnitude matching (with an unrelated sensory standard) and the gLMS (with labels in a different frame of reference) to make valid across-group comparisons; we have been particularly successful in this area because we have made significant efforts to demonstrate that our standards are, on average, unrelated to PROP taster status.

The gLMS and magnitude matching (with appropriate standards) can allow meaningful across-group comparisons; labeled scales like the VAS do not. Accordingly, a large proportion of the literature on human sensation and hedonics is compromised by invalid comparisons. We acknowledge that good science may be eclipsed by flawed methods in some of these cases, so we offer a strategy for assessing the validity of conclusions derived from such methods. Further, for those who swear by the simplicity of the VAS, we offer a simple correction that should enable across-group analysis for most domains. Finally, as we have suggested, magnitude matching and the gLMS are not the only possibilities for meaningful comparisons of experience between people. In fact, the use of remembered sensations as standards paves the way for new scaling methods that require minimal assumptions. Such methods may permit comparisons among individuals, which would both obviate the need for grouped data and allow the full range of individual differences to emerge. Moreover, by defining sensory range with multiple modalities of remembered sensation, we avoid the elasticity of descriptors and edge closer to a universal sensory ruler.

We are concerned about the widespread effects of invalid sensory and hedonic comparisons across groups. Our work on genetic variation in taste has required us to explore these effects; this work has revealed associations between oral sensation and food preferences, which in turn contribute to the health risks associated with dietary choice (e.g., Bartoshuk *et al.*, 2004; Duffy *et al.*, 2004). Some of these relationships are quite subtle, and only with advances in scaling have we been able to identify them. We have described here the evolution of the psychophysical methods we use in our research. Recent advances allow these tools to address broad-based needs for sensory and hedonic assessment whenever across-group comparisons are necessary.

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Caloric Homeostasis

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6

Our Evolving Understanding of Peptides and the Control of Food Intake

Randy J. Seeley

Introduction: The Changing Landscape

The last *Handbook* volume on ingestive behavior was published while I was a graduate student and it had (and still does have) an enormous impact on how I viewed the organization of the control of food intake. However, looking back at that book, it is remarkable how much has changed in the intervening 14 years. This is particularly true when it comes to the peptides that have a role in the control of food intake and body weight.

One thing that has changed is the context in which this research is done. Even as recently as 14 years ago, the study of the brain's control of food intake was largely an academic exercise. Industrial involvement certainly existed, but now virtually all of the major pharmaceutical houses include scientific efforts aimed at developing treatments for obesity. Additionally, numerous small biotech companies include efforts aimed at identifying systems within the central nervous system (CNS) that regulate energy balance. The reasons for this are multifold. The first and most important is the crushing need for therapeutic strategies that can stem the rising tide of obesity in the United States and across the developed and developing world. This need has made understanding the neural circuits that control food intake an

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Neurobiology of Food and Fluid Intake, 2nd Ed., Volume 14 of *Handbook of Behavioral Neurobiology,* edited by Edward Stricker and Stephen Woods, Kluwer Academic / Plenum Publishers, New York, 2004.

important scientific priority. Without additional therapies, the 300,000 deaths and $117 billion in healthcare costs in the United States attributed to obesity each year will continue to increase (Satcher, 2002). Second, the Food and Drug Administration (FDA) has altered the basic premise of what obesity therapeutics should look like. Previously, the FDA expected that a drug could be taken only for a 6-month period, and that weight loss would be maintained even after discontinuation of the drug. This unrealistic expectation kept many pharmaceutical houses from investing in the research necessary to bring a drug to market. The FDA now considers obesity a chronic condition that needs to be managed for a lifetime, which opens up the possibility for effective (and lucrative) treatment strategies. With such incentive, both biotechnology and pharmaceutical companies have been expanding the list of neurochemicals linked to the control of food intake.

The second change has been in focus, from the function of specific nuclei within the hypothalamus to the function of specific neurotransmitter systems within the hypothalamus (with the majority of that attention going toward neuropeptides). Historically, the picture of how the hypothalamus controlled food intake was dominated by the "dual-centers" hypothesis, proposed in 1954 by Eliot Stellar in a highly influential article (Stellar, 1954). Stellar eloquently argued that the hypothalamus contained two populations of neurons that provided opposing influences on food intake. The first set of neurons was in the ventromedial hypothalamus (VMH) and was thought to control "satiety." Consistent with this formulation, rats with lesions of the VMH showed marked increases in food intake whereas electrical stimulation of the VMH decreased food intake. The second population of neurons, in the lateral hypothalamus (LH), was believed to comprise a "hunger" center. Consistent with this hypothesis, rats with lesions of the LH showed decreased food intake whereas stimulation of the LH increased food intake.

The dual-centers perspective was eroded by several lines of research. One was work by Grill and colleagues demonstrating an important role of caudal brainstem structures to control important aspects of ingestion (Grill & Kaplan, 2002; Grill & Norgren, 1978). Thus, the hypothalamus is now recognized as but one component of a distributed network that controls multiple aspects of ingestive behavior. That topic is covered in detail in Chapter 9 by Berthoud in this volume.

A second major challenge to Stellar's formulation was the rejection of anatomical locations as the appropriate level of analysis. After all, both lesions and electrical stimulation act upon numerous neurochemicals within a single nucleus. Given that these nonspecific techniques provided much of the support for the dual-centers hypothesis, the new emphasis on neurochemical pathways made attributions of function to specific nuclei not only inappropriate but incomplete. The critical questions became which neurochemicals act on which receptors, and what are the sources of these ligands that serve to control ingestive behavior.

The revolution in molecular biology has brought an enormous set of new tools to answer these questions. First, it has made possible the discovery of numerous new neurochemicals that have been linked to the control of food intake and the development of new pharmacological agents that can be administered to influence ingestive behavior. Literally thousands of articles over the past decade have used this strategy to reveal the function of these peptides. Thus, the central challenge is no longer to assess the competing influences of two brain regions in the control of food intake but rather to assess the respective roles of dozens of neuropeptides. While there is little doubt that the identification of these peptides is a significant contribution to our understanding, a new conceptual framework is required to synthesize the roles of these peptides into a coherent and unified system.

Adiposity Signals: Linking Energy Balance to the Regulation of Food Intake

To make sense of the multiple peptide systems involved in the system that controls food intake requires a hypothesis about its central purpose. Our work has been built upon the hypothesis that these systems are there to maintain adequate stores of fuel so that the organism can survive periods of low food availability. To maintain adequate energy stores requires the matching of caloric intake to caloric expenditure over time. Gordon Kennedy (1953) hypothesized that this matching was accomplished by monitoring total body fat. Since body fat represents the difference between caloric intake and caloric expenditure, if total body fat could be monitored, food intake and energy expenditure could be adjusted such that energy stores could be maintained. Thus, one reasonable hypothesis states that a critical purpose of the multitude of peptides involved in the control of food intake is that it uses information about peripheral energy stores (primarily in the form of adipose tissue) to adjust caloric intake to match caloric expenditure over time.

To accomplish this, the brain must be informed about the status of peripheral energy stores. Several lines of evidence now indicate that this information is derived from hormones that can be termed "adiposity signals." Although many hormones have been hypothesized to function as adiposity signals, it is outside the scope of this chapter to discuss them all. However, two such hormones have received the most attention. The first is leptin, the hormone derived from adipocytes. Leptin is secreted in proportion to body fat, with more body fat associated with higher circulating levels of leptin in almost every species in which this relation has been investigated (Friedman, 2002). The significance of this adiposity signal is easily demonstrated in mice that either do not make a biologically active form of leptin or do not make its associated receptor. These mice have increased food intake, decreased energy expenditure, and, as a result, a markedly positive energy balance associated with the deposition of large triglyceride stores in adipose tissue (Leibel, Chung, & Chua, 1997). Thus, even in the face of enormous energy reserves, these mice continue to increment their positive energy balance because they lack a critical adiposity signal. While made in the periphery, leptin appears to be transported across the blood–brain barrier and into the brain via a saturable, receptor-mediated transport system where it can interact with leptin receptors found in several brain regions including, most prominently, the hypothalamus (Schwartz, Peskind *et al.*, 1996; Schwartz, Seeley, Campfield, Burn, & Baskin, 1996).

A parallel case can be made for the pancreatic hormone insulin. Insulin also circulates in proportion to total adipose mass and therefore could inform the brain of the status of peripheral energy balance (Polonsky, Given, & Carter, 1988). Like leptin, insulin is transported into the brain by a saturable, receptor-mediated process, and insulin receptors are found in many of the same regions of the hypothalamus as are leptin receptors (Baskin *et al.*, 1990; Schwartz *et al.*, 1990). The case for insulin acting in the brain as an adiposity signal that lowers body fat is more complex than that of leptin because of the important role of insulin action in peripheral tissues to regulate glucose homeostasis and to promote fat accumulation. Further, because complete genetic disruption of insulin signaling is not compatible with life, alternative approaches have been taken to provide evidence that endogenous insulin acts in the brain as an adiposity signal. Mice with reduced insulin receptor expression in the brain show increased food intake and body

weight (Brüning *et al.*, 2000; Obici, Zhang *et al.*, 2002). Both are consistent with previous research in which insulin antibodies administered locally to the brain increased food intake and body weight (McGowan, Andrews, & Grossman, 1992).

Hypothalamic Peptides: From "Alphabet Soup" to Effector Pathways

Rapid advances in molecular biology have made possible the identification of numerous novel peptide systems involved in the control of food intake and body weight. This "alphabet soup" of newly discovered peptides is both confusing and daunting, in part because the neuroanatomical connections among these peptides are numerous and complex (Elmquist, Maratos-Flier, Saper, & Flier, 1998; Saper, Chou, & Elmquist, 2002). In order to unravel the functional circuits that control ingestive behavior, I will begin with the ones that are directly regulated by adiposity signals. After all, if the central purpose of the system is to maintain normal energy homeostasis by regulating the amount of peripheral adipose tissue, the actions of these adiposity signals define one starting point from which to describe the circuit.

Functional adiposity signals should interact with two categories of peptide systems within the brain. Much like the scheme articulated by Stellar, systems can be divided into those that increase food intake and those that decrease it. Rather than terming these "hunger" and "satiety" systems, however, we refer to them as "anabolic" and "catabolic" effector systems, respectively (Schwartz & Seeley, 1997; Schwartz, Woods, Porte, Seeley, & Baskin, 2000; Woods, Seeley, Porte, & Schwartz, 1998). The terms anabolic and catabolic are meant to emphasize that these peptide systems do not simply influence the onset or size of individual meals but more generally affect long-term energy balance. Thus anabolic systems are those that when activated produce an increase in stored energy and body weight by increasing food intake, decreasing energy expenditure, or, as is often the case, both simultaneously. Catabolic systems are those that when activated produce decreases in stored energy and body weight by decreasing food intake, increasing energy expenditure, or both.

In order to match intake to expenditure and maintain constant energy stores, the activity of these anabolic and catabolic systems are tied to the levels of adiposity signals, including leptin and insulin. Adiposity signals inhibit anabolic effector systems and stimulate catabolic effector systems when an animal is in positive energy balance, leading to a lowering of energy stores. Conversely, when an organism experiences negative energy balance, anabolic systems that are normally inhibited by adiposity signals become disinhibited while the activation of catabolic systems is decreased. This reciprocal combination drives the animal to increase energy intake and decrease energy expenditure resulting in the restoration of peripheral energy stores.

The Arcuate Nucleus

The Melanocortin System. A logical place to begin untangling the highly interconnected circuits that control energy balance is the arcuate nucleus of the hypothalamus. The arcuate is located in the most medial and basal portion of the hypothalamus and contains numerous peptides including several related to the melanocortin (MC) system. Traditionally, the MCs have been studied for their critical role in the periphery to regulate skin and hair pigmentation. However, diverse lines of evidence also link brain MCs to the control of food intake (Cone, 1999). In particular, two G-protein-coupled MC receptors have been found within the brain. The MC3 and MC4 receptors are both found in the hypothalamus but the MC4

receptor also has prominent expression levels in other regions of the brain including the caudal brainstem and the nucleus accumbens. Targeted genetic disruption of the MC3 receptor results in a mild increase in body fat with little or no change in overall body weight, food intake, or energy expenditure (Butler *et al.*, 2000; Chen *et al.*, 2000). In contrast, targeted disruption of the MC4 receptor results in a large increase in body weight, adipose tissue, and food intake (Huszar *et al.*, 1997). Thus, it would appear that the MC4 receptor activity provides crucial inhibitory tone that acts to prevent further accumulation of energy stores.

Activity at the MC4 receptor is regulated at several levels. First is the release of the endogenous agonist that is encoded in a large precursor peptide termed proopiomelanocortin (POMC). This precursor is then processed within neurons to make several specific peptides including MC receptor ligands. As the name implies, this precursor also encodes the opioid receptor agonist, β-endorphin. Since β-endorphin increases food intake, much of the speculation about the function of POMC producing cells in the arcuate nucleus were tied to the opioid system (Glass, Billington, & Levine, 1999, 2000). However, the regulation of POMC gene expression in the arcuate did not fit an anabolic system. Rather, POMC gene expression is decreased by negative energy balance and increased by positive energy balance (Bergendahl, Wiemann, Clifton, Huhtaniemi, & Steiner, 1992; Hagan *et al.*, 1999). Moreover, both leptin and insulin receptors are found on POMC neurons and both have been shown to stimulate POMC gene expression (Mizuno *et al.*, 1998; Schwartz *et al.*, 1997). Thus, the POMC neurons appear to be a key component of the catabolic system that decreases food intake and lowers body energy stores.

α-Melanocyte Stimulating Hormone (α-MSH) is an agonist for both MC3 and MC4 receptors and is a product of the processing of the POMC precursor. In the periphery, α-MSH is an agonist for the MC1 receptor where it regulates melanin and thereby controls pigmentation (Barsh, Farooqi, & O'Rahilly, 2000). α-MSH was shown to reduce food intake in the 1980s, and recent work with a number of synthetic analogues of α-MSH have shown this to be a potent and long-lasting effect that reduces energy stores (McMinn, Wilkinson, Havel, Woods, & Schwartz, 2000; Shimizu, Shargill, Bray, Yen, & Gesellchen, 1989; Tsujii & Bray, 1989).

A second level of regulation of MC activity occurs at MC receptors. In the periphery, it had been shown that whereas MC activity could be regulated α-MSH, the magnitude of the effect could be decreased by interaction with Agouti Signaling Protein (ASP). ASP is both a traditional competitive antagonist for α-MSH as well as an inverse agonist (Yen, Gill, Frigeri, Barsh, & Wolff, 1994). That is, ASP appears to suppress MC receptor induced activity even in the absence of α-MSH. Overexpression of ASP in agouti mice results in a yellow coat-color as a result of ASP action on MC1 receptors. However, these mice also are obese in consequence of increased food intake and decreased energy expenditure. This obesity is the result of ASP being inappropriately expressed in the brain where it interacts with MC3 and MC4 receptors (Cone *et al.*, 1996; Fan, Boston, Kesterson, Hruby, & Cone, 1997). The relevance of these obese, yellow mice to the normal control of food intake was quite unclear until new tools were developed that allowed identifying the genetic sequence of the mutation in these mice and comparing it to other pieces of the genome. This process led to the discovery of a gene with a very similar sequence as ASP, but that is normally expressed in the brain (Shutter *et al.*, 1997). The gene encodes a peptide termed Agouti-Related Protein (AgRP), and it is found exclusively in the arcuate nucleus. Interestingly, AgRP is not co-localized with POMC but exists in a distinct population of neurons that are regulated in

a reciprocal manner. Thus, negative energy balance causes a robust increase in AgRP mRNA whereas leptin inhibits AgRP expression (Hahn, Breininger, Baskin, & Schwartz, 1998; Ollmann *et al.*, 1997).

Administration of AgRP into the brain causes a marked increase in food intake and rapid weight gain. In fact, a single dose of AgRP can increase food intake for periods as long as 6 days. The mechanism for this unique long-term effect is unknown but it would appear not to be mediated by continued MC receptor antagonism (Hagan *et al.*, 2000, 2001). Overexpression of AgRP results in an obese phenotype similar to that of the agouti mouse (except without the yellow coat-color since AgRP does not interact with peripheral MC1 receptors [Ollmann *et al.*, 1997]). These powerful effects to increase body fat stores by inhibiting the activity of an adiposity signal make AgRP a classic anabolic effector. Thus, within just the brain MC system, there would appear to be both catabolic and anabolic effectors, with the balance of activity between them determining the amount of inhibitory tone that restrains subsequent food intake and the accumulation of additional adipose stores.

The complexity of this system was revealed by recent observations that MC receptor signaling in the brain is influenced by a class of heparin-sulfated proteoglycans termed syndecans. Syndecan-3, in particular, is expressed at high levels within the brain in regions with heavy expression of MC receptors such as the paraventricular nucleus of the hypothalamus (Reizes *et al.*, 2001). When attached to the cell surface, syndecan-3 appears to act as a specific "coreceptor" for AgRP that increases its ability to bind and reduce MC receptor activity. However, syndecan-3 also can be cleaved from the cell surface. When this occurs, "shed" syndecan-3 binds AgRP, thus inhibiting AgRP's ability to bind to MC receptors, and consequently reducing AgRP's ability to reduce MC receptor activity (Reizes *et al.*, 2001). Thus, both expression of syndecan-3 and whether it is shed can influence the actions of the endogenous antagonist and thereby provide yet another mechanism by which MC tone is regulated.

In addition to adiposity signals, a number of other systems influence MC signaling (Cowley *et al.*, 2001). From the periphery, two gut hormones seem to have important and direct actions on the MC system. One is the gut hormone PYY that circulates in the form of PYY (3–36) and is secreted into the circulation after meals. PYY (3–36) is an agonist for Neuropeptide Y (NPY) Y2 receptors that are found in the arcuate nucleus. PYY (3–36) administered either peripherally or directly into the arcuate nucleus has been reported to cause a reduction in food intake that is associated with increased firing of POMC neurons (Batterham *et al.*, 2002). Ghrelin is a hormone released from the stomach and also made within the hypothalamus. Either peripheral or central administration of ghrelin increases food intake, and this effect is associated with decreased activity of POMC neurons and activation of AgRP neurons (Cone *et al.*, 2001; Cowley *et al.*, 2003; Tschop *et al.*, 2000). Finally, administration of serotonin agonists reduces food intake and body weight. These drugs, like PYY (3–36), increase the firing rate of POMC neurons, and their effects on food intake can be blocked by MC receptor antagonists (Heisler *et al.*, 2002). Collectively, these data point to the MC system as a nodal point that integrates various peripheral and central signals to regulate energy balance.

Neuropeptide Y. The MC system provides an important example that highlights how recent research has revealed the redundant and over-determined system by which the brain matches intake to expenditure. Needless to say, the MC system

is but one of many peptide systems that act in the hypothalamus and other brain locations to regulate energy stores. The system that has received the most attention in this regard is NPY. NPY is an abundant brain peptide whose genetic sequence is very similar across a wide range of species (Boswell *et al.*, 1993). Administration of NPY into the brain produces a vigorous feeding response, and repeated administration results in positive energy balance and an increase in body weight (Clark, Kalra, Crowley, & Kalra, 1984; Stanley & Leibowitz, 1984). Consistent with a role of NPY as an anabolic effector, leptin receptors are found on NPY neurons within the arcuate, and leptin inhibits NPY gene expression in the arcuate (Schwartz, Baskin *et al.*, 1996; Schwartz, Seeley *et al.*, 1996).

The arcuate, however, is not the only source of NPY even within the hypothalamus, and NPY made within the dorsomedial nucleus of the hypothalamus (DMH) appears to be regulated differentially than in the arcuate. NPY in the DMH is elevated in a number of genetic models of obesity whereas NPY levels in the arcuate are either unaffected or actually decreased (Guan *et al.*, 1998; Kesterson, Huszar, Lynch, Simerly, & Cone, 1997). One model, OLETF rats, is particularly interesting. These animals lack receptors for the cholecystokinin (CCK)-1 receptor, and CCK is an intestinal peptide that promotes satiety. CCK-1 receptors have been found on NPY producing neurons in the DMH (Bi, Ladenheim, & Moran, 2000). Thus, both arcuate and DMH populations of NPY neurons likely contribute to NPY signaling within the hypothalamus, but the exact role of each population is as yet unclear.

Mice with a targeted genetic disruption of the NPY gene have been generated. If NPY plays a key role as an anabolic effector critical to the regulation of energy balance, then such mice might be expected to be lean and not respond appropriately to negative energy balance. Both of those expectations are incorrect (Erickson, Clegg, & Palmiter, 1996). However, when NPY-deficient mice are crossed with obese leptin-deficient mice, the offspring are considerably leaner than leptin-deficient mice with an intact NPY gene (Erickson, Hollopeter, & Palmiter, 1996). Thus, part of the response to leptin deficiency does depend on elevated levels of NPY that result from leptin deficiency.

Several lines of evidence are consistent with the hypothesis that NPY serves not simply to increase food intake but to provide the anticipatory responses necessary when large meals are consumed (Woods *et al.*, 1998). Large meals necessarily disrupt a number of homeostatically regulated variables, including blood glucose and body temperature (Woods & Strubbe, 1994). Animals learn to anticipate large meals and initiate a variety of physiological responses that minimize the resulting perturbations. For example, in anticipation of a rise in blood glucose from ingested food, animals and humans secrete insulin and thereby minimize the rise in blood glucose (Berthoud, Bereiter, Trimble, Siegel, & Jeanrenaud, 1981; Teff & Engelman, 1996). Interestingly, central administration of NPY will produce significant insulin secretion even when food is not available (Billington, Briggs, Grace, & Levine, 1991; Billington, Briggs, Harker, Grace, & Levine, 1994). In addition, NPY expression in the arcuate of rats peaks prior to the onset of the dark phase, which is when the largest meals tend to be consumed (Kalra, Dube, Sahu, Phelps, & Kalra, 1991; Sahu, White, Kalra, & Kalra, 1992). Moreover, when animals have access to food for only a short period at the same time each day, NPY mRNA and protein levels peak just prior to that large meal. Thus, NPY might respond to classically conditioned cues that signal the occurrence of a large meal and thereby limit the perturbations it might cause in various physiological functions. This hypothesis predicts that mice lacking the gene for NPY may not have a deficit in the control of

their overall energy balance but may eat smaller and more frequent meals than control animals.

A Working Model of the Arcuate Nucleus. The combination of transgenic technology with electrophysiological recordings has made it possible to create a detailed model of the neuroanatomical wiring and interactions within the arcuate nucleus of the hypothalamus (Cowley *et al.*, 2001; see Figure 1). The picture that emerges is one in which two distinct populations of cells reside within the arcuate: catabolic cells that express POMC and release α-MSH, and anabolic cells that express and release both NPY and AgRP. Both sets of cells are influenced in opposite directions by a number of factors extrinsic to the arcuate nucleus. Leptin, insulin, and serotonin all stimulate POMC neurons while inhibiting NPY/AgRP neurons. Ghrelin, on the other hand, stimulates NPY/AgRP neurons while inhibiting POMC neurons.

In addition to these coordinated responses to ligands originating outside the arcuate nucleus, the two populations of neurons influence each other directly. POMC neurons contain MC3 receptors and thus could be inhibited by the AgRP from the adjacent cells. POMC cells are also inhibited by gamma-aminobutyric acid (GABA) secreted from NPY/AgRP cells within the arcuate. Finally, the NPY Y2 receptor has been hypothesized to be an inhibitory autoreceptor on NPY neurons including those in the arcuate (Schober, Gackenheimer, & Gehlert, 1996).

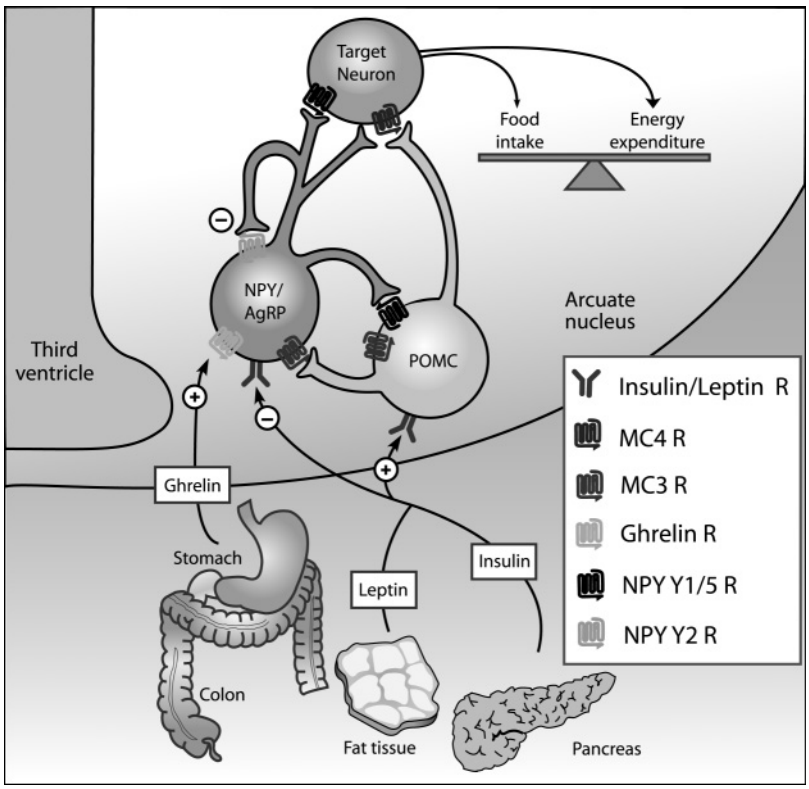

Figure 1. Schematic of the proposed anabolic and catabolic circuitry of the arcuate nucleus of the hypothalamus. (Adapted from Barsh & Schwartz, 2002.)

Thus, the effects of the Y2 agonist, PYY(3–36), to reduce food intake might result from inhibiting the release of GABA from NPY/AgRP neurons within the arcuate, thereby disinhibiting POMC neurons (Batterham *et al.*, 2002). While some details of this model are unsettled, converging evidence from anatomical, gene expression, mouse transgenic models, electrophysiological, and pharmacological studies have pointed to the nodal control of the anabolic and catabolic effectors in the arcuate nucleus as they integrate a wide range of inputs to guide both the ingestion of calories and their rate of expenditure.

Lateral Hypothalamic Peptides

Melanin Concentrating Hormone. The exploration of peptides involved in the control of ingestion has not been limited to the arcuate nucleus. Stellar and many others had identified the LH as a "hunger center" since electrical stimulation there elicited a robust feeding response while lesions produced hypophagia and weight loss (Stellar, 1954; Teitelbaum & Epstein, 1962). Therefore it would make sense to find anabolic effector pathways within this area, thus providing a possible underlying neurochemical basis for these phenomena. Using molecular biology tools, two peptides localized almost exclusively to the LH have been discovered. One is melanin concentrating hormone (MCH), which was identified as a gene whose expression was elevated in the hypothalamus of leptin-deficient mice (Qu *et al.*, 1996). Thus, like an anabolic effector, MCH expression was inhibited by an adiposity signal. Consistent with this hypothesis, intracranial administration of MCH produces a reliable increase in food intake (Rossi *et al.*, 1997; Sanchez, Baker, & Celis, 1997). Mice with targeted disruption of the MCH gene consume fewer calories, have higher metabolic rates, and have less body fat than their wild-type controls (Ludwig *et al.*, 2001; Shimada, Tritos, Lowell, Flier, & Maratos-Flier, 1998). MCH has very broad and diffuse projections throughout the forebrain such that its function is not likely to be limited to the control of food ingestion. For example, like lateral hypothalamic stimulation, administration of MCH induces water consumption independent of its effects on food intake (Clegg *et al.*, 2003).

Orexin/Hypocretins. Other peptide-containing neurons within the LH are differentially regulated by changes in energy balance (Broberger, De Lecea, Sutcliffe, & Hokfelt, 1998; Peyron*et al.*, 1998). These peptides were contemporaneously discovered by two separate groups of investigators. One group termed them hypocretin 1 and 2 while the other termed them orexin-A and B (de Lecea *et al.*, 1998; Sakurai *et al.*, 1998). Like MCH, prepro-orexin is inhibited by leptin while administration of orexin-A increases food intake. Also like MCH, projections from orexin-containing neurons are quite diffuse, and a role for the orexin system in regulating sleep and waking has been identified. In fact, genetic narcolepsy in dogs is caused by a targeted genetic deletion in one of the two identified orexin receptors (Lin *et al.*, 1999). Regardless, the destruction of neurons containing MCH and orexin likely contributes to the well-known effects of lateral hypothalamic lesions and stimulation on food intake and body weight as well as other aspects of the LH syndrome.

The Future: Conundrums and Opportunities

This chapter has provided a broad overview of how recent progress in the biology of neuropeptides has advanced our understanding of ingestive behavior and the

overall regulation of energy balance. However, the chapter does not present a complete review of hypothalamic peptides that influence food intake (see Table 1). In fact, the recent pace of discovery in this field has made a single comprehensive review nearly impossible. The rather lengthy list in Table 1 has led some scientists to attempt to identify which of these peptides is the *most* important. This desire is understandable as academic scientists need to focus on the most critical aspects of this increasingly complex system, and industrial scientists need to focus on the parts of the system that are the most promising for having large therapeutic effects. Unfortunately there is no simple way by which the most critical pieces of the system can be identified.

For example, let's compare the relative potency of NPY, AgRP, and MCH in increasing food intake. When rats are fed ad lib over a relatively short time period (e.g., 2 hr), NPY is the most potent of these three orexigenic peptides, and it is followed closely by AgRP with MCH coming in a distant third. The conclusion one might draw would be that NPY is therefore the most important. However, when intake is considered over 24 hr, AgRP is far more potent than either NPY or MCH, and the consequent conclusion would be that AgRP is the most important. Hence, there are fundamental differences among these peptides with regard to the duration of their action. At another level, it could be argued that looking at food intake after a single bolus administration of these peptides does not address the critical issue. After all, such a bolus injection simulates neither the way these peptides are secreted nor how they are likely to be used in a therapeutic setting. As an alternative, one could compare the effect of each peptide when administered either as

Table 1. Partial List of Hormones and Neurotransmitters Reported to Act on the Hypothalamus to Alter Food Intake and/or Body Weight

Anabolic	Catabolic
Agouti-Related Protein	α-Melanocyte Stimulating Hormone
Beacon	Amylin
β-Endorphin	Ciliary Neurotrophic Factor
Corticosterone	Cocaine- and Amphetamine-Related Transcript (CART)[a]
Dopamine	Corticotropin Releasing Hormone
Dynorphin	Galanin-Like Peptide
Endocannibinoids	Glucagon-Like Peptide 1
Ghrelin	Glucagon-Like Peptide 2
Interleukin-1 Receptor Antagonist	Histamine
Melanin Concentrating Hormone	Insulin
Neuropeptide Y	Interleukin-1
Norepinephrine	Interleukin-2
Orexins/Hypocretins	Leptin
	Neurotensin
	Oxytocin
	Prolactin-Releasing Peptide
	Serotonin
	Tumor Necrosis Factor-α
	Urocortin
	Urocortin II
	Urocortin III

Catabolic refers to compounds that suppress food intake and produce weight loss while anabolic refers to compounds that increase food intake and body weight.
[a]CART has additionally been shown to increase food intake when administered directly into the arcuate nucleus of the hypothalamus.

repeated daily doses or as a continuous infusion over several days. In that situation, AgRP and NPY have comparable potency and animals become obese rather quickly. MCH, on the other hand, is relatively ineffective at changing body weight. The conclusion from these observations would not only be that AgRP and NPY are more important than MCH, but that MCH has little or no role in the control of energy balance. Thus, how the experiment is designed has a large impact on the conclusion about which peptide system is *most* important.

Of course these pharmacological experiments are not the only way to approach the issue. One could assess their importance by comparing the phenotype of mice with targeted deletion of their respective genes. MCH knockout mice have large reductions in body fat stores while neither NPY nor AgRP knockouts show any diminution of body fat stores. Consequently, those data would lead to the conclusion that MCH is critical to long-term energy balance regulation and both NPY and AgRP have little or no role. Taken at face value, there is no straightforward hypothesis that unites these observations, and they have led to considerable debate over the relative merits of the different approaches. Needless to say, as the list grows from three peptides to three dozen, the problem of assigning relative importance becomes even more arduous.

My own conclusion is that trying to rank order these systems is ultimately a futile exercise. After all, these complex and incestuous peptide systems presumably evolved to confer additional reproductive fitness for animals living in complex and sometimes unpredictable environments. The regulation of energy balance is vitally important in enabling an organism to pass along genes to their offspring. Neither rats nor mice living in simple laboratory environments with ad lib access to a single, nutritionally complete diet reflects the complexities for which these systems evolved. Thus, the answer to which system is most important is an emphatic "it depends." Any rank ordering of these systems in terms of their importance will differ greatly depending on the problem facing the animal, with specific peptides being more critical in some situations than in others. The implication is that assessing the contribution of any one system to the increasingly widespread incidence of human obesity and its potential utilization for therapy may depend greatly on external variables that we have yet to assess in these animal models.

The identification of these peptides is only the first critical step in a long and difficult process of understanding how the system is actually put together. Powerful gene and protein expression profiling will continue to add to the list of peptides and other proteins associated with the regulation of energy balance. Fortunately, that list is finite and the emphasis on linking these peptides into functional circuits no doubt will continue to rise. While the anatomical description is necessary, these functional circuits will require using a variety of developing tools to look at patterns of changing activity in various neuronal populations during and after manipulations of energy balance. This chapter has tried to provide one way to begin building those functional circuits by emphasizing the actions of adiposity signals. Needless to say, this orientation depends on the implicit assumption that by using these adiposity signals as the starting point, we are more likely to uncover those aspects of this system critical for the maintenance of long-term energy balance. This assumption, while defensible, may turn out to be incorrect.

In addition to building functional circuits, future research is likely to emphasize two areas that have not yet received much attention. The first of these is the advancing knowledge of how individual cells sense their fuel status. Conventional wisdom held that under normal conditions neurons use only carbohydrates as fuel.

However, recent findings that implicate a role for fatty acid synthesis and oxidation in select populations of neurons are challenging that assumption (Loftus *et al.*, 2000; Obici *et al.*, 2002). How ongoing fuel sensing is integrated with information about the status of stored fuel to influence peptide networks is a critical question whose answer holds great promise to increase our understanding of how energy balance is regulated.

The second area that is likely to receive increased attention is the application of our progressing knowledge of cellular signaling to these hypothalamic peptide circuits. While cell signaling work has traditionally been studied in *in vitro* experiments, these tools and techniques are increasingly being applied *in vivo*. For example, recent work has shown that inhibition of either phosphatidylinositol 3-kinase or phosphodiesterase 3 blocks the effect of leptin and insulin to reduce food intake *in vivo* (Niswender *et al.*, 2001, 2003, Zhao, Huan, Gupta, Pal, & Sahu, 2002). These advances will continue to make it possible to explore how various receptor-mediated intracellular cascades might be integrated within particular populations of neurons via specific intracellular signaling pathways (see Bates *et al.*, 2003 for another example). Assessing the importance of these signaling pathways to the actions of specific peptides will provide much needed insight into how these neurochemical signals are translated into changes in activity and new protein synthesis. Like the revelation of new metabolic pathways, identifying these signaling pathways will provide new possibilities for interventions that could be used to treat obesity and add tremendously to our understanding of how peptides serve to regulate energy balance.

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7

Brain Insulin and Obesity: From Man to *C. elegans*

A Personal View

Daniel Porte, Jr.

Introduction

This chapter reviews development of a concept first proposed by Dr. Stephen Woods and me in 1976, that plasma insulin provides a critical feedback signal to the Central Nervous System (CNS) for the biological regulation of body adiposity. It is a personal history and describes how an interest in the role of the CNS to regulate insulin secretion was discovered and eventually led us to propose this feedback system for the regulation of energy balance. The subsequent expansion of this idea is then described and many of our key findings are commented upon. The potential impact of this system on the problem of obesity is discussed and some hypotheses are suggested to indicate its potential importance to human disease. Also reviewed are the more recent neuroendocrine studies, which describe some of the major hypothalamic pathways and intracellular signaling mechanisms that now provide possibilities for new treatments. Finally, the recent remarkable realization that this energy signaling system is present and regulates reproduction and life span in *C. elegans* and *Drosophila* is described. The impact of these findings on understanding mammalian physiology is introduced to indicate the widening implications of the brain insulin system that has held my interest for more than 35 years.

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Neurobiology of Food and Fluid Intake, 2nd Ed., Volume 14 of *Handbook of Behavioral Neurobiology,* edited by Edward Stricker and Stephen Woods, Kluwer Academic / Plenum Publishers, New York, 2004.

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My introduction to insulin secretion began by measuring plasma levels using the relatively new insulin immunoassay in 1966. I was studying the lipolytic effect of epinephrine in man and had found an unusual free fatty acid (FFA) response pattern, which suggested counter-regulation of fatty acid mobilization. This pattern could be explained if epinephrine altered insulin secretion, but at that time insulin was believed to be regulated only by plasma glucose. The concept of its modulation by any other factor was not seriously considered. Therefore it was a great surprise to discover that epinephrine and norepinephrine were major inhibitors of insulin secretion in man (Porte, Graber, Kuzuya, & Williams, 1966; Porte & Williams, 1966).

This finding led me to focus on insulin secretion and how it contributed to metabolic disease. I soon turned to study the mechanisms for hyperglycemia in type 2 diabetes. The earliest experiments were performed in normal and diabetic individuals who had varying body weight, because we knew obesity was a major contributor to the metabolic abnormalities of diabetes. We were fortunate to have picked a range of body weights for both our control and diabetic populations that matched reasonably well. We found that insulin levels were much higher in obese normal glucose-tolerant subjects compared to lean subjects (Figure 1), and concluded, therefore, that obesity was a major independent contributor to circulating insulin levels (Bagdade, Bierman, & Porte, 1967). Furthermore, in 1967 we observed that basal insulin levels predict the insulin response to a standardized glucose challenge. Thus, when expressed as a percent of basal values, the insulin responses were similar between lean and obese normal glucose-tolerant subjects. By this method it was possible to eliminate the obesity factor by analyzing the insulin release data as the percent of basal or relative response. We then compared insulin responses in individuals with type 2 diabetes and glucose-tolerant controls and found that while obesity was associated with hyperinsulinemia, type 2 diabetic subjects had insulin responses that were always lower than those of controls when appropriately matched for body adiposity, or expressed as a percent of basal, which we termed the relative insulin response. We therefore concluded that an elevated basal insulin level is the primary abnormality of insulin secretion in obesity and represents a total adjustment of the endocrine pancreas to the obese state (Porte & Bagdade, 1970).

This phenomenon of elevated basal insulin levels in obesity was eventually shown to be largely related to insulin resistance, which remained to be quantified by others in the future. We did however comment in a review that obesity and diabetes were an "odd couple" in that while most patients with type 2 diabetes are obese, most obese individuals never develop type 2 diabetes. We pointed out that it was also "odd" that obesity is associated with hyperinsulinemia while diabetes is associated with reduced relative insulin responses (Bierman, Bagdade, & Porte, 1968). Thus, my interest in understanding the pathophysiology of obesity and its association with other diseases, particularly type 2 diabetes, developed at the same time as my interest in the action of epinephrine on the secretion of insulin.

After recognizing the effects of epinephrine and norepinephrine on insulin secretion in man, studies of the neural regulation of insulin secretion were transferred to animals, and we identified several pathological states related to stress-induced hyperglycemia that were characterized by impaired insulin secretion. The mechanism of the impairment was activation of the α-adrenergic receptor on

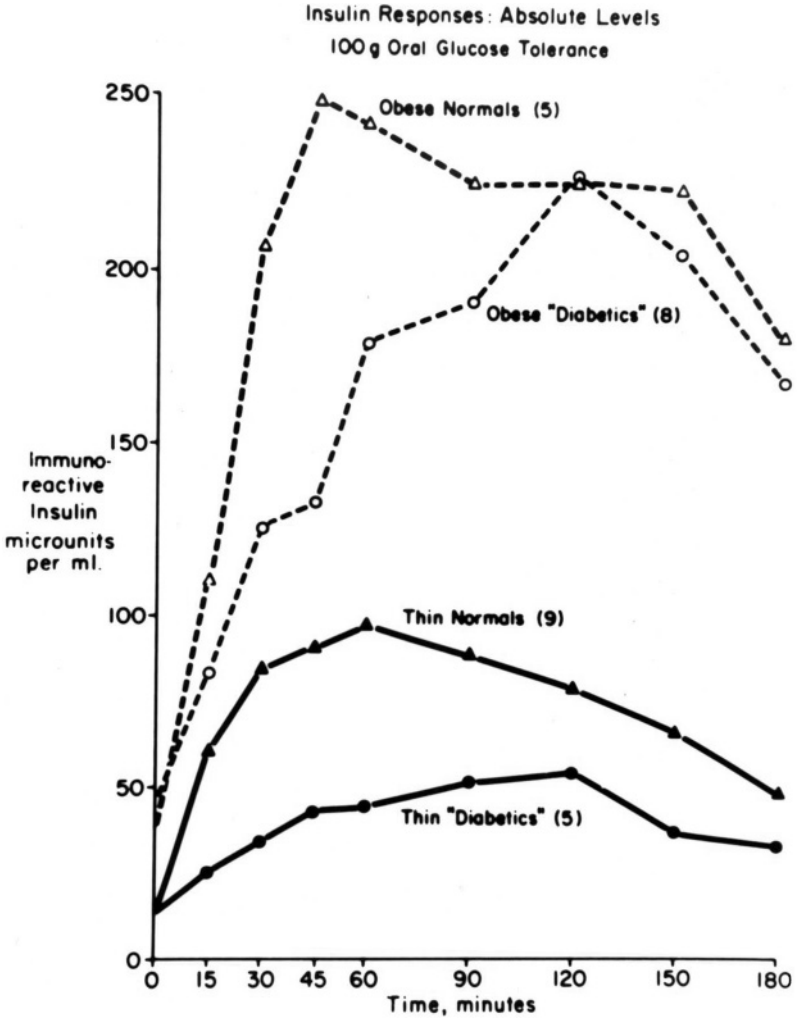

Figure 1. Insulin levels before and after 100 g oral glucose in lean and obese, normal and type 2 diabetes. Note the hyperinsulinemia basal and post challenge in the obese and the impaired responses in type 2 diabetes when matched for body weight. (From Bagdade *et al.*, 1967.)

endocrine pancreatic β-cells by endogenous epinephrine and norepinephrine. While both of these catecholamines were known to circulate in plasma, the question arose as to whether the sympathetic nervous system, which was known to be the major source of circulating norepinephrine, might play a direct role to regulate insulin secretion from the endocrine pancreas. This question was addressed during a sabbatical year at the University of Geneva because of a willingness of colleagues with neurophysiological and biochemical expertise to collaborate in the development of a suitable animal model for the assessment of what was at the time thought to be an unlikely possibility, that the nervous system regulated the endocrine system directly to control plasma insulin. However, during that year we developed an appropriate model in the dog to demonstrate regulation of both insulin and glucagon secretion by the sympathetic nerves that accompany the small arteries

supplying the pancreas (Marliss *et al.*, 1973; Porte, Girardier, Seydoux, Kanazawa, & Posternak, 1973).

Upon my return to the United States I was asked to collaborate on a project designed to assess the potential role of the parasympathetic nervous system to regulate insulin secretion. The model chosen was conditioned hypoglycemia, which Dr. Woods had demonstrated by pairing insulin-induced hypoglycemia with a novel odorant, and then demonstrating hypoglycemia when the odorant was presented to the conditioned animal (Woods & Shogren, 1972). Because insulin had been used to produce the conditioning, it was not possible to assess insulin levels using the immunoreactive insulin assay technology. Therefore, we developed a model using tolbutamide, a well-recognized insulin secretogogue, to produce the same type of conditioned hypoglycemia (Woods, Alexander, & Porte, 1972), and showed that under these conditions, the conditioned hypoglycemia was blocked by vagotomy. This finding suggested that parasympathetic input into the endocrine pancreas was the responsible mechanism (Woods, 1972). Simple assessment of circulating insulin levels during the conditioned hypoglycemia and comparison with appropriate controls and cholinergic blockers demonstrated quite clearly that conditioned insulin secretion was related to parasympathetic neurotransmitter activation.

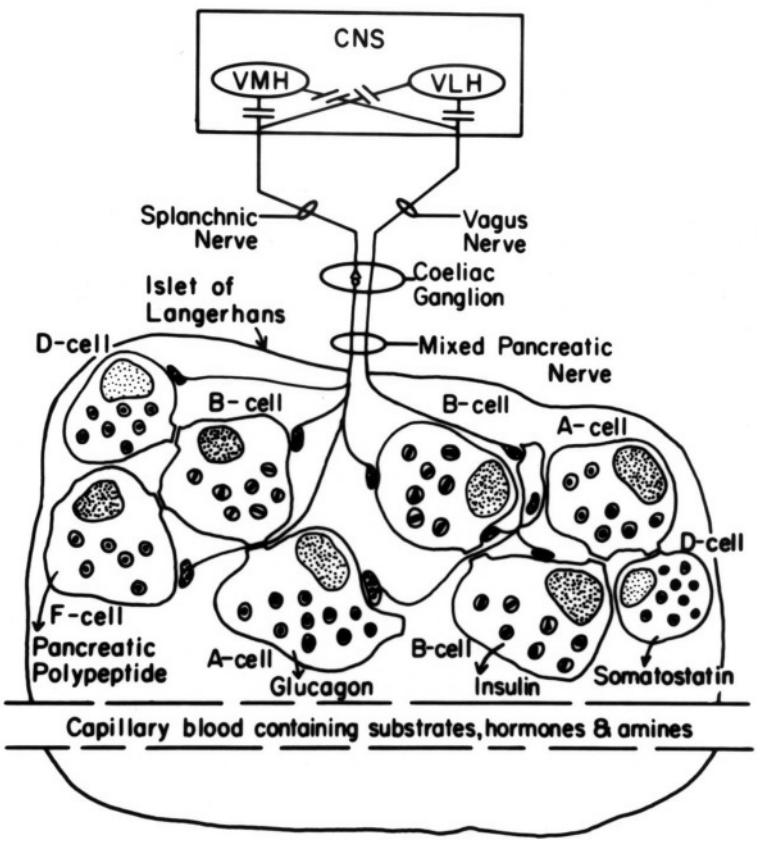

Figure 2. Diagrammatic representation of the autonomic regulation of the endocrine pancreas. Note that the VMH (ventromedial hyperthalamus) and VLH (ventrolateral hypothalamus) contribute neural signals to all cell types and provide both sympathetic and parasympathetic neural regulation. Neuropeptides that are species-specific are also present in postganglionic neurons and contribute to inputs from the same efferent neurons. (From Woods & Porte, 1978.)

These findings led to a major review on the neural regulation of insulin secretion (Woods & Porte, 1974), and a second review on the role of the nervous system in the pathophysiology of stress-induced hyperglycemia and its potential contribution to diabetes mellitus in man (Porte & Robertson, 1973). In considering the central neural inputs to the endocrine pancreas, we became aware of a puzzling paradox. Although stimulation of the ventromedial and ventrolateral hypothalamic regions of the brain individually produce inhibition and stimulation of insulin secretion (Figure 2), bilateral lesioning of these brain sites leads to a sustained reregulation of body weight and no change in plasma glucose. These hypothalamic areas were therefore thought to be associated with regulation of food intake, and through that mechanism, to modulate body adiposity (Mayer & Thomas, 1967).

During the mid-1970s I was still pursuing human research on obesity and the mechanism of its association with hyperinsulinemia. As part of our common interest, the Woods group performed a study in four groups of rats. One group was underfed 20% of calories by tube feeding and a second group was overfed 50% of calories by the same mechanism. They were compared with spontaneously free-feeding animals and animals tube-fed to match the free-fed group. The purpose of the experiment was to determine whether hyperinsulinemia is a result or a cause of obesity. The experiment worked as planned; the overfed animals became obese, the underfed animals became lean, and the pair-fed animals gained weight at the usual rate for these relatively young rodents. After approximately 100 days, plasma insulin levels were measured and compared. As expected, insulin levels were elevated in the obese animals and reduced in the lean animals, and there was a good correlation between body weight (and therefore, presumably, adiposity) and circulating plasma insulin levels (Bernstein, Lotter, Kulkosky, Porte, & Woods, 1975). Fortunately, the student who was performing the study continued to observe the animals, all of which were then allowed to feed freely. Remarkably, the previously overweight animals exhibited a reduction in food intake, while the underweight animals showed the opposite response. Therefore, within several weeks, the body weights of all the animals was very similar (Figure 3). This observation, coupled

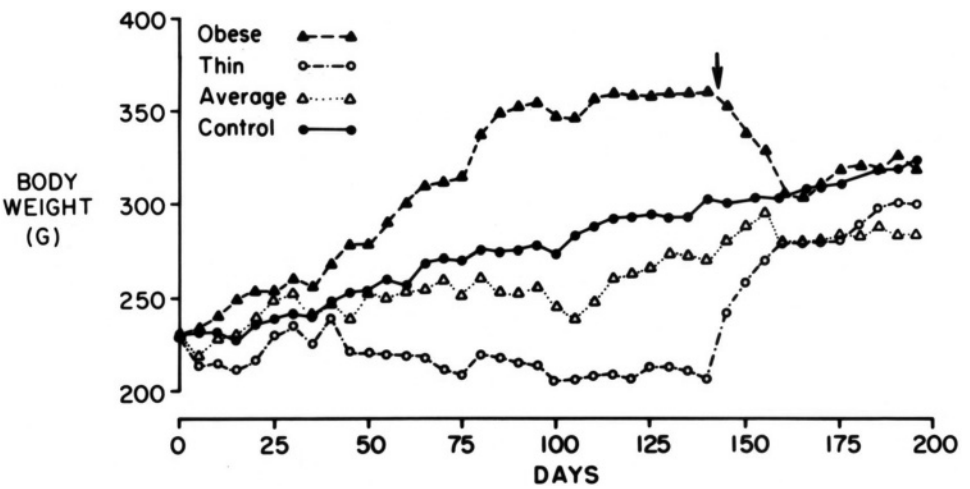

Figure 3. The effect of forced oral feeding, 50%, and under feeding, 20%, of daily calories to normal rats for 140 days compared with free feeding controls and a pair-fed (average) group. At (↓) the experiment was terminated and all animals were free-fed for 60 days. Note that all groups spontaneously returned to the same weights as free-fed controls. (From Bernstein *et al.*, 1975.)

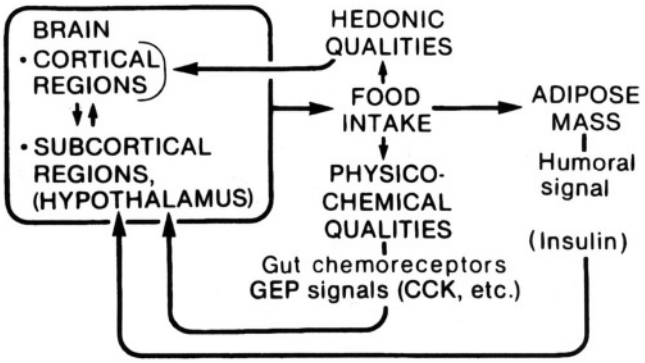

Figure 4. The initial diagrammatic representation of the hypothesis for body weight regulation developed by Woods and Porte. (From Porte & Woods, 1981.)

with our studies of conditioned hypoglycemia, neural regulation of the endocrine pancreas by autonomic nerves, and the recognition that the central sites of these neural signals were associated with alterations in body adiposity, led to a major change in our thinking about the nature of obesity and its pathophysiology (Woods & Porte, 1976) (Figure 4).

In this realignment of our concepts, we were influenced by a paper from Kennedy who suggested in 1953 that hypothalamic mechanisms for the regulation of food intake were sensitive to body fat stores (Kennedy, 1953). Coleman's observations that parabiotic connections between lean and obese animals (e.g., *ob/ob* or *db/db* mice) affect body weight of the parabiotic partner further suggested that the "adiposity signal" was a circulating molecule rather than a neural input (Coleman, 1973; Coleman & Hummel, 1969). Our studies at that time were focused on insulin as a molecule secreted over a 24-hr period in proportion to the quantity of body fat stores. Despite major changes related to meals and other factors important to plasma glucose regulation, average plasma insulin levels over a 24-hr period correlated well with body adiposity, primarily because metabolism during the major part of the day was metabolically similar to the basal state. Thus, mean plasma glucose levels and mean plasma insulin levels were very similar to values obtained in the basal state. These observations suggested the hypothesis that insulin might be the circulating "adiposity signal" that provides information to the CNS for the regulation of food intake and body weight. This hypothesis required both integration of the signal in the CNS over time and a mechanism for the insulin signal to have access to the CNS, a possibility considered unlikely at that time because of the 6,000-Da molecular weight of the insulin peptide. In addition, it was thought that the CNS was not responsive to insulin signaling because, while CNS insulin receptors had been demonstrated (Havrankova, Roth, & Browstein, 1978), exposure of neural tissue to insulin had not been shown to increase glucose uptake into neurons. Furthermore, the insulin-responsive glucose transporter was subsequently found to be largely absent from neuronal tissue. These findings suggested that insulin action in the brain related to food intake and body weight must involve a mechanism independent of glucose uptake into neurons.

The problem of demonstrating insulin's effects on the CNS regulation of energy homeostasis was approached in two ways. First, the hypothesis that insulin has access to the CNS through cerebrospinal fluid (CSF) was addressed. To analyze insulin levels in CSF, we refined the insulin RIA to measure very low insulin

concentrations with improved accuracy. Then we developed a method for serial removal of CSF in an intact animal during the intravenous (IV) infusion of insulin. These methodologies were first developed for studies in dogs, and enabled us to demonstrate the uptake of plasma insulin into CSF in 1977 (Woods & Porte, 1977). Then we developed an animal model in which insulin could be infused into the CNS to test the ability of CNS insulin to affect food intake and body weight. A primate model was chosen because of their availability at the University of Washington, and our belief that this hypothesis would seem more relevant to human physiology and pathophysiology if it could be demonstrated in an animal model close to man. Methods for assessment of food intake and for continuous access to the intraventricular (IVT) cavity thus were established in baboons. In 1979, insulin was infused into the lateral cerebral ventricle of baboons at three different doses over a period of several weeks and compared with an equimolar infusion of the peptide glucagon. Insulin, but not glucagon, induced a dose-dependent suppression of food intake, which took approximately a week or more to reach a maximum. Similarly, recovery of normal intake occurred over a period of 3–7 days at the middle dose (10 μU/kg/day) and over a period of several weeks at the highest dose (100 μU/kg/day). We therefore concluded that circulating insulin must have access to CSF, and that ICV infusion of insulin at doses too low to affect circulating levels acts as a signal to reduce food intake and body adiposity (Woods, Lotter, McKay, & Porte, 1979). Combined with the evidence of body weight regulation (e.g., the rodent study in which overfed and underfed animals returned to the weight of control animals who were allowed to freely feed), we concluded that there was a biological system for the regulation of body adiposity in which insulin was capable of providing long-term feedback from adipose tissue stores to CNS centers controlling food intake (Porte & Woods, 1981). Because the hypothalamus was known to regulate insulin secretion, we hypothesized that insulin acts in the ventral hypothalamus as part of this feedback loop. To further test this hypothesis, we subsequently increased plasma insulin by infusing IV glucose with or without insulin to elevate insulin levels to a variable degree and demonstrated a dose-dependent suppression of food intake as a result of hyperinsulinemia during intravenous nutrient administration (Woods, Stein, McKay, & Porte, 1984). The ability of a hyperinsulinemic euglycemic infusion of insulin and glucose to suppress food intake supported our conclusion that insulin rather than glucose was the relevant signal to the CNS. Subsequently, it was shown that lean *Fa/Fa* rats respond by suppressing food intake and body weight during IVT insulin whereas obese *fa/fa* Zucker fatty rats do not (Ikeda *et al.*, 1986), suggesting a potential role for impaired insulin action in obesity.

Further studies demonstrated that when IVT insulin was infused continuously in rats to reduce their food intake over a 24-hr period they still ate intermittent meals. Food intake was reduced because their meals were smaller and therefore a reduced number of calories was ingested over a 24-hr period. Contemporary studies showed that a variety of gut peptides inhibit food intake in rats. The first of these, cholecystokinin (CCK), was shown to produce the complete behavioral sequence of satiety, apparently differentiating this food reduction from sickness (Gibbs, Young, & Smith, 1973; Smith, Jerome, Cushin, Eterno, & Simansky, 1981). When this research was reviewed in 1981, it was pointed out that some other peptides increase meal duration and therefore meal size. They were known to be produced in the gastrointestinal tract also and it was suggested that both meal stimulation and inhibition was regulated by gut peptides during normal feeding

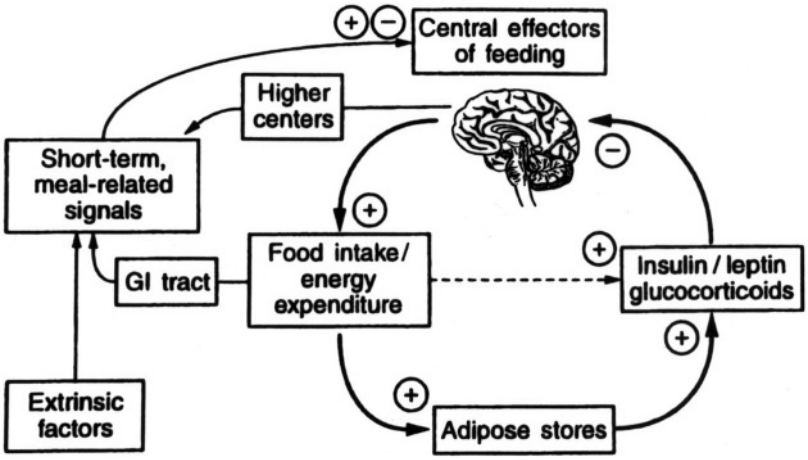

Figure 5. More modern view of body weight regulation. Central effectors contribute to both food intake and energy expenditure. Circulating signals for long-term regulation expanded to include leptin and glucocorticoids in addition to insulin. Meal-related signals interact with long-term signals to integrate day-to-day intake with energy stores. (From Porte *et al.*, 1998.)

(Woods *et al.*, 1981). Their specific role was clarified in a study utilizing a computer-controlled paradigm in 1984 to administer CCK with every meal. This test demonstrated that while there was a reduction in the size of every meal, body weight was only transiently reduced as there was an immediate compensatory increase in meal frequency (West, Fey, & Woods, 1984). Therefore, within a few days, body adiposity stabilized at a value only slightly lower than control animals. This finding supported the idea that gut peptides play a key role in meal size, but also showed they were not direct sensors or regulators of body adiposity. Rather, they appeared to act as short-term regulators of food intake by regulating single-meal size and not as long-term regulators of body adiposity, like insulin. In the mid-1980s we demonstrated that the effectiveness of a short-term regulator, CCK (given peripherally) to reduce meal size, was potentiated by a long-term regulator, insulin (given IVT) in baboons (Figlewicz, Stein, West, Porte, & Woods, 1986). This finding led to the concept of an interaction between single-meal satiety factors to regulate individual meal size with long-term body weight regulating factors, of which insulin was the only one known at that time. The hypothesis that insulin increased the sensitivity of each single-meal satiety factor to reduce single-meal food intake provides a mechanism to explain how single-meal intake is integrated with long-term body weight regulation over time (Figure 5).

Central Neuronal Mechanisms Responsive to Insulin

The CNS site and mechanism of interaction between insulin and the regulation of food intake and body weight remained a mystery until Williams reported in the late 1980s that among a variety of hypothalamic peptides measured in rats with streptozotocin-induced diabetes, the content of neuropeptide Y (NPY) was selectively increased (Williams *et al.*, 1988). Since NPY was known to increase food intake when infused IVT (Clark, Kalra, Crowley, & Kalra, 1984), this response could explain the mechanism of CNS insulin action and the hyperphagia of diabetes.

This increase in NPY was subsequently shown by others to be associated with a diabetes-induced increase in the messenger RNA (mRNA) for NPY in the arcuate nucleus, which was reversed by systemic insulin treatment (White, Olchovsky, Kershaw, & Berelowitz, 1990). Using these findings, we determined whether the insulin effect on expression of NPY mRNA and peptide was direct or indirect by injecting insulin IVT into control-fed rats and 48-hr fasted rats, and comparing it with control CSF injections. The CSF-injected rats showed a marked increase in arcuate nucleus NPY mRNA and hypothalamic NPY content during a fast that was completely blocked by IVT insulin (Schwartz, Sipols *et al.*, 1992). Subsequently, this study was repeated using diabetic animals. As expected, NPY mRNA in the arcuate nucleus was increased in diabetes and was associated with hyperglycemia and hyperphagia. When insulin was given IVT, there was a reduction in arcuate nucleus NPY mRNA compared with controls. While there was no change in hyperglycemia or polydipsia, both food intake and body weight were reduced. Based on these studies it was concluded that arcuate nucleus neurons containing NPY are a major site of insulin action in the CNS and that diabetic hyperphagia was the result, at least in part, of CNS insulin insufficiency (Sipols, Baskin, & Schwartz, 1995).

At about that time, insulin uptake into CSF was quantified in a dog model and the system responsible for this uptake was characterized. Uptake was first shown to be delayed and curvilinear, consistent with a transport mechanism rather than simple diffusion (Schwartz *et al.*, 1990, 1991). *In vitro* studies had demonstrated insulin receptor-based transport across endothelial cells without degradation of the internalized insulin (King & Johnson, 1985). We therefore hypothesized that a similar system was responsible for insulin transport across cerebral microvessel endothelium. Mathematical modeling of the kinetics of insulin uptake from plasma into CSF of dogs required three compartments to explain the dynamics, consistent with the idea that uptake occurred from plasma into brain interstitial fluid and then into CSF rather than directly from plasma into CSF. This uptake pattern also was consistent with a saturable mechanism with an apparent K_M comparable to the K_D of the insulin receptor (Baura *et al.*, 1993). According to this model, the high concentration of insulin receptors found in the choroid plexus prevents insulin access from plasma by degrading it, as in the liver, rather than transporting it into CSF like the cerebral microvessel insulin receptors. It was therefore concluded that insulin receptors on cerebral microvessel endothelial cells transport insulin across the blood-brain barrier directly into brain interstitial fluid with subsequent entry into CSF via bulk flow.

The demonstration of high insulin receptor number and insulin receptor mRNA concentrations in brain regions important to food intake, the regulated uptake of insulin from plasma to brain interstitial fluid, the lack of significant neuronal insulin content or synthesis, and the ability of exogenous insulin to suppress food intake and arcuate nucleus expression of NPY peptide, the most hyperphagic peptide known when injected into the CNS, made a compelling story for a biologically regulated body weight system with insulin as a key feedback controller. Since many of these findings had been contentiously debated for several years, a comprehensive review summarizing the relevant evidence was published in 1992 (Schwartz, Figlewicz, Baskin, Woods, & Porte, 1992). In that review, evidence was presented that energy expenditure also was an effector regulated by this feedback mechanism.

A further analysis in an updated review 2 years later summarized data suggesting an interaction between insulin and adrenal steroids in the regulation of

arcuate nucleus NPY neurons, thereby adding glucocorticoids as a second long-term regulator of body adiposity (Schwartz, Figlewicz, Baskin, Woods, & Porte, 1994). Not long afterwards, further work on glucocorticoids demonstrated an interaction with long-term body weight regulation systems in another way. It had been known for a long time that adrenal glucocorticoid insufficiency was associated with leanness, and glucocorticoid excess with an increase in adipose mass, although the distribution of the adipose tissue was quite different from that seen in ordinary obesity. The new finding was that in addition to regulation of NPY centrally, insulin uptake into the CNS was inhibited by the administration of glucocorticoids. This finding indicated that the insulin uptake system was sensitive to adrenal steroids and could be regulated (Baura *et al.*, 1996). A paper in a group of Pima Indians also was summarized that showed relatively low insulin responses were predictive of weight gain 5 years later (Schwartz, Boyko, Kahn, Ravussin, & Bogardus, 1995). These data supported the concept that impaired insulin secretion contributes to the development of obesity.

Leptin and Its Interaction with Insulin in the CNS

In 1994 the *ob* gene was cloned and quickly demonstrated to produce a peptide secreted by adipose tissue, later named leptin, with dramatic anorectic and weight-reducing effects in rodents (Zhang *et al.*, 1994). Furthermore, it was clear that lack of leptin was the defect responsible for obesity in the *ob/ob* mouse. Not long afterwards it was documented that obesity in both the *db/db* mouse and *fa/fa* rat is caused by mutations of the receptor for leptin (Chen *et al.*, 1996; Chua *et al.*, 1996). Since leptin administration to normal or obese *ob/ob* animals is much more effective when given IVT than when given systemically (Campfield, Smith, Gulsez, Devos, & Burn, 1995), the brain was implicated as a key target of leptin action. Consistent with this idea, the elevation of NPY in the hypothalamus of the *ob/ob* animal was exquisitely sensitive to the administration of leptin (Schwartz, Seeley, Campfield, Burn, & Baskin, 1996). After the demonstration of a receptor-mediated CNS uptake mechanism for leptin and its binding to cerebral microvessel leptin receptors, it was concluded that leptin, like insulin, has access via transport across the blood–brain barrier to hypothalamic neurons expressing NPY mRNA and peptide (Banks, Kastin, Huang, Jaspan, & Maness, 1996; Golden, Maccagnan, & Pardridge, 1997). As expected, *db/db* animals with a defect in the leptin receptor did not respond to leptin (Vaisse *et al.*, 1996).

Thus, almost from its discovery, it was assumed that leptin's effects on body weight are mediated by the CNS. We were gratified to see that a mechanism similar to our insulin adiposity feedback model was used to explain the long-term regulation of body adiposity by leptin (Campfield, Smith, & Burn, 1996) (Figure 6). What fascinated our group, considering the many years of contentious debate we had experienced surrounding our hypothesis for the CNS uptake of 6,000-Da molecular weight insulin, was the almost instant acceptance of the concept that a peptide of 16,000-Da molecular weight would be easily taken up and have access to appropriate CNS leptin receptors in the arcuate nucleus. Such receptors were eventually demonstrated to be present along with insulin receptors (Baskin, Breininger, & Schwartz, 1999; Baskin, Figlewicz *et al.*, 1999; Baskin & Schwartz, 1999; Baskin, Schwartz *et al.*, 1999; Schwartz, Figlewicz *et al.*, 1992).

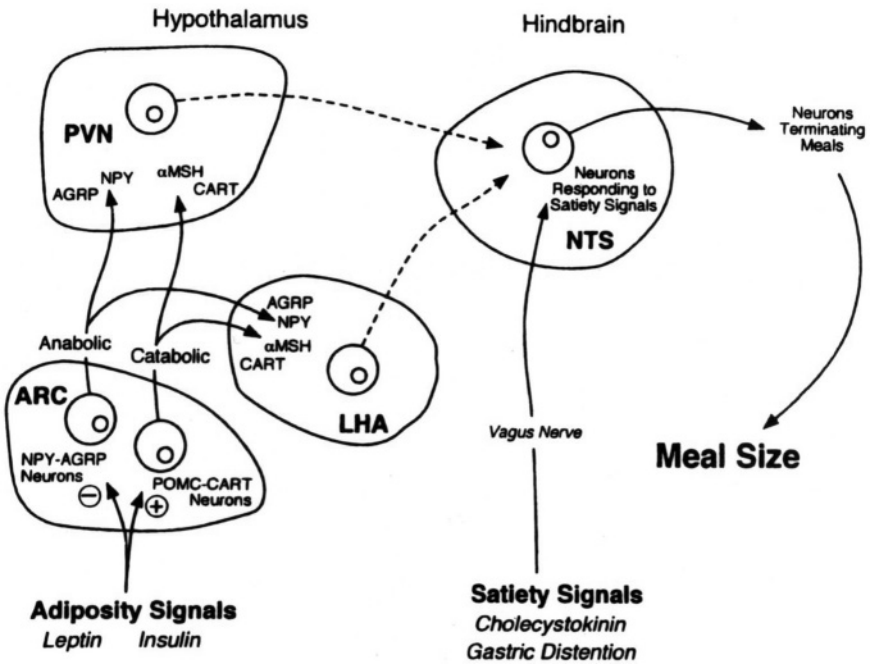

Figure 6. Detailed overview of central neural-signaling systems to integrate meal signals with long-term body weight regulation. Some of the better-defined neuropeptide contributors and their neural locations are indicated. (From Porte, Baskin, & Schwartz, 2002.)

The finding that CSF leptin levels in humans were curvilinearly related to plasma levels suggested that CNS leptin uptake in humans is also mediated by a saturable receptor-based transport system, as was shown for insulin (Schwartz, Peskind *et al.*, 1996). The association with adiposity of both these insulin and leptin signals was demonstrated by showing that plasma leptin and insulin are elevated in individuals with an increase in total body fat mass. However, plasma insulin levels appeared to be regulated by adipose tissue mass primarily because of its effect on insulin sensitivity, insulin secretion being responsive to that feedback mechanism. In contrast, plasma leptin levels were shown to be regulated primarily by the size of the total adipose tissue mass and were relatively independent of insulin sensitivity (Schwartz *et al.*, 1997).

By the time these studies were done in the mid to late 1990s, it had become clear that insulin sensitivity, while correlated with body adiposity, is most strongly associated with the presence of intra-abdominal cavity fat and central subcutaneous fat, and is relatively insensitive to changes in peripheral adipose tissue stores (Cnop *et al.*, 2002). Therefore, even individuals who are quite lean but have an increased intra-abdominal fat mass are insulin resistant and have increased insulin levels. Consequently, I suggest that hyperinsulinemia predominantly indicates an increase in intra-abdominal fat mass and/or central subcutaneous fat, while individuals who store equivalent amounts of fat in a predominantly peripheral subcutaneous distribution have a greater increase in leptin levels with minimal change in insulin levels as a result of the modest change in insulin sensitivity associated with fat deposition in peripheral subcutaneous sites. Thus, while both leptin and insulin provide information to the CNS about the state of stored calories in adipose tissue, the information

appears to be qualitatively different. This difference makes for a much more sophisticated feedback signal for adiposity regulation than was originally understood.

It must also be remembered that both hormones are affected by a variety of other factors such that neither insulin nor leptin can be viewed as providing information solely about either insulin sensitivity (visceral adipose mass) or total adipose tissue mass. Insulin secretion is regulated by nutrient administration on a very short-term scale and by almost all of the nutrients and neural factors known (Kahn & Porte, 2003). The pancreatic β-cell is in fact a metabolic integrator whose sensitivity to these inputs over the short term is modulated by long-term changes in insulin sensitivity, just as the sensitivity to short-term feeding-related signals are regulated by long-term adiposity signals. Thus, I suggest there is a parallel between the short-term and long-term mechanisms for regulating plasma insulin in relation to metabolic status just as there is a relation between long-term and short-term meal-related signals in the regulation of total caloric intake. These types of interactive regulatory processes should be kept in mind when analyzing other metabolic regulatory systems in the future.

Insulin and the Interaction Between Type 2 Diabetes and Obesity

When the interaction between obesity and diabetes was reviewed again by our group (Porte *et al.*, 1998), these adiposity-signaling systems provided a plausible mechanism to explain the association of the "odd couple." By then, the importance of the CNS to body weight regulation and its interaction with the periphery through circulating long-term signals was well described and accepted. That insulin, leptin, and the gut hormones and neural signals were important to the regulation of plasma glucose through a separate system of interacting feedback loops also was well described. Since type 2 diabetes is associated always with relatively impaired insulin secretion, it became evident that this pathophysiology predisposes to both glucose intolerance and weight gain (Porte *et al.*, 1998). It was concluded that the dependence of both body weight regulation and glucose homeostasis on the same circulating hormonal regulatory systems, particularly insulin, leptin, and adrenal steroids, and their common use of the same hypothalamic amine and neuropeptide signaling systems, suggested that this commonality was the basis for their frequent association.

At its simplest, reduced insulin secretion, reduced blood–brain barrier transport, or reduced neuronal responsiveness to insulin are mechanisms that should lead to an increase in caloric intake and storage. Therefore, any deficiency in the secretion of insulin would be expected to produce obesity, all other factors remaining unchanged, and this is what has been observed. Similarly, deficient insulin signaling would tend to increase hepatic glucose production and decrease glucose utilization in peripheral tissues, favoring hyperglycemia and diabetes. In patients with rare genetic defects such as maturity onset diabetes of the young (MODY) in which only insulin secretion is reduced, a mild impairment of insulin secretion is compensated for by hyperglycemia quite quickly and almost completely, at least initially in most cases, and therefore the degree of hypoinsulinemia is modest and difficult to detect (Kahn & Porte, 2003). In these cases the potential contribution of an isolated impairment of insulin secretion to the development of obesity is

modest. However, as demand on the islet increases from any dietary, lifestyle, or genetic propensity to develop obesity, the need to increase insulin secretion rises in a curvilinear fashion to compensate for the associated insulin resistance (Kahn *et al.*, 1993). This interaction means the deficiency is more and more difficult to completely compensate, and increased food intake and obesity will ensue to overcome the relative hypoinsulinemia so that resistance can elevate plasma insulin levels. As the β-cell compensation is often incomplete, hyperglycemia develops and provides an additional stimulus to the β-cell, contributing to the association of diabetes and obesity.

Similarly, defective insulin action in the CNS is predicted to increase food intake and obesity, and as demand increases, hyperglycemia develops unless the β-cell output completely compensates. In fact, recent studies demonstrate that reduced hypothalamic neuronal insulin signaling causes insulin resistance, thereby augmenting hyperglycemia directly (Obici, Feng, Karkanias, Baskin, & Rossetti, 2002; Obici, Zhang, Karkanias, & Rossetti, 2002). If the β-cell functions normally, then increased insulin output may compensate completely or almost completely for insulin resistance. If there is any limitation to β-cell compensation, there will be an associated hyperglycemia as insulin secretion is inadequate to maintain a normal glucose. In this case, the propensity for obesity will be exaggerated by the impairment of β-cell function. Thus, whether an individual starts out predominantly with defective insulin secretion or defective CNS insulin action, the development of hyperglycemia indicates impaired insulin secretory compensation. A diminished action of insulin in the regulation of both plasma glucose and body weight is thus proposed to be a potentially important part of the explanation for an increase in body weight.

In our society the rapid increase in body adiposity over the last century with its exacerbation within the past 10–15 years, and the associated increase in the prevalence of type 2 diabetes mellitus, has been hypothesized to be due to dietary obesity secondary to the ready availability of high-density, highly palatable foods of relatively low cost (Kawamori, 2002; van Dam, Rimm, Willett, Stampfer, & Hu, 2002). The opportunity to study a population of Japanese Americans who have maintained their genetic identity by intermarriage into the second and third generation in Seattle allowed us to evaluate this hypothesis. This population has a high predisposition to central obesity and type 2 diabetes, and was being followed prospectively to assess important risk factors for the development of type 2 diabetes and cardiovascular disease (Fujimoto *et al.*, 2000). Among these risk factors was a polymorphism in the β-cell glucokinase promoter that was common (frequency 25%), associated with a 30% reduction in the early insulin response to glucose and a significantly increased risk of impaired glucose tolerance (Stone, Kahn, Deeb, Fujimoto, & Porte, 1994, 1996). In a subgroup of the population entered into a long-term epidemiology trial, those who progressed to type 2 diabetes were compared with those who did not, both at baseline and after 2.5 and 5 years of observation. While both progressor groups had impaired early insulin secretion compared with the non-progressors at baseline, only those who progressed at 2.5 years had increased abdominal obesity at baseline while those who developed diabetes at 5 years did not develop excess intra-abdominal fat until that assessment (Chen *et al.*, 1995; Fujimoto *et al.*, 2000). Thus, this population was characterized by a genetic risk factor for impaired insulin secretion, and their progression from normal glucose tolerance to diabetes was associated with impaired insulin secretion 5 years prior to the development of intra-abdominal fat (Figure 7), the best correlate of insulin resistance so far identified (Cnop *et al.*, 2002).

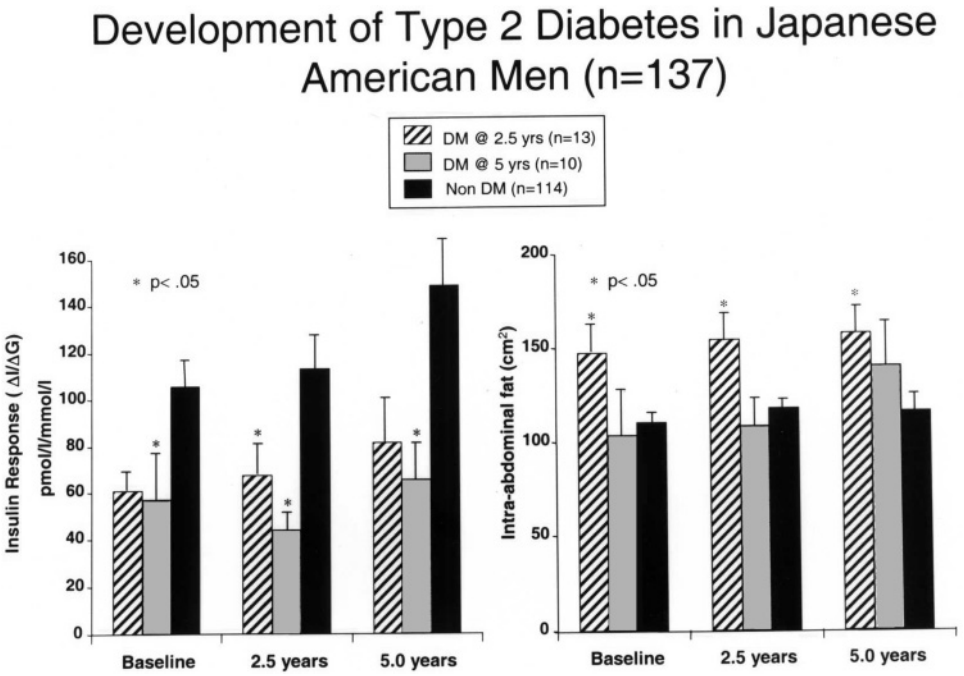

Figure 7. Decreased insulin secretion precedes intra-abdominal fat accumulation in "prediabetic" Japanese Americans. Insulin secretion and abdominal fat in nondiabetic Japanese Americans at baseline, 2.5 and 5 years, was assessed and compared in those who never developed type 2 diabetes with those who developed diabetes after 2.5 and 5 years of observation. Note that both groups who developed diabetes had reduced insulin secretion at baseline and that this was maintained throughout the study. Increased intra-abdominal fat was also present at baseline in those who developed diabetes after 2.5 years, but the group who developed diabetes at 5 years did not show increased intra-abdominal fat until the 5-year exam. Thus decreased insulin secretion preceded the accumulation of intra-abdominal fat. This finding is consistent with the hypothesized contribution of insulin to the regulation of body adiposity. (Adapted from Chen *et al.*, 1995.)

The high prevalence of the glucokinase promoter polymorphism in this population suggests that a high proportion of the population inherited a mild defect of insulin secretion that was not expressed clinically as long as body weight was not excessive. As the population aged and increasingly consumed a high-fat, palatable American diet, the frequency of impaired glucose tolerance increased. The increase in calorie ingestion in this population may have been facilitated by relatively impaired insulin secretion and appears to have led to the increased deposition of intra-abdominal fat, which is known to be associated with insulin resistance. This body fat increase therefore can be interpreted as compensation for the genetic defect in insulin secretion, increasing insulin levels until a new steady state of obesity and hyperglycemia is reached. Final progression from impaired glucose tolerance or early type 2 diabetes to overt clinical hyperglycemia is associated with a progressive further loss of β-cell function. This latter pathophysiological process is well described and may relate to toxic factors secondary to lipid (Unger, 2002) or glucose excess (Poitout & Robertson, 2002), and/or to the deposition of amyloid in the β-cells of the pancreas (Kahn, Andrikopoulos, & Verchere, 1999), leading to β-cell death and further impairment of insulin secretion despite treatment. Thus, I suggest that the association between diabetes and obesity is driven in part by abnormalities in the β-cell that predispose to both weight gain and hyperglycemia.

This progression is exacerbated by factors in the environment that favor an increase in body weight such as socioeconomic status, the availability of a high-fat diet, the high palatability of relatively inexpensive food stuffs, and a background of many common gene variants that predispose to the development of obesity (Figure 8). Future work focused on understanding the molecular mechanisms involved in the deregulation of insulin secretion should lead to discovery of common defects that are amenable to therapeutic interventions to improve both syndromes.

I suggest that the genetic defects that contribute to this interaction are likely to be modest, and that type 2 diabetes is a polygenic disorder. These polygenes interact with the environment to produce the obesity and insulin-resistance syndromes. Obviously, this interaction also could be driven by genes predisposing to insulin resistance that consequently increase the demand on the β-cell. The presence of environmental factors that predispose to β-cell stress and/or damage such as high-fat feeding (Unger, 2002) or mild chronic hyperglycemia (Poitout & Robertson, 2002) also could drive the interaction. Regardless, those individuals with impaired β-cell responses would tend to be the most obese and therefore have the greatest risk for the development of the common syndrome. From this point of view, preventive treatments that attack either insulin resistance or impaired insulin secretion should have beneficial effects on reducing the progression of hyperglycemia. While supporting this hypothesis, recent experience with the thiazolidinedione antidiabetic insulin-sensitizing drugs that reduce hyperglycemia and hyperinsulinemia demonstrate a key problem: a high prevalence of undesirable weight gain (Chilcott, Tappenden, Jones, & Wight, 2001). Although this outcome may be related to the propensity of these agents to differentiate pre-adipocytes into mature cells, the accompanying reduction in insulin levels would be expected to aggravate obesity by reducing adiposity-related signaling in the hypothalamus. Supporting this idea, studies many years ago found an increase in food intake when ventromedial hypothalamus (VMH) lesioned rats were made diabetic with streptozotocin, and this hyperphagia was partially reversed by pancreatic β-cell transplants

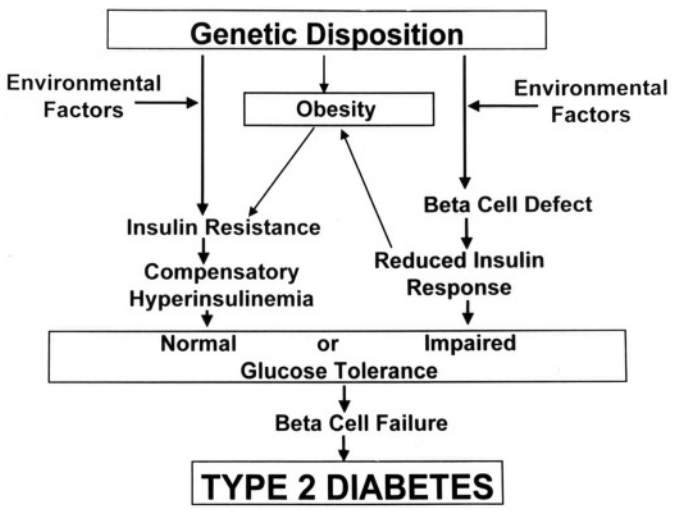

Figure 8. Pathophysiological description of the factors contributing to type 2 diabetes and obesity. The hypothesized contribution of impaired insulin secretion to obesity is also shown with its impact on insulin resistance.

(Inoue, Bray, & Mullen, 1978). Further understanding of the mechanisms involved in the signaling shared between these two syndromes in the CNS and islet that predispose to obesity and type 2 diabetes is clearly an area that deserves much further emphasis.

Cell Biology of Leptin and Insulin Action in the CNS

Within the past few years, support for overlapping CNS actions of insulin and leptin has grown using modern methods of molecular and cellular biology. One important finding is that selective knockout of the brain leptin receptor induces severe hyperphagia and obesity confirming a primary role for the CNS as a target of leptin action as far as its regulation of adipose mass is concerned (Cohen *et al.*, 2001). Similarly, knockout of the brain insulin receptor produces mice that are obese and, interestingly, also have impaired reproductive capacity with low pituitary LH and FSH secretion (Bruning *et al.*, 2000). This obesity has been replicated by selective deletion of the insulin receptor in the hypothalamus (Obici, Feng *et al.*, 2002). Since a reproductive defect has long been known to occur in leptin-deficient *ob/ob* and leptin-resistant *db/db* animals, there seems to be an association between the ability to store fat and the regulation of the reproductive axis by signals informing the CNS that fat has been stored. Associations between the ability of mammals (including man) to reproduce and store adipose mass have been reported for many years, but the mechanisms had been difficult to delineate (Frisch *et al.*, 1981; Frisch & McArthur, 1974). It would appear that signals such as insulin and leptin play an important role in this process, although recent studies suggest that they alone are not sufficient to explain the onset of reproductive competence at the time of puberty (Chehab, Qiu, Mounzih, Ewart-Toland, & Ogus, 2002; O'Rahilly, 2002). Thus, both signals appear to be necessary, but not sufficient.

With the identification of the "long" or "signaling" form of the leptin receptor and its presence in the arcuate nucleus along with the insulin receptor, studies have more recently begun to focus on the potential intracellular mechanisms for the effects observed on the regulation of neuropeptides such as NPY. The initial knockout of insulin receptor signaling (IRS-1), an early step in the insulin signaling process in peripheral tissues, did not demonstrate any change in food intake or body adiposity (Araki *et al.*, 1994; Tamemoto *et al.*, 1994). Thus, despite its presence in the hypothalamic arcuate nucleus (Baskin, Sipols, Schwartz, & White, 1994) and its general acceptance as the first step in insulin action, IRS-1 did not seem to be critical to the function of either insulin or leptin in the CNS. However, almost simultaneously with the study demonstrating the effect of genetic deletion of the brain insulin receptor, IRS-2, a second insulin receptor signaling molecule now recognized to be commonly associated with insulin action in tissues not originally thought to be insulin regulated (such as the β-cells of the pancreas), was knocked out. IRS-2 deficient mice were found to have a major reduction of β-cell mass and function, an increase in body adiposity, and an impairment of reproduction. This last effect occurred despite elevated circulating leptin levels, implicating IRS-2 in the action of both CNS insulin and leptin.

Studies following up on this possibility supported an interaction between insulin and leptin signaling in the CNS when it was initially demonstrated that either hormone given IVT suppressed elevated levels of NPY in fasted rats (Schwartz, Seeley *et al.*, 1996; Schwartz, Sipols *et al.*, 1992). Similarly, the hyperphagia of

diabetes is partly or completely suppressed by the administration of either leptin systemically or insulin IVT (Sindelar *et al.*, 1999; Sipols *et al.*, 1995). Based in part on the more recent findings in the IRS-2 knockout mouse, there has been a reevaluation of the cellular-signaling mechanisms for the effects of leptin in the arcuate nucleus. Initially, it was thought that signaling by leptin and insulin utilizes totally independent systems. In peripheral tissues, insulin activation of its receptor tyrosine kinase phosphorylates a variety of intracellular mediators leading to the long-term regulation of growth through modulation of MAP kinase and metabolism via activation of phosphotidylinositol-3-kinase (PI3K). Leptin signaling also had been shown to require tyrosine phosphorylation, but the leptin receptor is not a tyrosine kinase. Rather, its activation by leptin activates an associated tyrosine kinase (Janus kinase), and, like other cytokine receptors, this interaction phosphorylates a particular member of the STAT family, STAT-3, which dimerizes and translocates to the nucleus where it regulates gene expression (Vaisse *et al.*, 1996) (Figure 9).

The demonstration that IRS-2 knockouts develop adiposity and a reproductive defect similar to that observed with knockout of the brain insulin receptor suggested an important role for IRS-2 in CNS insulin signaling. The apparent resistance of the IRS-2 knockout mouse to leptin raised the possibility that IRS-2 might be involved in leptin action as well. Recent findings strongly support such a hypothesis (Niswender & Schwartz, 2003). The first indication of a STAT-3 independent effect of leptin in the CNS was the demonstration of a rapid activation of neuronal ATP-sensitive potassium (K-ATP) channels when hypothalamic slices or membrane patches were exposed to leptin (Spanswick, Smith, Groppi, Logam, & Ashford,

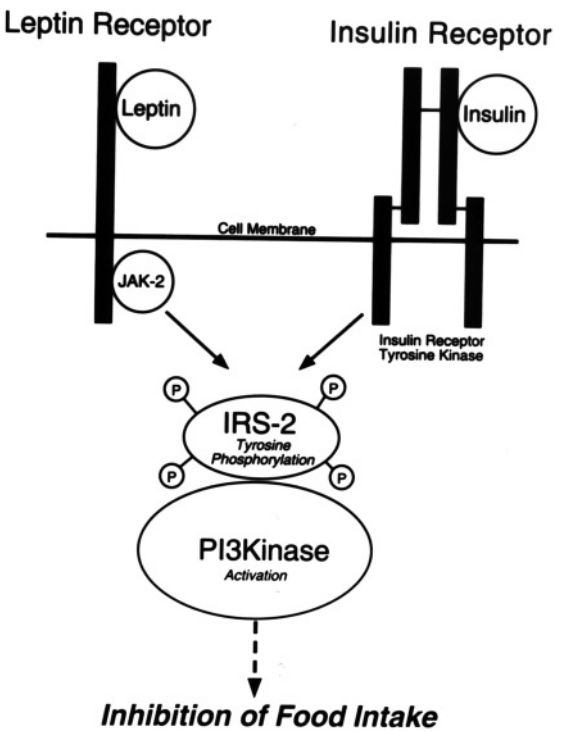

Figure 9. Proposed biochemical interaction between insulin and leptin action in the neural hypothalamus to regulate food intake. (From Porte *et al.*, 2002.)

1997). These channels, originally identified in pancreatic β-cells, had been demonstrated many years before to mediate inhibition of channel currents induced by the sulfonylureas, tolbutamide and glyburide (Ashford, Boden, & Treherne, 1990). These leptin-sensitive channels were shown to be present in the same glucose responsive neurons from hypothalamic tissue containing the ventromedial and arcuate nuclei. The response was far too rapid to be explained by the effect of a transcription factor such as STAT-3. A few years later, a similar activation of K-ATP channels by insulin in hypothalamic neurons of lean, but not obese, rats was demonstrated by the same group of investigators using similar techniques (Spanswick, Smith, Mirshamsi, Routh, & Ashford, 2000). These effects of insulin were reversed by tolbutamide and involved the same pool of glucose-responsive neurons sensitive to leptin. Since PI3K activation had been shown to be required in signal transduction between insulin receptor activation and cellular responses in peripheral tissues (Kanai *et al.*, 1993; Shepherd, Withers, & Siddle, 1998), the question was raised as to whether insulin action on K-ATP channels was PI3K dependent. Possible convergence between insulin and leptin on PI3K signaling was suggested by the ability of leptin to activate K-ATP channels in an insulin-secreting cell line involving a PI3K mechanism (Harvey *et al.*, 2000). Thus a PI3K inhibitor was tested in a hippocampal neuron preparation (Shanley, Irving, Rae, Ashford, & Harvey, 2002). The findings supported the concept of a common signaling pathway, since PI3K inhibition was shown to be effective in blocking leptin signaling of K-ATP channels in this system. The role of IRS-2 in the action of leptin on hypothalamic neurons was assessed shortly thereafter in studies of the food intake response to leptin using the same PI3K inhibitor and measuring short-term and 24-hr food intake as well as the phosphorylation of STAT-3 and IRS-2 associated PI3K activity. Both STAT-3 phosphorylation and IRS-2 associated PI3K activation were shown to be rapid (within 5–10 min) and this response was followed within 4 hr by suppression of food intake, which was maintained for 24 hr (Niswender *et al.*, 2001). The inhibition of both 4- and 24-hr food intake by leptin was prevented by the administration of IVT LY294002 or wortmanin, two different pharmacological inhibitors of PI3K activity. These compounds had no effect when given to control animals alone. It was shown also that administration of the anorexia-inducing neuropeptide α-MSH was unaffected by one of the PI3K inhibitors. Thus the suppression of leptin and insulin anorexia was not nonspecific.

Subsequently, a phosphodiesterase 3B (PDE3B) inhibitor was shown to block the effect of leptin to suppress food intake. Since this compound blocks the PI3K-dependent activation of PDE3B to reduce cyclic AMP, it was suggested that the next step in the leptin signaling pathway after PI3K may be mediated by induction of PDE3B with the subsequent reduction of intracellular cyclic AMP (Zhao, Huan, Gupta, Pal, & Sahu, 2002). Furthermore, it was demonstrated that within 45 min of IVT leptin, there was increased activity of hypothalamic PDE3B, which is known to be a PI3K-activated enzyme. IRS-1-associated PI3K activity also was increased. Of interest, STAT-3 phosphorylation also was blocked by the administration of a PDE3B inhibitor, suggesting that PDE3B activity stimulated by PI3K leads to the reduction of cyclic AMP and subsequently to the phosphorylation of STAT-3 and the inhibition of feeding. Since this cascade of events would not be expected from the IRS-2 studies, it is not clear whether there are several mechanisms for activating PI3K after leptin receptor activation, more than one PI3K that is involved, or some nonspecific effect of the PDE3B inhibitor. Regardless, it has become clear that the insulin receptor and the leptin receptor share IRS-2 and PI3K as common

downstream intracellular signaling proteins and that their activation is directly related to the inhibition of food intake. The potential role of STAT-3 activation in food intake regulation remains an area of active investigation (Niswender & Schwartz, 2003). A recent report using STAT-3 knockout mice suggests that leptin regulation of STAT-3 is important to its energy balance effects but not to its effect to regulate fertility (Bates *et al.*, 2003).

Brain Insulin in Worms and Flies: Biology Expanded

By 1997 two very simple organisms, the worm *C. elegans* and the fruit fly *Drosophila*, were discovered to contain insulin-like molecules and signaling systems homologous to the CNS insulin-signaling system in mammals (Garofalo, 2002; Guarente & Kenyon, 2000). These systems appear to function not only in the regulation of fat storage and reproduction in these primitive organisms, but surprisingly, as a determinant of the animal's life span. The initial discovery in *C. elegans* was the cloning of the molecule DAF-2, which turned out to be a homologue of the insulin receptor that contains ligand binding and tyrosine kinase domains (Kimura, Tissenbaum, Liu, & Ruvkun, 1997). It was well known in *C. elegans* that periods of reduced food availability caused the worm to enter a stage of diapause arrest called "dauer" in which reproduction is inhibited, metabolism is markedly reduced, and a state of suspended animation or hibernation is induced. It was known also that mutations in DAF-2 produced the "dauer" state, but the molecular mechanism had not been determined. In 1997, DAF-2 turned out to be the first step in a pathway present in neurons, GI tract cells, and the muscles of this organism, and each cell type was later shown to contain two other homologues of the insulin pathway in mammals. The first of these is a protein described in 1996 called AGE-1, a PI3K whose knockout led to the onset of a similar "dauer" stage phenotype with increased longevity (Kimura *et al.*, 1997). Not too long afterwards, DAF-16 was described, which turned out to be a fork-head transcription factor related to HNF-3 and Foxo1 that when knocked out reversed the DAF-2 and AGE-1 knockout phenotypes (Ogg *et al.*, 1997). Ruvkun and colleagues in their original paper (Kimura *et al.*, 1997) suggested that an insulin-like ligand bound to DAF-2, which under appropriate signaling conditions led to the phosphorylation and activation of AGE-1 (the PI3K-like molecule), activating downstream effectors similar to those observed for insulin in peripheral tissues of mammals. They pointed out that the increase in longevity associated with the DAF-2 knockout could be thought of as analogous to mammalian longevity associated with caloric restriction, a process that produces decreases of both circulating insulin and fertility. Three years later this group restored DAF-2 signaling specifically to neurons, muscle, or intestine using different promoters to selectively express DAF-2 cDNAs in each of these cell types (Wolkow, Kimura, Lee, & Ruvkun, 2000). Interestingly, only restoration of DAF-2 in neurons was sufficient to restore the wild-type life span and normal reproduction. Neuron-specific restoration of AGE-1 in its mutants produced the same effect. The intestinal triglyceride deposition pattern also was restored and the metabolic defects were reversed. Expression in muscle regulated metabolism but not the "dauer" stage or life span while restoration in the intestine had relatively minor effects. Thus, the critical pathway appears to be neuronal insulin-like signaling, and the metabolic and reproductive defects appear to be largely secondary to abnormalities specific to this pathway. Recently, KO of Foxo1 in the mouse has

reproduced the effects of KO of DAF-16 in *C. elegans* on insulin signaling (Kitamura *et al.*, 2002; Nakae *et al.*, 2002) indicating its strong homology and maintenance of this system over evolution.

In 2001, studies of the insulin-receptor-like signaling system in the fruit fly, *Drosophila*, demonstrated that mutations in either the insulin-like receptor substrate proteins or complex heterozygotes of the insulin-like receptor extended life span and reduced reproduction in a manner similar to that observed in DAF-2 mutants in *C. elegans* (Tatar *et al.*, 2001). While the insulin-like receptor had been known in *Drosophila* since 1996, knockouts were lethal and therefore some insulin-receptor-like activity was absolutely essential for life during development. The life span extension was associated with a general growth deficiency and a decrease in cell number and size. As in *C. elegans*, signaling depended on a PI3K function and, in the *Drosophila* system, an IRS-like molecule, CHICO, whose known mutations produced a similar phenotype with increased life span and triglyceride storage with decreased size (Bohni *et al.*, 1999). While the role of this system in the regulation of enzymes of glucose storage and mobilization has not been studied in *Drosophilia*, surgical removal of the medial neurosecretory glands in the blowfly brain, which contain an insulin-like peptide, leads to hyperglycemia (Rulifson, Kim, & Nusse, 2002). Since these cells also are present in the *Drosophila* brain, it appears that insulin-like molecules are made and secreted from neurosecretory cells and act on neuronal function to regulate life span and reproduction in flies, as in *C. elegans* (Figure 10). The findings in *C. elegans*, which have neither adipose cells nor leptin-like molecules, may mean that an insulin-signaling system for regulating energy

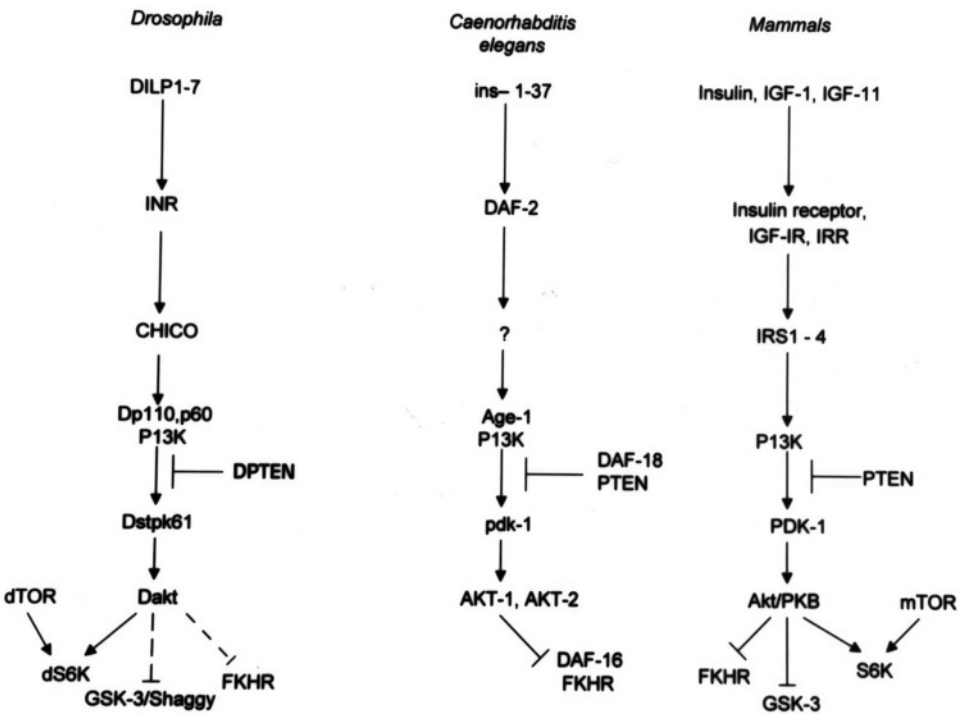

Figure 10. Parallel biochemical mechanisms for insulin and insulin-like molecules and receptors in *Drosophila*, *C. elegans*, and mammals. Note the conservation of signaling systems across extended evolutionary time. (Adapted from Garofalo, 2002.)

homeostasis is evolutionarily older than leptin. This suggests that its original role was to regulate metabolism through neuronal control of nutrient storage and to regulate reproduction and life span in relation to nutrient availability, and only later in evolution did its role in carbohydrate metabolism appear.

The description of insulin signaling in the CNS systems of mammals, including humans, seems to have many parallels, as for many years it has been recognized that caloric restriction in mammals is associated with reduced circulating insulin levels and inhibition of the secretion of the reproductive hormones FSH and LH, which, if prolonged, leads to an extension of life span (Weindruch & Sohal, 1997). It also has become clear that these nutritionally deficient states are associated with low adipose tissue mass and therefore low leptin levels. Since insulin plays a major role in the regulation of the biosynthesis and release of leptin, low insulin and low leptin usually are found together and are associated with similar outcomes. It had been recognized for many years that caloric restriction delayed the onset of puberty and that females participating in vigorous exercise, with its associated reduction in total body fat stores, experienced reductions of circulating leptin and insulin, reduced circulating levels of pituitary reproductive hormones, and reduced reproductive capacity (Frisch *et al.*, 1981; Frisch & McArthur, 1974). Therefore, genetic dissection of the insulin signaling system in *Drosophila* and *C. elegans* may lead to the identification of new metabolic end points, which might be targets for future therapeutic intervention in diabetes, obesity, and/or life span extension (Figure 11).

It can be pointed out that if central insulin resistance is induced or accompanies peripheral insulin resistance, the hypothalalmus and pituitary might then perform their evolutionarily conserved roles and adjust peripheral physiology to this perceived deficit in adiposity and increase energy storage while inhibiting

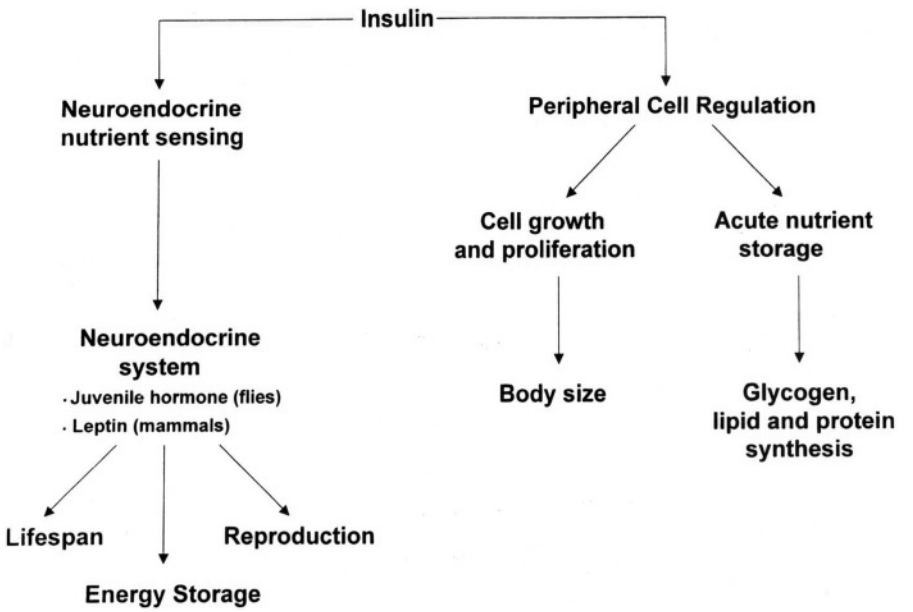

Figure 11. Insulin signaling and its impact on the CNS compared with its effect on peripheral tissues. Reproduction, energy (fat) storage, and life span are CNS-regulated events by insulin acting as a catabolic signal. Short-term nutrient storage and cell growth regulation are peripheral tissue effects of insulin acting as an anabolic signal. (Adapted from Garofalo, 2002.)

reproduction. While this response might serve the organism well in situations of low nutrient availability, reduced CNS insulin action also might contribute to weight gain and reproductive complications of the insulin-resistance syndromes such as polycystic ovarian disease (Dunaif & Thomas, 2001). Therefore, analysis of this system and dissection of its implications have achieved great importance during the last 2–3 years, and has implications far beyond those originally recognized. Most intriguing has been the report suggesting that the downstream pathways controlling longevity and reproduction may be independent of one another (Dillin, Crawford, & Kenyon, 2002). This conclusion was based on the use of RNA inhibitors in adult *C. elegans* to suppress DAF-2 activity after the development of reproductive capacity and demonstrate that this late suppression still can extend life span. Furthermore, it was shown that reproductive timing could be specified independent of the "dauer" decision, a finding that suggests the DAF-2 pathway may function late in development to affect the timing of reproduction (Dillin *et al.*, 2002). Thus, while it initially appeared that extension of longevity would invariably be associated with impaired growth or reproduction, it may be possible to manipulate this pathway to extend youthfulness and life span without affecting these other processes. The report (Flurkey, Papaconstantinou, & Harrison, 2002) and evaluation (Hsieh, DeFord, Flurkey, Harrison, & Papaconstantinou, 2002a, 2002b) of the Snell dwarf mouse suggest a similar extension of life span in this mammalian model, and its association with a reduced insulin/IGF-1 signaling additionally supports the life span mechanisms uncovered in *C. elegans* and *Drosophila*. Most recently, it has been found that this phenomenon can be seen without reduced food intake, as mice with selective knockout of the adipose tissue insulin receptor have normal food intake, a 25% reduction in adipose mass, elevated leptin for the lower fat mass, but a lower plasma insulin with normal fertility and an extended life span (Bluher, Kahn, & Kahn, 2003).

Thus what my colleagues and I initially discovered more than 30 years ago while trying to explain the association between diabetes and obesity in man, has now expanded to cover a wide range of neurobiology that is still developing and of potential significance to a host of human pathophysiology.

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REVIEW CHAPTERS

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Neural Circuits

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8

Postnatal Development of Central Feeding Circuits

Linda Rinaman

Introduction

It is increasingly evident that many overlapping neural systems—various "routes to action" (Winn, 1995)—are involved in controlling food intake in mature animals. Many components of these neural systems are operational at birth itself, while others gradually become functional during postnatal development. Theoretically, an ontogenetic approach to the analysis of neural substrates underlying ingestive behavior will permit the developmental emergence of new functions to be directly related to definable events in neural circuit maturation (Hall, 1985, 1989).

A large variety of interacting internal and external sensory signals modulate the output of central neural control pathways that stimulate, inhibit, or disinhibit food intake in mature animals (Watts, 2000). My goal in writing this chapter is to summarize current knowledge about the functional postnatal maturation of these neural systems in rats. This summary requires integration of three largely separate scientific literatures. The first is primarily behavioral and, to a lesser extent, physiological in nature—it describes the postnatal development in rat pups of ingestive responsiveness to stimuli and treatments that affect food intake in adult rats. This literature was expertly reviewed in a previous volume of this *Handbook* (Hall, 1990). This chapter emphasizes the most significant aspects of those studies from a neural systems perspective, and also incorporates some important new findings that have emerged since 1990.

The second literature to be considered provides a wealth of information about the anatomical and chemical features of hindbrain and hypothalamic circuits

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Neurobiology of Food and Fluid Intake, 2nd Ed., Volume 14 of *Handbook of Behavioral Neurobiology,* edited by Edward Stricker and Stephen Woods, Kluwer Academic / Plenum Publishers, New York, 2004.

implicated in controlling food intake in mature rats. Investigation of such issues has expanded markedly during the last decade, and has been the subject of several recent comprehensive reviews (Berthoud, 2002; Broberger & Hokfelt, 2001; Watts, 2000; Williams *et al.*, 2001). The first two literatures together provide a framework with which to consider the third, which comprises a relatively small number of studies attempting to correlate emerging ingestive responsiveness with developmental events in neural circuit maturation. An ontogenetic approach to the analysis of central ingestive control circuits was championed by Hall in 1990, when he noted,

> … there is little specific information regarding correlations of ingestive response development and neural maturation. Indeed, attempts at such developmental correlations have been limited and indirect … (p. 107)

It seems clear that research on neural circuit maturation has heuristic value for understanding how the brain controls ingestive behavior. The presence or absence of behavioral and physiological responsiveness to internal and external sensory events presumably reflects the functional integrity of neural circuits that receive and process the relevant stimuli, and those that organize and execute the responses. As new responses emerge during postnatal development, one may infer maturation of the underlying neural systems and their functional interactions.

What is Feeding Behavior?

Before discussing the functional development of central feeding circuits, we must first define "feeding behavior" (Hogan, 2001). We say that a rat is feeding when it performs voluntary motor functions that culminate in swallowing food. Feeding behavior can be studied directly by monitoring various aspects of these motor functions. More commonly, however, feeding behavior is studied indirectly by monitoring the number and size of feeding bouts that occur during a given period of time.

Motor Mechanisms. Feeding behavior in mature animals usually comprises appetitive and consummatory motor patterns that are easily recognizable, but which can vary considerably. For example, an adult rat in a laboratory setting may have to work hard to obtain small amounts of food, or it may consume food placed directly into its mouth. It may bite or tear small pieces of solid food from a larger chunk, or it may lick drops of liquid food from a drinking spout. In contrast to consummatory components, appetitive components of feeding are acquired with experience (Changizi, McGehee, & Hall, 2002; Craig, 1918; Hall, Arnold, & Myers, 2000). The motor patterns of feeding behavior vary according to the requirements for food acquisition and consumption; in all cases, however, the patterns accurately reflect activity in central motor circuits that generate the behavior (Hogan, 2001). As we shall see, the motor patterns that characterize adult-like feeding differ from those that characterize suckling, which is a developmentally unique mode of feeding.

Perceptual Mechanisms. Perceptual (i.e., sensory) neural circuits comprise the afferent side of the feeding system, and directly impact the initiation and termination of neural activity within feeding motor circuits (Hogan, 2001). Perceptual circuits monitor various qualities of food substances prior to and during their consumption, and also monitor ongoing environmental events and changes in the organism's internal physiological state. Perceptual circuits generally integrate

exteroceptive and interoceptive signals arising from several sensory modalities. In addition, experience-dependent memories and other learned associations can influence perceptual circuits and affect how sensory inputs are interpreted. For example, information about the flavor and familiarity of a particular food will be integrated with environmental cues that signify safety or danger, and with physiological feedback signals about gastric volume, plasma osmolality, and plasma insulin levels. As discussed further below, perceptual circuits that impact feeding motor output in adult rats seem to have little in common with those that affect suckling in neonates.

Executive Mechanisms. Some still undefined set or sets of executive neural mechanisms act to initiate, maintain, and terminate feeding motor outputs as a function of the integrated inputs received from perceptual circuits (Hogan, 2001). Portions of these executive circuits are responsible for the temporal patterning and sequencing of feeding motor outputs, and for determining and modulating the animal's overall behavioral state (i.e., sleeping, aroused, quiescent) and hence the likelihood that feeding will occur. The net effect of activity within these complex central circuits defines motivational states such as "hunger" and "satiety," which can be inferred only from the animal's behavioral motor output.

Thus, neural circuits that control feeding behavior include (1) *motor circuits* for obtaining and ingesting food, (2) *perceptual circuits* for recognizing various food qualities and for monitoring and integrating environmental events with the animal's internal physiological state, and (3) *executive circuits* that modulate the saliency of integrated perceptual signals in a context- and state-dependent manner, and which ultimately initiate, maintain, and terminate neural activity within the motor output circuits. Mounting evidence indicates that key components of these neural circuits already are operational at birth, whereas others are functionally immature until later in postnatal development. By probing and documenting ingestive responses in rats at progressive developmental stages, newly emerging responses may be identified that directly relate to definable events in neural system maturation.

Development of Feeding Behavior

Motor, perceptual, and executive neural mechanisms are the building blocks from which feeding behavior is formed. By the time of birth itself, the CNS is highly differentiated, and the basic organization of neural systems and connections is determined. However, much refinement of chemical signaling pathways and synaptic circuitry takes place after birth. A developmental analysis of the neural control of food intake requires examination of how the motor, perceptual, and executive building blocks develop, and how functional connections among them become established. There are several levels of motor, perceptual, and executive mechanisms, and the connections among them are quite complex. Nevertheless, it is useful to ask: What are the neural systems that control food intake? In mammals, the answer to this question changes over the course of development, as ingestive mode changes from suckling to independent ingestion, and as the neural underpinnings of each become increasingly complex.

The ability of newborn mammals to suckle milk within moments after birth demonstrates that the necessary and sufficient neural circuits for this mode of

ingestion are operational already. However, a surprising fact about the feeding behavior of many neonatal animals is that ingestive motor outputs occur relatively independently of perceptual factors associated with food deprivation, and that suckling behavior is influenced very little by the amount of food obtained (Hall, 1990; Hinde, 1970). Indeed, and as discussed further below, the perceptual and motor controls of suckling in neonatal rats share little in common with those that control feeding behavior in mature animals. The anorexia (*anx*) mutation in mice provides a striking example that supports this general conclusion. Homozygous *anx/anx* mice are asymptomatic during early development, and exhibit normal suckling intake and body weight gain during the first 9–10 days postnatal. However, independent ingestion away from the dam fails to develop, and *anx/anx* mice die of starvation 3–4 weeks after birth (Broberger & Hokfelt, 2001; Broberger *et al.*, 1999; Jahng, Houpt, Kim, Joh, & Son, 1998; Maltais, Lane, & Beamer, 1984).

Suckling versus Mature Feeding—Different Motor Mechanisms

At any age, a given factor can control food intake only if it affects activity in brain circuits that initiate or terminate consummatory ingestive movements. A common feature of such movements is their rhythmicity, evident both in the suckling infant rat and in the chewing or licking adult. Commands for rhythmic consummatory movements arise in central pattern generators (CPGs) whose component neurons occupy the brainstem magnocellular reticular formation (Nakamura, Katakura, & Nakajima, 1999). Brainstem CPGs transform nonrhythmic neural inputs into rhythmical suckling, chewing, or licking outputs, which are then delivered to premotor neurons in the parvocellular reticular formation, positioned dorsolateral to the gigantocellular nuclei. Separate and coordinated rhythmical activities in the trigeminal, facial, and hypoglossal nerves are produced by distinct CPG and premotor neuron pools, which are located segmentally at the respective level of the cranial motor nuclei (Fay & Norgren, 1997a, 1997b, 1997c; Nakamura *et al.*, 1999; Travers & Rinaman, 2002).

At all ages, then, the final components of rhythmic ingestive movements are the product of specific interactions among brainstem CPGs and motor output circuits. However, despite these general similarities in motor mechanisms, suckling is a highly specialized form of ingestive behavior, an "ontogenetic adaptation" (Oppenheim, 1981) that is not developmentally continuous with adult-like feeding either in its motor components or, as discussed further below, in its central integrative controls. Suckling is an inborn, single-patterned stereotyped motor output of jaw, tongue, lips, and cheeks. It features a simple vertical jaw movement that generates negative pressure at the nipple to allow efficient extraction of milk, whose physical features do not change before it is swallowed. Conversely, solid foods vary considerably in their composition, and their physical properties change in the mouth during the course of mastication. Chewing comprises a complex constellation of learned motor patterns characterized by coordinated movements of the jaw, tongue, lips, and cheeks. These motor patterns are adjusted continuously based on sensory feedback from the oral cavity, and can vary markedly depending on the type of food being ingested (Nakamura *et al.*, 1999). In rats, rhythmic chewing movements require descending cortical drive to brainstem CPGs (Nozaki, Iriki, & Nakamura, 1986), whereas rhythmic suckling movements do not (Thexton & Griffiths, 1979). Chewing movements begin near the end of the second postnatal week of development, coincident with tooth eruption but not dependent upon it

(Iriki, Nozaki, & Nakamura, 1988). Importantly, initiation of chewing movements corresponds to a period during which corticobulbar projection systems are becoming increasingly myelinated (Iriki *et al.*, 1988; Sarnat, 1989), and still are establishing new synaptic connections with brainstem target neurons (Martin, Cabana, Culberson, Curry, & Tschismadia, 1980; Rinaman, Roesch, & Card, 1999; Rinaman, Levitt, & Card, 2000).

Suckling versus Mature Feeding—Different Perceptual and Executive Mechanisms

Beyond differences in motor patterns and the necessity for cortical input, suckling and adult-like modes of ingestive motor output also are modulated by different perceptual mechanisms. During the first 10–12 days postpartum, the "appetitive" rooting and nipple-attachment components of suckling behavior are facilitated by olfactory and tactile cues originating from the dam, but occur largely independent of the pup's internal state (Hall, Cramer, & Blass, 1975; Henning, Chang, & Gisel, 1979). Young pups spend the majority of their time attached to the nipple, even while sleeping. That nipple attachment *per se* may be a reinforcing behavior even in the absence of milk availability is supported by findings that rats as young as 7 days of age will learn a spatial discrimination task that provides them with access to a non-lactating nipple (Blass, Hall, & Teicher, 1979b). However, other evidence indicates that the prior pairing of nipple-related sensory cues with the sweet taste of milk provides a powerful conditioning effect that supports nipple attachment (Cheslock, Varlinskaya, Petrov, & Spear, 2000). Internal sensory cues related to hydration, to gastric fill, and to metabolic state do not reliably control nipple attachment or intake volume until 10–12 days postnatal. At this time, nipple attachment becomes closely related to deprivational state, and nondeprived pups either do not attach or do so in a "leisurely manner" (Blass *et al.*, 1979b).

Increasing the amount of milk available to young rat pups (i.e., less than 10 days old) during suckling leads to increased consumption regardless of the pup's deprivation state. For example, milk intake increases when nondeprived pups are furnished with several milk-laden dams in succession, to remove any maternally imposed ceiling effect on intake (Friedman, 1975). Thus, milk availability appears to be the most important determinant of milk intake in young suckling rats, leading to the prediction that young rat pups might suckle milk continuously if given the opportunity to do so. In one relevant experiment, a constant supply of milk was delivered through posterior intraoral cannulas in young rat pups while they were suckling the dam. Pups consumed the infused milk continuously to the point of "virtual drowning" and imminent gastric rupture (Hall & Rosenblatt, 1977), leading to the conclusion that intake volume is not controlled when milk is present within the young pup's oral cavity (Hall, 1990). The apparent absence of a direct inhibitory control of intake in this situation makes young rat pups seem even less physiologically sophisticated than blowflies, in which feeding is terminated by neural signals generated by foregut and crop distension (Dethier, 1976). It is important to note, however, that the posterior position of the intraoral cannulas in the cited study (Hall & Rosenblatt, 1977) likely promoted reflexive swallowing that was beyond the voluntary control of the pups.

In contrast to the apparent lack of feedback control over intake when milk is delivered into the posterior oral cavity, several studies have shown that, under normal conditions, suckling intake by young rat pups is modulated by experiential

factors associated with maternal deprivation and gastric fill (Blass *et al.*, 1979b; Friedman, 1975; Houpt & Epstein, 1973; Houpt & Houpt, 1975). Pups left with a non-lactating foster dam for 8 hr subsequently suckle more milk than nondeprived or 2-hr deprived littermates, when three milk-laden dams are provided in succession (Friedman, 1975). Moreover, pups consume less milk from the second and third dam than from the first, suggesting that there is an inhibitory control of intake operating in 10-day-old suckling rats (Friedman, 1975).

The intermittent availability of milk during suckling implies that the perceptual mechanisms that influence natural milk intake, at least in young rat pups, are not directly comparable to those that influence adult-like ingestion. In animals ingesting independently, intake is fully determined by each animal's own appetitive behavior during food acquisition. Conversely, in pups suckling milk from their dam, milk intake is a consummatory reflex-like response to milk letdown. The pup's consummatory response depends entirely on its behavioral state and the vigor of its ingestive responses during short periods of milk availability (Hall, 1985). Intake during suckling requires the pup to actively extract milk from the nipple. When milk is not present within the nipple's ducts and cisterns, young pups suckle intermittently in random arrhythmic and rhythmic patterns. Milk letdown occurs during brief episodes lasting approximately 10 s, during which nipple turgor increases. This event arouses attached pups, which begin to actively extract the milk from the nipple by suckling in a steady and rhythmic manner. Since milk intake is not a passive process, it is reasonable to expect intake volume to be affected by physiological or experimental stimuli that affect the pup's behavioral state and sucking vigor (Blass *et al.*, 1979b; Brake, Shair, & Hofer, 1988; Hall, 1990). Indeed, milk-deprived pups are more alert and behaviorally active compared to their nondeprived littermates (Blass *et al.*, 1979b; Hall, 1979a; Hofer & Shair, 1982; Moorcroft, Lytle, & Campbell, 1971), and milk deprivation increases the frequency of spontaneous mouthing and probing behaviors (Hall, 1979a) and of ultrasonic "distress call" emissions (Weller & Blass, 1988). Conversely, rat pups with stomachs full of milk appear lethargic and sleepy (Hall & Rosenblatt, 1977).

In human babies, a similar connection between suckling and behavioral state is evidenced by the common observation that successful nursing episodes often presage the baby falling asleep. As a suckling episode concludes, the infant's muscle tone gradually decreases while its posterior cortical electroencephalogram activity amplitude increases, indicative of a more relaxed behavioral state (Lehtonen *et al.*, 1998). In rat pups, nutritive or nonnutritive gastric loads that approach 4% of body weight significantly increase the amount of time pups spend in paradoxical sleep (Lorenz *et al.*, 1998), during which oromotor suckling and other movements are inhibited. The pronounced effect of gastric distension on the pup's behavioral state is consistent with the ability of nutritive or nonnutritive intragastric loads of 4–6% body weight to inhibit suckling intake (Drewett, 1978; Drewett & Cordall, 1976; Houpt & Epstein, 1973; Houpt & Houpt, 1975), whereas intake is not significantly affected by smaller gastric loads (Friedman, 1975).

In addition to having lower intragastric volumes, milk-deprived pups become hypovolemic in as little as 2 hr (Friedman, 1975; Heller, 1949). Colloid-induced hypovolemia increases suckling intake in a linear fashion in 10-day-old rats (Friedman, 1975), suggesting that deprivation-related increases in suckling intake might be related, at least in part, to a perceptual signal associated with dehydration. It is unclear whether dehydration affects general behavioral state in neonatal rats; if so, it might be expected to counteract the "somnolent" effects of gastric

distension. In this regard, the effect of amphetamine on suckling intake in young rat pups may be illuminating. In older rats, systemically administered amphetamine produces marked behavioral arousal, but inhibits food intake. The anorexigenic effect of amphetamine is exerted on the appetitive phase of feeding, with a much weaker effect on the consummatory phase (Wolgin, 2002). Thus, amphetamine appears to primarily disrupt the adult rat's ability to locate food or orient toward it, but has little effect on actual food consumption. Conversely, amphetamine apparently does not interfere with the ability of neonatal rats to locate and attach to the nipple. In 5-day-old rat pups deprived of milk and mother for 4 hr, amphetamine-induced behavioral arousal is associated with increased milk intake during suckling (Leshem, 1981). Thus, stimulatory and inhibitory controls of suckling intake in young rat pups may depend largely on modifications of overall behavioral state. An association between hypothalamic hypocretin/orexin neural systems that regulate feeding and sleep–wakefulness has been described in adults (Willie, Chemelli, Sinton, & Yanagisawa, 2001), although it is unclear whether similar mechanisms underlie the apparent association in neonates.

Interestingly, the effects of milk deprivation on consumatory suckling intake can be completely dissociated from deprivation effects on appetitive rooting and nipple-attachment behaviors in pups younger than 10 days of age. Results from many studies indicate that internal physiological cues related to deprivation state begin to influence nipple attachment in rats only after postnatal day 10. Older pups gradually develop the capacity to regulate both nipple attachment and suckling intake in response to gastric and postgastric cues, including caloric and metabolic signals (Cramer & Blass, 1985; Drewett & Cordall, 1976; Hall & Rosenblatt, 1978; Henning, Chang, & Gisel, 1979; Lorenz, 1983). Around the time of weaning (i.e., approximately 20 days postnatal), rats attach to the nipple and suckle rhythmically only when they have been deprived, and the amount of milk consumed during suckling is appropriate to their degree of deprivation. Further, the amount consumed by older pups during suckling is adjusted in an adult-like manner following nutritive and nonnutritive gastric preloads, which do not affect nipple attachment or suckling intake in young rat pups (Hall & Rosenblatt, 1978; Westneat & Hall, 1992).

Such findings can be interpreted as evidence that the suckling mode of ingestion in pups younger than 10 days is subject to a different set of perceptual and executive control mechanisms than those that control suckling in older pups. As in mature rats, the new controls reflect pronounced effects of gastric distension, hydration, and metabolic state on both appetitive and consumatory phases of ingestive motor output. Conversely, physiological cues that contribute to behavioral evidence of "hunger" and "satiety" in young rat pups appear not to directly affect the suckling mode of ingestion, but rather to modulate intake indirectly by affecting the pup's overall behavioral state. The behavioral effects of the gut hormone, cholecystokinin octapeptide (CCK), provide an example. Suckling elicits increased plasma levels of CCK in human newborns (Uvnas-Moberg, Marchini, & Winberg, 1993) and in neonatal rats (Weller *et al.*, 1992). Exogenously administered CCK has calming effects in neonatal rats (Blass & Shide, 1993; Weller & Blass, 1988), and functional antagonism of CCK receptors counteracts the calming effect of intraoral milk infusion in neonates (Blass & Shide, 1993). CCK also has been reported to elicit and prolong rest behavior and sleep in adult rats (Mansbach & Lorenz, 1983). These effects of exogenous CCK are similar to those produced in pups by intragastric saline infusion, which reduces ultrasonic vocalizations in maternally deprived

neonates (Nelson & Alberts, 2002). The calming effects of milk or saline in rat pups could be due to gastric distension or to rehydration. During suckling, the somnolent or calming effects of milk may be supplemented by additional effects of endogenously released CCK to modulate the pup's behavioral state and, consequently, the amount of milk it extracts from the nipple during episodic milk let-down.

In adult rats, the inhibitory effects on feeding produced by gastric fill or CCK depend on vagal viscerosensory inputs to the caudal medulla. Convergent anatomical and functional evidence indicates that this vagal afferent pathway is operational already in newborn rats, as discussed further below. Interestingly, however, exogenously administered CCK does not significantly affect milk intake during suckling in neonatal rats (Blass, Beardsley, & Hall, 1979a). This finding has been taken as further evidence that the neural controls of suckling differ from those of adult-like feeding. However, if CCK has calming or somnolent effects in rat pups, it is unclear why exogenously administered CCK does not reduce suckling intake. One possibility is that the short bioactive half-life of synthetic CCK precludes an inhibitory effect on suckling, since the availability of milk during suckling is controlled by the dam and may not coincide with CCK's pharmacological time course of action.

Independent Ingestion in Neonatal Rats

As summarized above and emphasized previously by others (Hall, 1990), suckling and adult-like ingestion appear to be two separate behaviors that comprise only partially overlapping motor, perceptual, and executive central neural circuits. Interestingly, these two behaviors have been shown to coexist simultaneously in neonatal rats. Only suckling is expressed initially in the natural situation. In the proper experimental environment, however, rat pups have the behavioral capacity to ingest milk independent of the dam, and this capacity is evident as early as 1 day after birth. The early controls of such independent ingestion are different from those that control suckling (summarized in Table 1), and appear instead to be

Table 1. General Summary of the Physiological Controls of Suckling and Independent Ingestive Behaviors in Rats During Early Postnatal Development

	Age of pup	
Feeding mode	0 to 8–9 days	9–10 days and older
Suckling	*Nipple attachment* controlled by olfactory and tactile cues from the dam; independent of pup's internal state	*Nipple attachment* modified by pup's internal state (i.e., length of prior deprivation, amount of gastric fill)
	Intake volume modulated by pup's behavioral state (i.e., aroused or asleep); pup's behavioral state *may* be affected by gastric fill and/or hydrational state	*Intake volume* decreased by gastric distension, increased by hypovolemia
Independent ingestion	*Intake volume* inhibited solely by gastric distension; stimulated solely by osmotic or volemic dehydration	*Intake volume* modulated by pup's metabolic state; inhibited by gastric distension; milk intake stimulated by dehydration until day 15 but thereafter inhibited by dehydration

developmentally continuous with more complex controls of adult-like feeding that emerge later. The early existence of an independent, adult-like ingestive system in neonatal rats presents a unique opportunity to examine the neural underpinnings of this system as it matures during the course of postnatal development.

Experiments investigating independent ingestion in neonatal rats typically are designed so that pups ingest milk or other liquid substances from saturated towels placed beneath them, or from intraoral catheters implanted near the front of their tongue (Hall & Bryan, 1980). A warm ambient temperature (i.e., 33–35°C) is critical to the success of such experiments; pups fail to independently consume significant amounts when tested in cooler ambient temperatures, even when a warm core body temperature is maintained (Hall, 1979b). Independent ingestion from the floor requires the pups to establish and maintain contact with the liquid food during ingestion, and thus involves more appetitive response components than does intake via intraoral infusion (Hall, 1985). It could be argued that floor feeding is a rather unsophisticated reflection of appetitive behavior, because a pup's random movements could bring its snout into contact with liquid on the floor. However, it has been demonstrated that rat pups can direct their ingestive behavior to a restricted food source as early as postnatal day 3 (Phifer, Denzinger, & Hall, 1991b). Such appetitive behavior is not displayed by surgically decerebrated pups and, therefore, appears to be controlled by central neural mechanisms that include connections between the hindbrain and forebrain. However, decerebrate pups can ingest liquid food via intraoral catheters, a behavior that does not require the pups to approach or contact food but which does include the final consumatory component of ingestion (Swithers-Mulvey & Hall, 1993; Swithers-Mulvey, Mishu, & Hall, 1992). Thus, neural circuits contained entirely within the caudal brainstem are sufficient to support the consumatory component of ingestion in neonatal rats, as in adult rats (Grill & Kaplan, 2002). As in adult rats, the neural circuits that control appetitive and consumatory components of independent ingestion in neonates are at least partially distinct. For example, centrally acting dopamine receptor antagonists dose-dependently suppress intake of 10% sucrose from the floor in 7-day-old rats without affecting their overall activity, but those drugs do not inhibit sucrose intake from intraoral catheters (Tyrka & Smith, 1991; Tyrka, Gayle, & Smith, 1992). A similar behavioral dissociation has been reported in older pups after blockade of fatty acid oxidation using 2-mercaptoacetate (Swithers, Peters, & Shin, 1999).

In the absence of pharmacological manipulation, neurologically intact pups ingest similar volumes in both floor and intraoral intake paradigms, and the amount consumed is modified by length of prior deprivation and by gastric preloads as early as postnatal day 1 (Hall, 1990). In the earliest report of this phenomenon (Hall, 1979b), 3-day-old pups deprived for 30 min, 7 hr, or 22 hr consumed milk from the floor in amounts that corresponded, respectively, to 1.5%, 3.0%, and 5.3% of their body weight in a 30-min test. Thus, both appetitive and consumatory components of independent ingestion are modified by factors associated with deprivation state in young rat pups, in striking contrast to the lack of deprivation effect in modulating the appetitive phase of suckling behavior at the same developmental period.

Independent ingestion by neonatal rats also appears to be motorically similar to the feeding behaviors exhibited by mature rats (Hall, 1990). For example, mouthing motor patterns are similar in pups and adults during ingestion via intraoral infusions. These oromotor movements feature a simultaneous activation of digastric and genioglossus lingual muscles (Swithers, Westneat, & Hall, 1998;

Travers & Norgren, 1986) and a rhythmic, alternating activity in masseter and digastric muscles (Swithers *et al.*, 1998). Further, oral habituation during the course of sequential intraoral infusion trials occurs in rats at all ages examined, and is modulated by the physiological state (e.g., hydration state, gastric fill) as early as postnatal day 6 (Swithers, 1995; Swithers & Hall, 1994; Swithers-Mulvey & Hall, 1993). The independent modulation of individual masticatory and lingual muscles during oral habituation reflects altered functions of the brainstem CPGs that synchronize the activity of these muscles in young rat pups (Swithers *et al.*, 1998), as in adult rats.

The biological necessity of making the transition from suckling to independent ingestion may dictate that neural components of adult-like ingestive behaviors are functional prior to weaning (Epstein, 1976), as has been demonstrated for other behavioral systems whose components can be elicited experimentally before they are spontaneously expressed. For example, 3-day-old rat pups display organized behavioral outputs that include licking, pawing, and gaping in response to electrical stimulation of the medial forebrain bundle (Moran, Schwartz, & Blass, 1983), although these behavior patterns are not expressed in their normal functional context until later in development. Similar findings have been described for locomotion behaviors (Altman & Sudarshan, 1975). The precocial, prefunctional appearance of an independent ingestive system in neonatal rats allows us to study relationships between neural and behavioral development by experimentally tapping into the system in its immature state.

Physiological Controls of Early Independent Ingestion

Although gastric preloads do not affect appetitive rooting and nipple-attachment behaviors in young rat pups, preloads effectively suppress both appetitive and consumatory phases of independent ingestion in rats as early as postnatal day 1 (Hall, 1990). Results from experiments using floor or intraoral intake tests in pups younger than 9–10 days of age after nutritive and nonnutritive gastric preloads indicate that gastric distension provides the sole inhibitory control over both appetitive and consumatory phases. Suppression of independent ingestion in young pups occurs when loaded or ingested gastric contents reach approximately 4% of body weight, independent of the nutritional content of the gastric load. Similar findings have been reported in adult decerebrate rats (Grill & Kaplan, 2002), suggesting that hindbrain mechanisms are sufficient for receiving and processing the gastric volume signal, and using it to inhibit ingestive motor outputs. Rats 9 days of age and older terminate intake at lower levels of gastric fill after receiving nutritive gastric preloads (Phifer & Hall, 1988; Phifer, Browde, & Hall, 1986; Swithers & Hall, 1989; Weller, 2000), indicating a newly emergent influence of a nutritive or metabolic signal that operates in addition to gastric fill to inhibit food intake.

Despite the inhibitory influence of gastric fill on independent ingestion, deprived or nondeprived neonatal rats will "over-fill" their stomachs by independently consuming relatively large quantities of milk if they are acutely dehydrated (Bruno, 1981; Callahan & Rinaman, 1998). Indeed, gastric preloads of calorie-free, hypotonic solutions that empty from the stomach prior to a feeding test significantly attenuate subsequent independent ingestion in young milk-deprived rat pups (Friedman, 1975), presumably because the water content of the load is absorbed into the circulation and rehydrates the animals. Similarly, deprivation-related

increases in independent ingestion that normally are observed in 6-day-old rat pups are eliminated when dehydration is prevented during the deprivation period (Phifer, Ladd, & Hall, 1991b). Conversely, hydrational maintenance during deprivation does not reduce deprivation-related increases in milk intake in 15-day-old rats (Phifer *et al.*, 1991b) or in adult rats. Thus, the ingestive behavior of rat pups during the first 9 days postnatal appears to be driven only by perceptual mechanisms associated with dehydration, and not by mechanisms associated with an empty stomach or caloric deficits. A general summary of these findings is provided in Table 1.

Behavioral Capacities of Neonatal Rats and Adult Decerebrate Rats

The similarities and differences between ingestive controls operating in independently feeding rats younger than 9 days of age and in adult decerebrate rats are worth exploring, because such comparisons focus attention on the necessary and sufficient neural mechanisms that underlie those controls. For example, young rat pups and decerebrate adults appear to lack any nutritive or metabolic control over food intake, but both are responsive to inhibitory signals produced by gastric distension and the gut hormone CCK (Grill & Kaplan, 2002; Hall, 1990; Robinson, Moran, & McHugh, 1988). A potential explanation of these similarities is that metabolic controls of feeding require neural circuits with forebrain components that are surgically interrupted in adult decerebrate rats and functionally immature in neonates. Correspondingly, the inhibitory influences caused by gastric fill or CCK are apparently mediated by circuits contained entirely within the caudal brainstem and spinal cord. These circuits are preserved in adult decerebrate rats, and they are already functional in neonates.

Despite the similarities just mentioned, further comparison of neonatal and adult decerebrate rats reveals some telling differences in their behavioral response capacities. Internal and external sensory cues that affect ingestive behavior in neonatal rats but not in adult decerebrates include olfactory stimuli, ambient temperature, and dehydration (discussed further below). Neonatal rats can develop lithium chloride-induced conditioned taste aversions by postnatal day 12 (Vogt & Rudy, 1984), whereas adult decerebrate rats cannot (Grill & Kaplan, 2002). Perhaps most importantly, decerebrate rats lack appetitive drive, but neonatal rats do not. This finding indicates that rostral brainstem transection disrupts central mechanisms that underlie motivational states such as "hunger," and central mechanisms that link such states to behavioral output. Although decerebrate rats can locomote and even groom, they do not approach or otherwise voluntarily contact food or water, and they appear incapable of learning any operant behaviors to receive food or water. Conversely, appetitive-motivated ingestive behaviors are expressed by rat pups as early as 1 day after birth (Hall, 1979b, 1990; Johanson & Hall, 1979). Independent ingestion from the floor of a testing chamber requires the pup to initiate contact with milk or other ingesta, and to bring the ingesta into the oral cavity for consumption. Thus, the floor test measures both appetitive and consumatory phases of ingestion. One-day-old rat pups efficiently feed from the floor, whereas adult decerebrate rats do not.

Although intake of milk through an intraoral cannula measures only the consumatory phase of ingestion, Johanson and Hall demonstrated that 1-day-old rats

can learn to perform a discriminatory motor task in order to receive intraoral infusions of milk (Johanson & Hall, 1979). The ability of pups to learn and perform this task provides additional evidence that neural substrates underlying appetitive drive and motivated behavior are functional at an early age. Further evidence comes from work demonstrating that 3-day-old rats learn to perform an operant task that delivers electrical stimulation to the medial forebrain bundle (Moran, Lew, & Blass, 1981). In addition, young rat pups are behaviorally activated by intraoral infusions (Hall, 1979b; Specht, Burright, & Spear, 1996), whereas decerebrate neonatal and adult rats are not (Kornblith & Hall, 1979) (S. E. Swithers and J. M. Kaplan, personal communications). The behavioral activation observed in intact newborn pups during intraoral infusions seems to extend to the entire body and to other behaviors such as anogenital licking, and may be related to developing neural arousal and reward systems associated with consumatory behaviors in general (Hall, 1979b). Considered together, these findings suggest that feeding-related arousal, motivational, and learning systems already are operational in neonatal rats, but are no longer functional in adult decerebrates.

Central Feeding Circuits

Modern views of behavioral control circuits incorporate ideas of parallel processing, interactive neural systems, and multiple central integrators (Berthoud, 2002; Levine & Billington, 1997). Behavioral control systems evolved over millions of years as adaptive mechanisms that serve specific biological functions under highly variable environmental conditions. Similarly, there may be several parallel central integrators that control ingestive behavior and responsiveness, each exerting influence over behavioral output in different ways and under different circumstances (Berthoud, 2002; Levine & Billington, 1997). Analyses in rats and other experimental animals indicate that mature feeding behavior is the functional output of distributed neural networks whose individual components are found in multiple regions of the forebrain, midbrain, hindbrain, and spinal cord. Forebrain regions implicated in food intake control include the cortex, hippocampus, hypothalamus, thalamus, extended amygdala, striatum, and septal nuclei. These telencephalic and diencephalic regions include identified neural substrates for processing exterosensory signals (i.e., photic, auditory, somatosensory) and interoceptive signals (i.e., endocrine, viscerosensory) that affect feeding behavior, and for providing circadian control and other timing features that codetermine the occurrence and shape of feeding bouts. Less well-defined forebrain regions comprise the distributed executive neural mechanisms that determine motivational drive, and give ingestive behaviors their overall goal-directed character and complexity (Watts, 2000). At "lower" levels of the central neuraxis, feeding-related hindbrain and spinal regions contain the motor and premotor nuclei that control ingestive and digestive somatic and visceral motor outputs. Direct and relayed humoral, somatosensory, and viscerosensory inputs to these hindbrain and spinal nuclei are critical components of these distributed feeding networks, together with brainstem relay centers (i.e., solitary and parabrachial nuclei, ventrolateral medulla, raphé nuclei, periacqueductal gray) that link ingestive and digestive sensorimotor functions to "higher" forebrain centers.

An attempt to summarize all that is currently known about neural connections within this multitude of feeding-relevant regions would soon approximate a wiring

diagram of the CNS. The picture becomes even more complex when one adds in multiple blood-borne signals (e.g., leptin, insulin, glucocorticoids) that are known to affect transmission within these neural circuits and thereby modify feeding-related physiological and behavioral outputs. The remainder of this chapter focuses on some essential components of these feeding control systems, and reviews what is known about the functional development of these components and their interconnections. For this purpose, Smith's theory of the direct and indirect controls of feeding (Smith, 1996, 2000) has been adapted as an organizational approach to the problem. His theory is described briefly in the following section, and then a new working hypothesis regarding the essential role of the hindbrain dorsal vagal complex (DVC) in the central neural control of food intake is introduced.

Direct and Indirect Controls of Feeding

As postulated by Smith (1996, 2000), the direct controls of feeding comprise sensory signals relayed to the brain as a direct consequence of food-induced stimulation of mucosal receptors in the nasal cavity and along the digestive tract, from the tip of the tongue through the small intestine. Direct controls include the smell, temperature, taste, and texture of food, and also include postingestive but preabsorptive gastric distension and release of certain gut hormones. Thus, direct controls of feeding provide CNS perceptual circuits with sensory information about ingesta or (in the case of olfactory signals alone) potential ingesta. Olfactory, orosensory, and gastrointestinal viscerosensory signals that stimulate and maintain food intake provide "positive feedback" control, whereas signals that inhibit or terminate food intake provide "negative feedback" control. For example, a direct positive feedback control over food intake is provided by sucrose stimulating specific classes of oral and nasal chemoreceptors; the resulting "flavor" neural signal is relayed to the olfactory bulb and caudal brainstem via sensory afferents traveling in the olfactory, trigeminal, facial, glossopharyngeal, and vagus nerves. An example of a direct negative feedback control of food intake is gastric distension, supplemented by food-induced intestinal release of CCK; in this case, the resulting neural signal is delivered to the brain via mechanoreceptive, and perhaps chemoreceptive, afferents traveling primarily in the vagus nerve and secondarily through spinal afferents.

Once initiated, food intake proceeds until the relative potency of negative feedback signals equals or outweighs the potency of positive feedback signals. The sensory feedback signals are received, processed, and integrated by central perceptual and executive circuits that provide the necessary "comparator function" to determine whether and how the animal's behavioral and physiological motor outputs will be modified. Somatic motor pathways control the behavioral appetitive and consummatory movements, and visceral pathways control interrelated autonomic and endocrine digestive processes. The somatic and visceral outputs that are generated directly affect the nature of the feedback signals from mucosal receptors, thus completing the behavioral control loop.

According to Smith's criteria, all other feeding controls are indirect, and affect intake by modulating the potency of the direct controls (i.e., the positive and negative preabsorptive feedback signals). For example, estrogen is considered to provide an indirect control of feeding, because it acts to increase the satiating potency of CCK, a direct control of feeding (Asarian & Geary, 1999; Eckel & Geary, 1999;

Geary, 2001). Other indirect controls of feeding include rhythmic, metabolic, thermal, hydrational, conditioned, and cognitive factors (Smith, 1996, 2000).

The two general categories of feeding controls correspond to two grossly defined neuroanatomical loci that are proposed to mediate their effects on physiological and behavioral output (Smith, 1996, 2000). As defined, the direct controls of feeding engage neural circuits whose necessary components are contained entirely within the hindbrain and spinal cord. Conversely, the indirect controls of feeding engage descending and long-loop reciprocal connections between telencephalic and diencephalic regions that interact with hindbrain and spinal output circuits. Supportive evidence for this hypothesis comes from studies in decerebrate adult rats, in which intraoral intake is modulated by orosensory and gastrointestinal viscerosensory direct controls, but is not modulated by rhythmic, metabolic, hormonal, hydrational, thermal, conditioned, cognitive, or adiposity-related indirect controls (Grill & Kaplan, 2002). For example, food deprivation alters taste reactivity in neurologically intact adult rats, but not in decerebrates (Kaplan, Roitman, & Grill, 2000).

When considered in the context of Smith's theoretical framework, studies of independent ingestion in neonatal rats indicate that olfactory, orosensory, and gastrointestinal viscerosensory direct controls of feeding are operational as early as postnatal day 1, with later additional sensory refinement (Ackerman, Albert, Shindledecker, Gayle, & Smith, 1992; Hall & Bryan, 1981; Hudson & Distel, 1999; Kehoe & Blass, 1985). Thus, the basic neural circuits through which these direct controls affect feeding must already be functional in neonatal rats. Conversely, while some indirect controls (e.g., thermal, hydrational) are known to affect feeding in neonatal rats, the stimulatory or inhibitory effect of these controls shifts during development, and other indirect controls (e.g., metabolic, rhythmic) initially are absent altogether.

The results of anatomical and functional studies have led me to develop my own working hypothesis about the central control of food intake in mature and developing animals. It is consistent with Smith's theory of direct and indirect controls, but it goes further by postulating a common hindbrain effector site at which the direct and indirect controls exert their influence. The hypothesis also provides a framework in which to understand postnatal changes in ingestive behavioral responsiveness. Specifically, I propose that feeding behavior is a function of neural activity within the hindbrain DVC, and that the ability of any internal or external factor to modulate food intake depends on its ability to affect DVC neural processing. This working hypothesis is open to experimentation, and therefore, to refinement or rebuttal. I believe that it provides useful insights into understanding how complex endocrine and neural signaling mechanisms acting at multiple peripheral and central sites come to exert their controlling influence on ingestive behavior. Questions about postnatal changes in behavioral responsiveness to various feeding controls can then be rephrased to ask the following: When, and by what mechanisms, do these controls gain access to DVC neural circuits?

The DVC as an Ingestive Control Center

Anatomical, physiological, and behavioral data support the view that the central control of ingestive behavior is largely achieved by modulating neural signaling in the hindbrain DVC, which comprises the dorsal motor nucleus of the vagus (DMV),

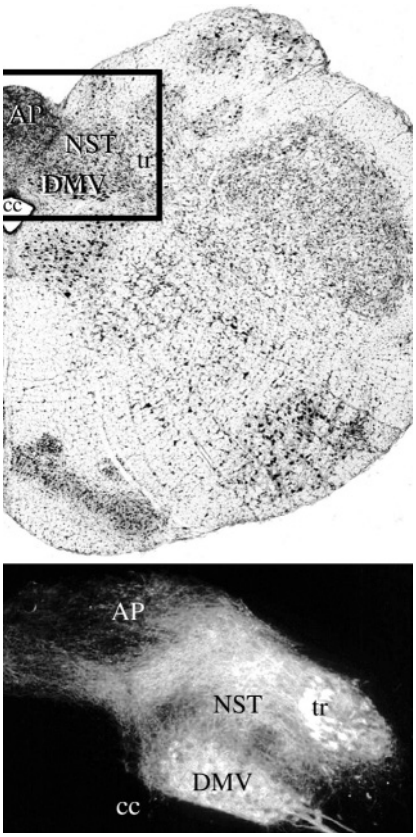

Figure 1. The anatomical organization of the dorsal vagal complex (DVC) is illustrated in a Nissl-stained tissue section (top panel). The DVC is located within the caudal dorsomedial medulla (boxed region), and comprises the area postrema (AP), nucleus of the solitary tract (NST), and dorsal motor nucleus of the vagus (DMV). Injection of neural tracer into the cervical vagus nerve in a newborn rat (bottom panel) reveals an adult-like distribution of anterogradely labeled vagal sensory afferents in the solitary tract (tr), medial NST, and AP (Rinaman & Levitt, 1993). Retrogradely labeled vagal motor neurons occupy the DMV, and their dendrites extend dorsally into the NST. cc, central canal.

nucleus of the solitary tract (NST), and area postrema (AP) (Figure 1). Several features of the DVC make it ideally suited as a brainstem integration and command center for ingestive behavior:

1. DVC neurons receive direct and relayed synaptic input from olfactory, glossopharyngeal, facial, trigeminal, vagal, and spinal viscerosensory afferents. These inputs convey information to DVC neurons about the chemical and mechanical properties of food, comprising the direct positive and negative feedback controls of food intake.
2. A significant portion of the medial DVC contains fenestrated capillaries, allowing blood-borne factors (e.g., toxins, cytokines, hormones, and osmolytes) that affect food intake to access the local brain parenchyma and affect DVC neural activity.
3. DVC neurons are interconnected with brainstem CPG, premotor, and motor nuclei that organize and execute ingestive movements such as licking, biting, chewing, and swallowing (Streefland & Jansen, 1999). For example,

neurons in viscerosensory subregions of the medial DVC are infected in adult rats after a retrograde viral tracer is injected into masticatory, facial, or lingual muscles, evidence that DVC neurons are synaptically connected to the somatic motor circuits that control these muscles (Fay & Norgren, 1997a, 1997b, 1997c; Travers & Rinaman, 2002).

4. The DVC contains vagal parasympathetic motor neurons that regulate digestive secretory and motor functions (i.e., gastric, intestinal, pancreatic, and hepatic). Vagal motor output to these digestive viscera shapes the way in which ingesta are handled and metabolically processed, and thus shapes many of the direct and indirect controls of food intake.
5. Reciprocal neural pathways exist between the DVC and the insular cortex, medial prefrontal cortex, amygdala, BNST, striatum, hypothalamus, thalamus, and other forebrain regions. The neural interaction of these brain regions with the DVC may allow emotional, cognitive, metabolic, rhythmic, thermal, conditioned, and other events to serve as indirect controls of feeding.

Bearing these properties in mind, one can imagine how hypophagic and hyperphagic responses to disparate physiological and experiential conditions might be accomplished through common DVC neural circuits, which are accessed by numerous peripheral and central afferent routes (Figure 2). The following section reviews what is known currently about the development of key signaling pathways

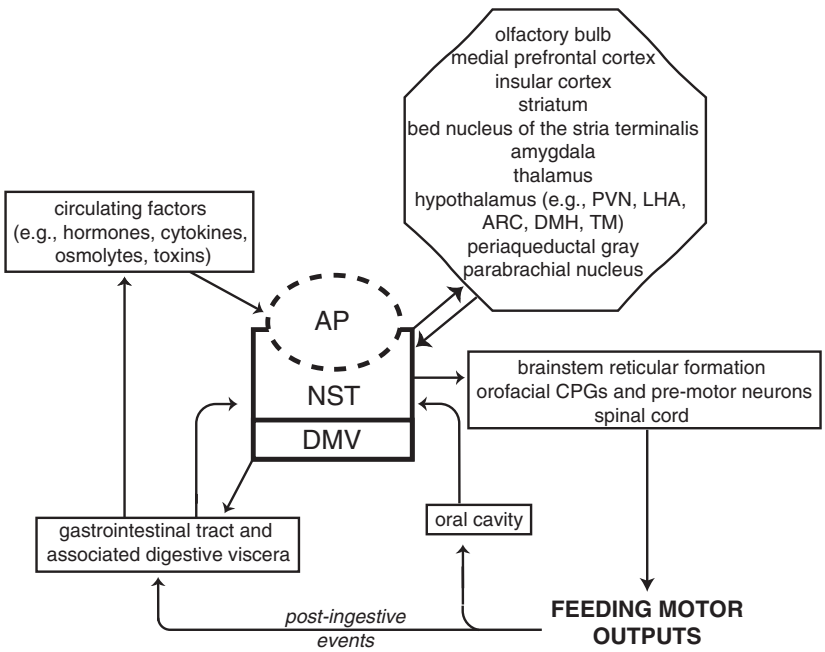

Figure 2. Schematic of feeding-related inputs to, and outputs from, the hindbrain dorsal vagal complex (AP, NST, and DMV). The dashed line around the AP signifies the absence of a blood–brain barrier. Arrows indicate the presence of known direct connections; indirect connections among these CNS regions also exist but are not illustrated. AP, area postrema; ARC, arcuate nucleus; CPG, central pattern generator; DMH, dorsomedial nucleus; DMV, dorsal motor nucleus of the vagus; LHA, lateral hypothalamic area; NST, nucleus of the solitary tract; PVN, paraventricular nucleus; TM, tuberomammillary nucleus.

through which the direct and indirect controls can access DVC neural circuits and thereby affect food intake, beginning with an example of a well-known direct negative feedback control by CCK.

Inhibition of Feeding by CCK

Intestinal secretory cells release CCK into the local interstitial fluid and the circulation. The adequate natural stimulus for CCK release is provided preabsorptively by food particles contacting the intestinal mucosa (Richards, Hillsley, Eastwood, & Grundy, 1996). Evidence that endogenously released CCK contributes to meal-induced satiety comes from findings that pharmacological blockade of CCK receptors, or their congenital absence, is associated with significant increases in meal size (Moran, 2000; Reidelberger & O'Rourke, 1989). In adult rats, exogenously administered CCK increases the firing activity of gastrointestinal vagal sensory neurons that provide synaptic input to the DVC (Raybould, 1992; Schwartz, 2000; Schwartz, McHugh, & Moran, 1993). CCK-induced stimulation of this afferent pathway inhibits feeding and produces other physiological effects similar to those produced by gastric distension (Moran, 2000; Olson *et al.*, 1993; Rinaman, Baker, Hoffman, Striker, & Verbalis, 1998; Rinaman, Verbalis, Stricker, & Hoffman, 1993). All of these effects are eliminated after interruption of peripheral CCK receptor signaling, or by interruption of vagal sensory inputs to the DVC (Moran, 2000). Importantly, both exogenous CCK and gastric distension inhibit intraoral sucrose feeding in decerebrate adult rats (Grill & Kaplan, 2002; Grill & Smith, 1988). Thus, neural circuits contained within the isolated brainstem are sufficient for viscerosensory inputs to the DVC to gain executive control over the oral motor components of ingestive behavior.

As discussed previously in this chapter, gastric distension and CCK provide potent inhibitory feedback control over independent ingestion in neonatal rats (Hall, 1990; Robinson, Moran, & McHugh, 1985, 1988). In fact, the densest concentration and widest distribution of gastrointestinal CCK receptors in rats is observed at birth (Robinson, Moran, Goldrich, & McHugh, 1987), consistent with the lower threshold doses of exogenous CCK required to inhibit feeding in neonatal as compared to older rats (Robinson *et al.*, 1985, 1988). An inhibitory role for endogenous CCK also has been demonstrated in neonatal rats during tests of independent ingestion (Blass & Shide, 1993; Weller *et al.*, 1990; Weller, 1992).

Immunocytochemical detection of an immediate-early gene protein product, cFos, has been used to identify central neurons that are stimulated in 2-day-old rats after systemic administration of CCK (Rinaman, Hoffman, Stricker, & Verbalis, 1994). Hindbrain cFos expression was virtually identical in 2-day-old and adult rats after CCK treatment, with activated neurons located in specific subregions of the DVC that receive gastric vagal sensory input (Figure 3). A similar pattern of DVC activation was reported in neonatal rats after milk ingestion via suckling (Hironaka, Shirakawa, Toki, Kinoshita, & Oguchi, 2000). However, in striking contrast to results in adult rats, CCK treatment in 2-day-olds did not activate cFos expression in the hypothalamus or other forebrain regions, and did not stimulate pituitary hormone release (Rinaman *et al.*, 1994). Thus, viscerosensory activation of hindbrain circuits appears sufficient to mediate the inhibitory effects of exogenous CCK on independent ingestion in neonatal rats, as in adult decerebrate rats. The lack of hypothalamic activation in neonates after CCK treatment is consistent with other evidence for delayed postnatal maturation of ascending projections from the DVC

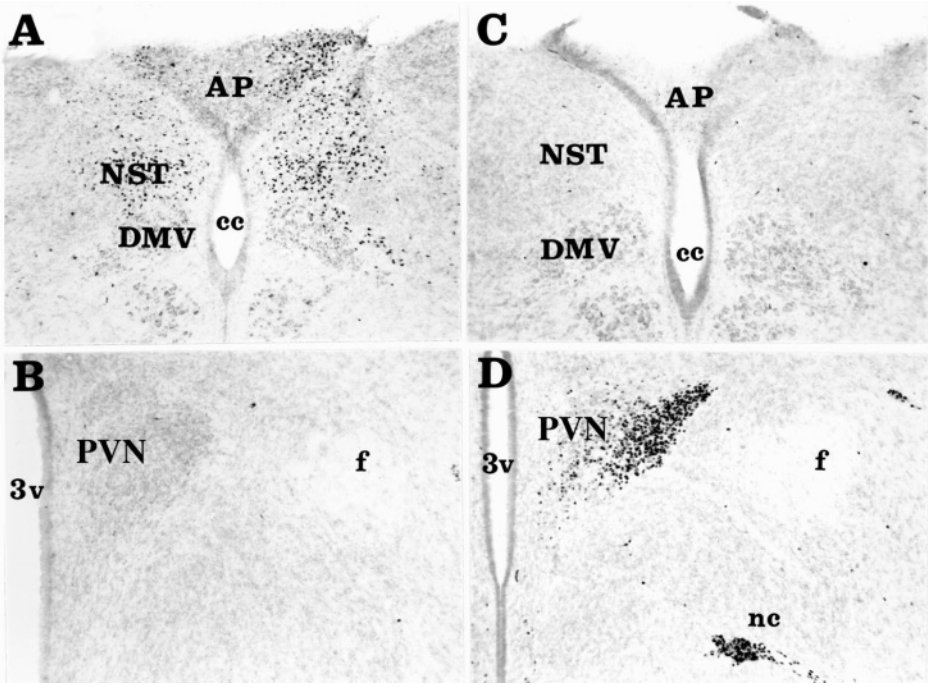

Figure 3. Central cFos immunolabeling in 2-day-old rats after treatment with synthetic cholecystokinin-8 (CCK; 0.1 μg/10 g BW, i.p.; panels A and B), or 2.0 M NaCl solution (0.1 μ1/10 g BW, s.c.; panels C and D). CCK treatment suppresses independent ingestion in 2-day-old rats, and activates cFos expression in the dorsal vagal complex (A) but not in the hypothalamus (B) (Rinaman *et al.*, 1994). Conversely, hypertonic NaCl, which does not suppress independent ingestion in 2-day-old rats, activates cFos expression in the hypothalamus (D) but not in the dorsal vagal complex (C) (Rinaman *et al.*, 1997). AP, area postrema; cc, central canal; DMV, dorsal motor nucleus of the vagus; f, fornix; NST, nucleus of the solitary tract; PVN, paraventricular nucleus of the hypothalamus; 3v, third ventricle.

and ventrolateral medulla that transmit viscerosensory information to the hypothalamus and other forebrain regions in adult rats (Ericsson, Kovacs, Sawchenko, 1994; Rinaman *et al.*, 1995; Sawchenko & Swanson, 1981, 1982).

The ability of exogenous CCK to engage DVC circuits and thereby affect hindbrain feeding motor outputs in neonatal rats means that the necessary afferent inputs and efferent outputs of the DVC are functional already at birth. Indeed, as summarized below, anatomical tracing studies in fetal and newborn rats reveal a precocious development of DVC motor outputs to the gastrointestinal tract, and a correspondingly early development of viscerosensory inputs to the DVC.

Early Development of the DVC

The influence of vagal parasympathetic output on food intake is indirect, but influential nonetheless. Vagally mediated control of various digestive motor and secretory functions produces moment-to-moment changes in the viscerosensory landscape, and thus shapes many of the neural and endocrine feedback signals that comprise the direct and indirect controls of feeding.

The final neural control of alimentary motor and secretory functions is provided by enteric neurons that occupy the gastrointestinal tract and associated

viscera. Substantial work has detailed the embryonic colonization of the gut by neural crest cells and the subsequent development of the enteric nervous system (Gabella, 1979; Rothman, Nilaver, Gershon, 1984). Parasympathetic vagal motor neurons provide essential modulatory control over the enteric system, and hence, over digestive functions. In rats, vagal motor fibers first reach the gut on embryonic day (E) 12 or E13 (Boekelaar Drukker, Groen, & Baljet, 1985; Rinaman & Levitt, 1993). The basic distribution pattern of vagal nerve branches is established by E15 and maintained through adulthood (Boekelaar *et al.*, 1985; Powley *et al.*, 2001).

In newborn rats, as in adults, the dendrites of gastric vagal motor neurons extend dorsally from the DMV into circumscribed NST subnuclei that are the primary termination sites for gastric vagal sensory afferents (Rinaman, Card, Schwaber, & Miselis, 1989; Rinaman & Levitt, 1993; Shapiro & Miselis, 1985) (Figure 1). Ultrastructural analyses in adult rats reveal that the codistributed gastric sensory afferents and motor neuron dendrites form synaptic contacts in a restricted subnucleus of the medial NST (Rinaman *et al.*, 1989), allowing monosynaptic viscerosensory modulation of vagal motor outflow that operates in addition to di- and polysynaptic vagal sensorimotor loops (Rogers & Hermann, 1992; Rogers McTigue & Hermann, 1995). The anatomical substrate for these vago-vagal sensorimotor circuits is already evident by E16, when the central processes of gastric vagal sensory neurons arborize within the DMV and NST in close proximity to the dendrites of gastric motor neurons (Rinaman & Levitt, 1993). By the time of birth, the anatomical organization and relationship of digestive vagal sensory and motor components of the DVC are remarkably mature (Figure 1). Other cranial nerve afferents to the DVC (i.e., facial, trigeminal, and glossopharyngeal) are similarly well established in rats by the time of birth (Zhang & Ashwell, 2001).

Delayed Maturation of Diencephalic and Telencephalic Inputs to the DVC

Despite evidence for early organization of primary sensory and motor components of the DVC, results from several studies suggest that other central neural inputs to the DVC undergo a significant amount of postnatal maturation in rats, together with significant remodeling of local DVC networks (Kawai & Senba, 2000; Rao, Jean, & Kessler, 1997; Vincent & Tell, 1999). The number of vesicle-containing axon terminals in the DVC more than doubles within the first 10 postnatal days (Miller, McKoon, Pinneau, & Silverstein, 1983). The volumetric fraction of DVC tissue occupied by synaptophysin, a component of synaptic vesicles, increases from 1% at birth to 5% after postnatal day 11, a proportion that is maintained into adulthood (Lasiter & Kachele, 1989; Rao *et al.*, 1997). The postnatal increase in synaptic terminals in the DVC parallels increases in the dendritic elaborations of DVC neurons (Lasiter, Wong, & Kachele, 1988). Since the overall density and distribution of vagal sensory inputs to the DVC is established already at birth (Rinaman & Levitt, 1993), the observed postnatal increase in axon terminals is consistent with an increasing innervation of the DVC by the hypothalamus and other central nuclei.

The structural maturation of hypothalamic inputs to the DVC in rats during postnatal development has been investigated. One study examined oxytocin (OT)-containing projections from the paraventricular nucleus of the hypothalamus (PVN) to the DVC (Rinaman, 1998). OT acts centrally to inhibit food intake in adult rats (Flanagan, Verbalis, & Stricker, 1989; Olson, Drutarosky, Stricker, & Verbalis, 1991; Rinaman & Rothe, 2002), perhaps by acting at OT receptor sites in

the DVC. OT-positive fibers within the DVC arise exclusively from the PVN, providing an unambiguous anatomical marker for this projection pathway. Mature, processed OT neuropeptide is not visible in hypothalamic neurons by immunocytochemical methods until the end of the first postnatal week, and OT-positive axons are not visible in the DVC until several days after that (Buijs, Velis, & Swaab, 1980). However, using a well-characterized monoclonal antibody (PS36) specific for OT-neurophysin prohormone that labels neurons and fibers in the rat hypothalamus as early as E16 (Whitnall, Key, Ben-Barak, Ozato, & Gainer, 1985), it has been shown that a few scattered PS36-positive fibers are present already in the newborn rat DVC (Rinaman, 1998). Retrograde tracing confirmed that these fibers originate from a small subset of PS36-positive neurons in the PVN. Quantitative analysis demonstrated that the cumulative length of immunopositive fibers in sampled DVC subregions increased markedly and progressively between birth and adulthood, attaining adult-like values by the time of weaning (Rinaman, 1998). These findings are consistent with a gradually increasing innervation of the DVC by chemically immature PVN neurons during the early neonatal period. The later appearance of processed OT in fibers within the DVC at the end of the second postnatal week may signify an important step in the functional maturation of this descending pathway.

Despite the early presence of PVN projections to the DVC, the establishment of synaptic contacts with DVC target neurons may be a temporally distinct event. Such a distinction is critical for determining the connectivity of developing neural circuits. To address this issue, pseudorabies virus (PRV) has been used for transneuronal tracing of developing neural circuits (Rinaman *et al.*, 1999, 2000). PRV is a neurotropic α-herpesvirus, and attenuated vaccine strains have utility for anatomical tracing of synaptically linked neural circuits after peripheral or central inoculation (Card, 1998). PRV transport between neurons occurs only at points of synaptic contact, and proceeds retrogradely from infected neurons to their sources of presynaptic input.

In order to identify central sources of synaptic input to the DVC at different stages of postnatal development, the ventral stomach wall was inoculated with PRV in rats on postnatal day 1, 4, or 8. Rats were sacrificed 60–64 hr later and their brains processed for immunocytochemical detection of transported virus (Rinaman *et al.*, 2000). In each age group, PRV was transported to similar autonomic and preautonomic regions of the spinal cord and brainstem, evidence for early synaptic connectivity within these regions. DVC infection patterns appeared identical at each age. However, in rats injected with virus on postnatal day 1, the only transneuronal labeling observed in the forebrain 2.5 days later was a relatively small number of infected neurons in the PVN and dorsomedial nucleus of the hypothalamus (DMH) (Figure 4). The complete absence of other forebrain labeling indicates a lack of other diencephalic and telencephalic inputs to the DVC in rats during the first few days postnatal. The first telencephalic neurons to become infected were located within the central nucleus of the amygdala (CeA) and bed nucleus of the stria terminalis (BNST) in rats injected with PRV on postnatal day 4 and sacrificed 2.5 days later. The lateral hypothalamic area (LHA) also contained infected neurons for the first time in this age group; however, infected cortical neurons were still absent.

The early absence of LHA inputs to the DVC is noteworthy. LHA neurons in newborn rats already display evoked responses and convergent inputs from visceral, osmosensory, gustatory, and glucosensory stimuli (Fisher & Almli, 1984). Conversely, LHA neurons in neonates do not respond to visual, auditory, tactile, or

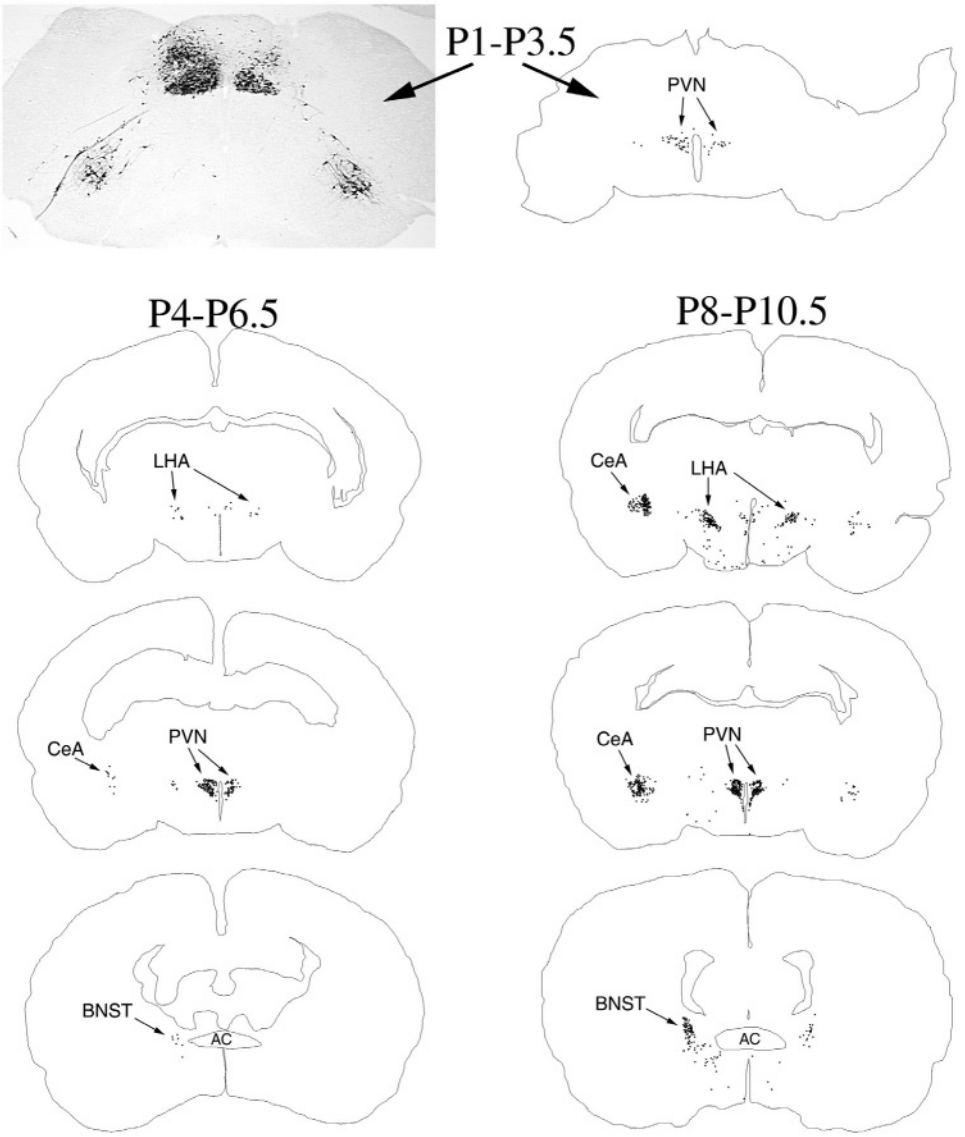

Figure 4. PRV immunolabeling in the medulla (top left), diencephalon and telencephalon 62–64 hr after inoculation of the ventral stomach wall in rats on postnatal day 1, 4, or 8 (Rinaman *et al.*, 2000). The photomicrograph shows medullary labeling in a rat injected with virus on postnatal day 1; similar medullary labeling is observed at later ages. Line drawings are computer-assisted tracings of single tissue sections in which the distribution of all PRV-labeled neurons is plotted. In rats injected on postnatal day 1, the only forebrain neurons to become infected are located in the PVN (top right). In pups injected on day 4, labeled forebrain neurons are present in the PVN, LHA, CeA, and BNST. Increased numbers of infected neurons are observed in these regions in pups injected on postnatal day 8; in addition, labeled neurons are found in the medial prefrontal cortex and insular cortex (not shown). AC, anterior commissure; BNST, bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala; LHA, lateral hypothalamic area; PVN, paraventricular nucleus of the hypothalamus.

olfactory inputs, all of which modulate LHA neuronal activity in adult rats. Other developmental changes in the LHA response properties indicate that these neurons undergo a significant amount of "fine tuning" during postnatal development (Fisher & Almli, 1984). Most telencephalic regions provide stronger connections to the LHA than to other feeding-related regions of the hypothalamus in adult rats, suggesting that the LHA is particularly important in mediating many of the volitional aspects of ingestive behavior in adult rats (Petrovich, Setlow, Holland, & Gallagher, 2002; Watts, 2000) that only emerge postnatally. The maturation of these processes may be related to the maturation of LHA intrinsic circuits and inputs (Fisher & Almli, 1984), as well as the maturation of descending LHA projections to the DVC (Rinaman *et al.*, 2000).

The overall distribution of transneuronally infected forebrain neurons in rats injected on postnatal day 8 and sacrificed 2.5 days later was similar to that observed in adult rats, and included neurons in the lateral and medial hypothalamus, CeA, BNST, and visceral cortices. Additional experiments involving intra-DVC injections of PRV or a standard retrograde neural tracer revealed that neurons in hypothalamic and limbic forebrain regions project to the DVC several days before establishing synaptic connections there with target neurons, and that cortical axons do not even reach the vicinity of the DVC until a few days postnatal (Rinaman *et al.*, 2000). These results demonstrated a novel strategy for evaluating synaptic connectivity in developing neural circuits, and showed a temporally segregated postnatal emergence of hypothalamic and telencephalic synaptic inputs to the DVC.

Dehydration-Induced Activation of DVC Neurons Increases Postnatally

The studies described above offer anatomical evidence that hypothalamic inputs to the DVC undergo a significant amount of structural maturation during early postnatal development. Thus, the ability of the hypothalamus to modulate DVC neural circuits may be functionally immature in neonatal rats. To test this hypothesis, central cFos expression patterns were compared in 2-day-old and adult rats after systemic administration of hypertonic saline (HS) (Rinaman, Stricker, Hoffman, & Verbalis, 1997). HS treatment produces hypernatremia, which increases the effective osmotic pressure of extracellular fluid and draws water out of the intracellular compartment (Gilman, 1937). In both neonates and adults, HS treatment generates pronounced hypernatremia, and induces similar cFos activation patterns in osmosensitive regions of the basal forebrain and hypothalamus, including magnocellular and parvocellular PVN neurons (Rinaman *et al.*, 1997). Thus, hypothalamic circuits in neonatal rats appear to respond in an adult-like manner to signals generated by plasma hyperosmolality. Conversely, very little cFos expression in the NST was observed in dehydrated neonates, as opposed to marked NST activation in dehydrated adults (Figure 3).

The lack of cFos expression in the NST in neonatal rats after HS treatment may be due to functional immaturity of descending projections from osmosensitive hypothalamic neurons, including OT neurons in the PVN. Since anorexia is known to accompany states of dehydration in adult rats, it is significant that this behavioral effect is blunted by central antagonism of OT receptors (Olson *et al.*, 1991). In adult rats, the OT-containing projection from the PVN to the DVC provides a tonic inhibitory influence over vagally mediated gastric motility that is further amplified by plasma hyperosmolality (Flanagan, Blackburn, Verbalis, & Stricker, 1992;

Rogers & Hermann, 1992). Microinjection of OT into the DVC increases the activity of NST neurons that receive synaptic input from gastric viscerosensory afferents (McCann & Rogers, 1990), consistent with the view that OT may act within the DVC as an indirect control of food intake. Thus, cFos activation in the NST of dehydrated adult rats is consistent with activation of an OT-containing projection pathway from the hypothalamus to the DVC. The lack of NST cFos activation in dehydrated neonates suggests that this descending OT signaling pathway is functionally immature, consistent with immunocytochemical and virus-tracing data indicating structural immaturity of this pathway. As discussed in the next section, the postnatal maturation of this signaling pathway to the DVC may be directly related to the postnatal emergence of dehydration anorexia.

Postnatal Emergence of Dehydration Anorexia

Centrally mediated responses to plasma hyperosmolality in adult rats include compensatory drinking (Fitzsimons, 1963), neurohypophyseal secretion of OT and vasopressin (Stricker & Verbalis, 1986), inhibition of vagally mediated gastric motility and emptying (Flanagan *et al.*, 1989; Flanagan *et al.*, 1992), and inhibition of food intake (Flanagan *et al.*, 1992). The first two responses also occur in neonatal rats (Almli, 1973; Bruno, 1981; Sinding, Robinson, & Seif, 1980), whereas the latter response, termed "dehydration anorexia," does not emerge until after the first 2 weeks of postnatal development (Bruno, 1981; Bruno & Hall, 1982). The inhibitory processes mediating dehydration anorexia appear to influence the appetitive approach to food, but do not inhibit consumatory responses to food delivered intraorally (Bruno, 1981; Bruno & Hall, 1982; however, see Flynn, Curtis, Verbalis, & Stricker, 1995; for a discussion of this issue, see Rinaman, 2003).

Mechanisms that underlie the postnatal emergence of dehydration anorexia may be related to a delayed maturation of osmotic influences on hindbrain neural circuits, as outlined above. Specifically, the lack of NST cFos expression in neonatal rats after HS treatment suggests that plasma hyperosmolality does not recruit DVC neural circuits in neonates, and raises the question of whether dehydration inhibits gastric emptying in neonatal rats. In fact, HS treatment does not delay gastric emptying in rats tested on postnatal days 4 or 11, but does inhibit gastric emptying on postnatal day 19 (Callahan & Rinaman, 1998). Thus, the inhibitory effect of dehydration on gastric emptying emerges sometime between postnatal days 11 and 19. Importantly, this is the same developmental period during which plasma hyperosmolality first begins to inhibit independent ingestion (Bruno, 1981; Bruno & Hall, 1982; Callahan & Rinaman, 1998). As noted above, this period is marked also by progressive maturation of OT inputs to the DVC (Rinaman, 1998). Thus, the ontogeny of dehydration anorexia and dehydration-induced inhibition of gastric emptying is correlated with the postnatal maturation of hypothalamic inputs to the DVC, suggesting that these developmental events are causally related.

Metabolic Controls of Food Intake may Require Hypothalamic–DVC Connections

As reviewed in preceding sections of this chapter, converging evidence indicates that hypothalamic and other forebrain inputs to the DVC undergo a significant amount of structural maturation in rats during the first 10 days postnatal.

Feeding behavior is proposed to be a function of neural activity within the DVC, such that the ability of any internal or external factor to modulate food intake depends on its ability to affect DVC neural processing. Thus, physiological or experimental controls of feeding that depend on forebrain mechanisms would not be expected to gain control over food intake until those mechanisms are able to engage DVC circuits.

Hall and his colleagues were the first to demonstrate that neonatal rats are behaviorally unresponsive to nutritional and metabolic factors that control feeding in adult rats (Hall, 1990; Phifer & Hall, 1988). This general finding has since been confirmed in other laboratories using different experimental models of nutritional or metabolic challenge (Swithers, 1997; Swithers & Hall, 1989; Weller, Gispan, & Smith, 1996). The consensus reached in these studies is that postabsorptive metabolic signals do not control meal size during independent ingestion tests in rats before postnatal day 9. Metabolic signals also are ineffective in controlling meal size in decerebrate adult rats (Grill & Kaplan, 2002), evidence that the central neural circuits necessary for the efficacy of such control are disrupted by disconnection of the forebrain from the caudal brainstem.

Experiments using acute metabolic challenges that increase food intake in intact adult rats (e.g., glucoprivation after systemic 2-deoxy-D-glucose) indicate that at least some of the metabolic signals relevant to the control of normal feeding are conveyed to the hypothalamus via ascending adrenergic and noradrenergic projections from the caudal brainstem, including projections from the DVC (Fraley & Ritter, 2003; Ritter & Dinh, 1984; Ritter, Bugarith, & Dinh, 2001). Ascending adrenergic and noradrenergic projections from the DVC and ventrolateral medulla to the hypothalamus are biochemically and functionally immature in neonatal rats (Rinaman, 2001; Rinaman *et al.*, 1994), consistent with neonatal insensitivity to the orexigenic effects of glucoprivation (Williams & Blass, 1987) and other acute metabolic challenges (Swithers, 1997, 2000).

Chronic metabolic signals involved in the physiological control of feeding include the hormones insulin and leptin (Seeley & Schwartz, 1999; Woods, Seeley, Porte, & Schwartz, 1998; Woods & Seeley, 2000). In adult rats and mice, circulating levels of insulin and leptin are proportional to the amount of stored fat; thus, both hormones can inform the brain about stored energy reserves. Insulin and leptin are categorized as indirect controls of feeding (Smith, 2000), based partly on evidence that they decrease food intake by increasing the inhibitory effect of direct controls such as CCK (Emond, Schwartz, Ladenheim, & Moran, 1999; Figlewicz *et al.*, 1996; Matson, Reid, Cannon, & Ritter, 2000).

Leptin. The functions of leptin have been particularly well studied since its discovery in 1994 (Williams *et al.*, 2001; Zhang *et al.*, 1994). Leptin is actively transported into the brain parenchyma via a saturable transport mechanism (Banks *et al.*, 1996). As fat mass increases, increased leptin signaling to the brain promotes negative energy balance and inhibits food intake. Conversely, decreased fat mass and leptin signaling promotes positive energy balance and stimulates or disinhibits food intake. Several brain regions express the long form of leptin receptor, Ob-Rb, which mediates the known physiological and behavioral effects of centrally acting leptin. Ob-Rb expression is especially dense in the arcuate nucleus of the hypothalamus (ARC), where the receptor has been localized to two distinct populations of neurons. One population is immunopositive for both pro-opiomelanocortin (POMC; the precursor to alpha melanocyte stimulating hormone [α-MSH]) and

cocaine-amphetamine-related transcript (CART). The second population is immunopositive for both neuropeptide Y (NPY) and agouti gene-related peptide (AGRP). Leptin exerts its inhibitory influence on ingestive behavior, at least in part, by upregulating and downregulating ARC neuronal gene expression and central release of ARC neuropeptides (Beck, 2000). NPY and AGRP act within the brain to stimulate or disinhibit food intake, whereas α-MSH and CART act centrally to inhibit food intake. Results from numerous studies indicate that the ARC and its neuropeptide transmitters comprise critical components of the central neural circuits that mediate leptin responsiveness, and relay leptin signals to other brain areas (Beck, 2000; Berthoud, 2002; Broberger & Hokfelt, 2001; Cone *et al.*, 2001). However, as discussed below, the ARC is not unique in this capacity.

Postnatal Development of ARC Signaling Pathways. In adult rats and mice, NPY/AGRP and POMC/CART neurons in the ARC innervate the PVN, DMH, and LHA (Berthoud, 2002; Williams *et al.*, 2001), and also project directly to the hindbrain DVC (Palkovits, 1999; Sim & Joseph, 1991). The intra- and extra-hypothalamic projections of ARC neurons deliver information about circulating levels of leptin and other hormones that affect food intake (i.e., growth hormone, glucocorticoids, and insulin). In mice, the intra-hypothalamic projection pathways develop postnatally within distinct temporal domains (Bouret, 2002; Bouret & Simerly, 2002; Bouret, Draper, Polston, & Simerly, 2001; Bouret, Draper, & Simerly, 2004). ARC inputs to the DMH in mice first appear at postnatal day 6, followed by inputs to the PVN on postnatal days 8–10, and inputs to the LHA on postnatal day 12 (Figure 5). Other tracing studies demonstrated that ARC efferent projections to each major hypothalamic target are reduced in homozygous *ob/ob* mice, which lack leptin. Thus, leptin signaling may be a key developmental event that affects the architecture of intra-hypothalamic feeding circuits (Bouret & Simerly, 2002). Similar neural tracing studies have not yet been performed in developing rats. However, the distribution of fibers immunopositive for AGRP, expressed only by neurons in the ARC, is consistent with a protracted development of ARC inputs to other hypothalamic nuclei over the first 2 weeks postnatal (Grove, Allen, Grayson, & Smith, 2003).

Although fat stores are low in neonatal rats, adipose metabolic activity and leptin mRNA expression is high; in fact, baseline circulating leptin levels are higher in neonates than in adults (Ahima, Prabhakaran, & Flier, 1998; Devaskar, Ollesch, Rajakumar, & Rajakumar, 1997; Rayner *et al.*, 1997). Exogenous systemic leptin alters ARC neuronal gene expression in neonatal mice and rats in a manner that is qualitatively similar to its effects in adult rats, including downregulation of NPY and upregulation of POMC mRNAs (Ahima & Hileman, 2000; Proulx, Richard, & Walker, 2002). Leptin administration during the first 3 days postnatal also causes a significant increase in LHA levels of prepro-orexin mRNA measured on postnatal days 5 and 10 (van den Pol, Patrylo, Ghosh, & Gao, 2001; Yamamoto *et al.*, 2000), again similar to the effect of exogenous leptin in adult rats. Maternal deprivation and refeeding have been reported to upregulate and downregulate NPY gene expression, respectively, in the neonatal ARC (Kowalski *et al.*, 1998b), although 24-hr deprivation does not affect LHA levels of prepro-orexin mRNA in rats 10 days of age or younger (Yamamoto *et al.*, 2000). Thus, both ARC and LHA neurons are responsive to exogenous leptin very early in development. However, the physiological effects of deprivation and refeeding appear to alter ARC gene expression without affecting LHA gene expression in young rat pups, perhaps due to immaturity of ARC projections to the LHA (Bouret, 2002; Grove *et al.*, 2003).

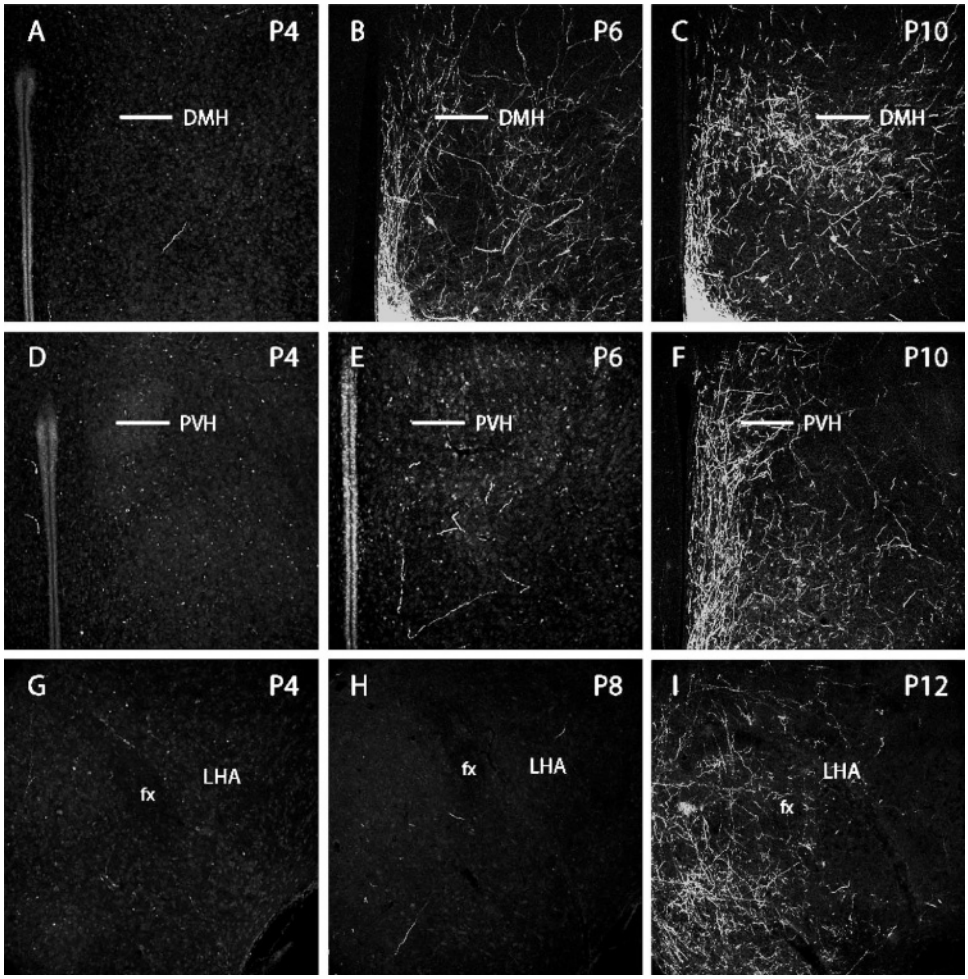

Figure 5. Fluorescent confocal images illustrating the development of projections from the arcuate nucleus to the dorsomedial nucleus of the hypothalamus (DMH), paraventricular nucleus of the hypothalamus (PVH; panels D–F), and the lateral hypothalamic area (LHA; panels G–I). A small crystal of the carbocyanine dye, DiI, was implanted into the arcuate nucleus in fixed brains from mice on postnatal day 4, 6, 8, 10, or 12. Neural projections from the arcuate nucleus develop postnatally within distinct temporal domains. DMH innervation is apparent on day 6, followed by PVH innvervation on day 10, and LHA innervation on day 12. Figure generously provided by Sebastien Bouret (Bouret, 2002; Bouret *et al.*, 2001; Bouret *et al.*, 2004).

Other evidence suggests that central NPY receptor expression (Tong, Dumont, Shen, & Quirion, 1997) develops well before NPY-containing ARC neurons innervate their postsynaptic targets. Significant alterations in hypothalamic NPY systems occur during early postnatal development in rats (Grove, Brogan, & Smith, 2001; Grove *et al.*, 2003; Kagotani *et al.*, 1989; Singer, Kuper, Brogan, Smith, & Grove, 2000), although other brain regions have not been as closely examined (but see Woodhams *et al.*, 1985). Mechanisms for behavioral responsiveness to exogenously administered NPY or other neurochemicals are likely to be present well in advance of a complete neurological system for that chemical's endogenous actions (Ellis, Axt, & Epstein, 1984; Epstein, 1984). Thus, although modulation of feeding by centrally administered agents does not by itself constitute evidence for a functional

neural system, it may indicate the presence of functional receptor-signaling pathways that are linked to behavioral output. In 2-day-old rats, for example, microinjection of NPY into the hypothalamus significantly increases both milk and water intake delivered by intraoral infusion (Capuano, Leibowitz, & Bare, 1993). NPY does not stimulate water intake in older rats, suggesting that central NPY infusion in 2-day-olds acts nonspecifically to increase swallowing responses to intraoral infusions. Nevertheless, a potential stimulatory effect of central NPY on milk intake in 2-day-olds could be partly due to stimulation of NPY receptors within the hypothalamus and subsequent signaling to the hindbrain DVC, since a subpopulation of PVN neurons already innervates the DVC early in postnatal development (Rinaman *et al.*, 2000). Alternatively, or in addition, the infused NPY could have entered the ventricular system and accessed NPY receptors present in the DVC or other caudal brain regions. Centrally administered NPY can act as an indirect control of food intake by attenuating DVC neuronal responses to gastric distension in adult rats (Schwartz & Moran, 2002), and it is possible that a similar effect is at work in 2-day-old rats. Further experiments are needed to determine the behavioral specificity and site(s) of action of central NPY in developing rats.

Despite the ability of exogenous leptin to alter hypothalamic gene expression in neonatal rats, it does not appear to modify either suckling (Proulx *et al.*, 2002; Stehling, Doring, Ertl, Preibisch, & Schmidt, 1996) or independent ingestion (Proulx *et al.*, 2002) until some time after 10 days postnatal. The lack of an effect of leptin on suckling intake is consistent with evidence reviewed previously that the controls of suckling are different from the controls of adult-like ingestion. In this regard, the apparent inability of exogenous leptin to control independent ingestion in neonates might be due to immaturity of intra- and extra-hypothalamic ARC projections. However, ARC neurons are not the only transducers for leptin's inhibitory effects on food intake. Many other central and peripheral regions express Ob-Rb in adult rodents, including neurons in the DVC and nodose ganglia (Burdyga *et al.*, 2002; Goldstone *et al.*, 1997; Grill *et al.*, 2002; Mercer, Moar, & Hoggard, 1998; Peiser *et al.*, 2002). Recent experiments in adult rats and mice have demonstrated that the brainstem is a direct target for leptin's actions (Grill *et al.*, 2002; Hosoi, Kawagishi, Okuma, Tanaka, & Nomura, 2002). Intra-DVC microinjection of leptin suppresses food intake at doses that are subthreshold when injected into the lateral ventricles (Grill & Kaplan, 2002), suggesting that endogenous leptin may control feeding by acting directly on the DVC. Whether or not exogenous leptin inhibits food intake in adult decerebrate rats has not been reported; this experiment would be important to assess whether the isolated brainstem is sufficient to mediate leptin's suppressive effects on feeding.

The development of Ob-Rb receptor expression in the DVC and nodose ganglia has not been reported. However, exogenous leptin activates DVC neurons and increases the response of vagal sensory neurons and DVC neurons to exogenous CCK in neonatal rats (Yuan, Attele, Dey, & Xie, 2000; Yuan, Attele, Wa, Zhang, & Shi, 1999). Thus, leptin administration might be expected to inhibit independent ingestion in neonates. In one study reporting that it does not, rat pups were injected with leptin or saline vehicle, maternally deprived for 3 hr, and then given access to milk above a warm heating pad (Proulx *et al.*, 2002). However, a warm ambient temperature, which is necessary for pups to consume significant volumes during tests of independent ingestion (Hall, 1979b), was not provided. Indeed, 30-min intake volumes in control pups amounted to only 1.2%–1.5% BW (Proulx *et al.*, 2002), which is less than the 2%–3% BW intake typically observed when pups

are tested in a warm ambient temperature after similar periods of deprivation (Kowalski, Ster, & Smith, 1998a; Tyrka & Smith, 1991). It is possible that the low baseline intakes contributed to a failure to observe leptin-induced inhibition of feeding; thus, additional experiments are necessary to confirm this finding. Studies are needed also to describe the development of Ob-Rb receptors in the nodose ganglia, DVC, and other central regions, and to determine the effects of leptin on neuronal gene expression in these regions.

Summary

There may be a genetically predetermined, inherent drive to eat that is only periodically neutralized by satiety, sleep, or other competing drives (Berthoud, 2002). In infants, and perhaps also in adults, disinhibition of feeding motor outputs by factors that remove, compete with, or otherwise neutralize inhibitory controls of feeding could be enough to initiate and maintain food intake without the need for special "hunger" stimuli (Stricker, 1984). Research reviewed in this chapter supports the view that behavioral responses to such direct and indirect controls of feeding might generally be effected through DVC neural circuits. The intrinsic components and output pathways of these circuits are accessed by numerous afferent inputs in mature rats, but by a more limited set of inputs in neonatal rats. Our understanding of these central neural systems will be enhanced by continued examination of behavioral and physiological responses to treatments that affect food intake differently in developing and mature animals. The presence or absence of responses to a given stimulus or control presumably reflects the functional integrity of neural circuits that receive and process the signal, and those that organize and execute the response. As new behavioral responses emerge during postnatal development, one may infer maturation of the neural systems and, importantly, the functional interactions among neural systems that support these responses.

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9

The Caudal Brainstem and the Control of Food Intake and Energy Balance

Hans-Rudolf Berthoud

Introduction

Obesity is now recognized as a major health problem in the United States. Genes that have been selected over millions of years of human evolution to handle scarcity of food and high levels of physical activity are suddenly faced with the easy availability and low cost of calories from sugar and fat and a sedentary lifestyle (Drewnowski, 2000; Neel, 1962). In many individuals this "obesigenic" environment results in a higher defended body weight and an associated higher risk for type 2 diabetes and heart disease (Ravussin & Bogardus, 2000). As environmental changes seem unlikely in the near future, exciting recent discoveries in the field of neural regulation of food intake and energy balance may be our strongest hope to combat the obesity epidemic.

Progress over the last two decades of research can be summarized by three major developments: (1) The discovery of leptin and its receptors, and the ensuing identification of leptin's downstream signaling pathways via hypothalamic peptidergic neuron populations involving both anabolic and catabolic processes. (2) The systematic investigation of the gut–brain axis, with its humoral and neural afferent pathways to the brain, including the hindbrain, and the focus on satiety. (3) The recognition of the importance of pleasure and reward with their neurological substrates in the prefrontal cortex, nucleus accumbens, and the

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Neurobiology of Food and Fluid Intake, 2nd Ed., Volume 14 of *Handbook of Behavioral Neurobiology*, edited by Edward Stricker and Stephen Woods, Kluwer Academic / Plenum Publishers, New York, 2004.

mesolimbic dopamine system. This chapter touches directly on the first two issues but not on the third (see Chapter 3 by Lattemann in this volume).

These new insights have on one hand led to a reconfirmation of the hypothalamus as a crucial player in energy homeostasis, and on the other hand strengthened the idea of a distributed system. In the *Handbook*'s last volume, published in 1991, Grill and Kaplan argued convincingly that the caudal brainstem participates in the distributed neural control of feeding (Grill & Kaplan, 1990). These same authors recently published a new review based on their continuing work demonstrating the presence of leptin receptors in the dorsal vagal complex, and effects on food intake following direct injections of leptin and other peptides into this brainstem area, functions previously thought to be organized exclusively in the hypothalamus (Grill & Kaplan, 2002). In addition, the reader should be aware of an excellent discussion of lower brainstem mechanisms involved in eating and metabolism published by Blessing in 1997 (Blessing, 1997).

In this review I first present an overview of chemical and functional anatomy of the brainstem neural circuits considered important for autonomic and behavioral control of food intake and energy balance. I then argue that although the caudal brainstem contains crucial circuits for activation of skeletal and autonomic effectors and for control of food intake and autonomic outflow, it has little or no direct role in the regulation of body weight and adiposity. We could compare it to the role of the spinal cord in an animal running from a predator. Although the spinal cord integrates sensory feedback and orchestrates complex muscle activation, it alone cannot save the animal's life. Only when it receives commands from higher brain areas is its true capacity revealed.

General Flow of Information Relevant for Energy Homeostasis

To understand the role of a particular part of the nervous system in food intake and energy homeostasis, we need to have an idea about the overall organization of the regulatory system and the general flow of information. By definition, an organism is in energy balance when the rate of energy input equals the rate of energy output. The rate of energy input is determined by the behavioral act of food intake and by mechanisms of energy assimilation in the alimentary canal (digestion, transport, absorption), as well as postabsorptive fuel partitioning. Energy expenditure is determined largely by thermogenesis, the cost of metabolic processing, and physical activity.

The hypothalamic "dual center" hypothesis has dominated research on food intake during much of the last half-century. However, with the advent of neuronal tracing in the 1970s, it became clear that the hypothalamus is well connected to most other areas of the brain and does not work in isolation. In particular its strong reciprocal connections with certain areas in the caudal brainstem and spinal cord involved in oromotor, locomotor, and autonomic control suggested a much wider distributed system. It has long been recognized that the medulla receives a stream of sensory neural and humoral information from the alimentary canal and its associated organs, and generates motor patterns for oropharyngeal and autonomic control (Figure 1).

The tissues and organs involved in energy assimilation and expenditure communicate with the nervous system via several routes. Primary afferent neurons of both spinal dorsal root and vagal origin (and other cranial nerves for oral signals) send relevant signals from various tissues to the spinal cord and caudal brainstem.

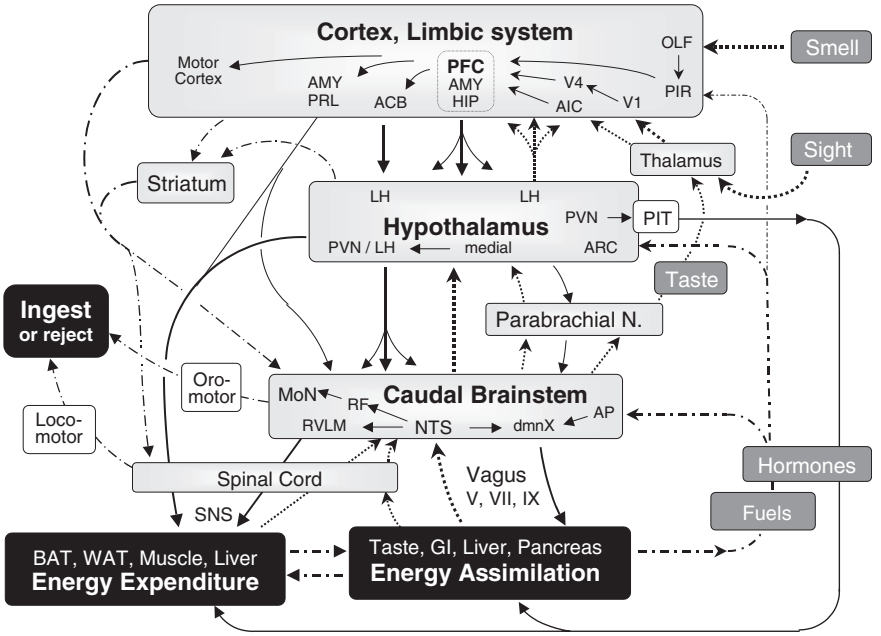

Figure 1. Schematic diagram depicting the multiple neural systems contributing to the control of food intake and energy balance. See text for details.

In addition, metabolites, fuels, and hormones secreted from such tissues circulate through the blood stream and can affect certain brain areas either because they do not have a blood-brain barrier, extend dendrites into such areas, or have active transport mechanisms for a given molecule. The area postrema and parts of the nucleus of the solitary tract lack a blood-brain barrier (Gross, Wall, Pang, Shaver, & Wainman, 1990; Gross, Wall, Wainman, & Shaver, 1991), and several circulating peptides have been shown to act directly on the area postrema (e.g., Riediger, Schmid, Lutz, & Simon, 2001). The mediobasal hypothalamus too, and in particular the arcuate nucleus, is thought to be sensitive to a variety of circulating hormones (e.g., Batterham *et al.*, 2002), although it is not clear whether this is due to a lack of blood-brain barrier or the presence of specific transport mechanisms. In addition, there is evidence that the piriform cortex is directly involved in the detection of circulating indispensable amino acids (Russell *et al.*, 2003).

In turn, the brain can modulate peripheral processes via the two limbs of the autonomic nervous system and pituitary hormones. Tissues and organs involved in energy assimilation are particularly well innervated by the vagus nerve (Berthoud & Neuhuber, 2000), and those involved in energy expenditure are preferentially innervated by sympathetic nerves. Motor neurons innervating the skeletal muscle groups responsible for approaching food are contained in the spinal cord, and those for ingesting food, and motor pattern generators orchestrating coherent movements, are located in the brainstem. These motor pattern generators are under continuous feedback control from interoceptors within the muscles and related tendons, and they receive descending command signals from the forebrain. Food-related signals from the environment are mediated via visual, olfactory, and gustatory pathways to the cortical association areas, where they converge with

interoceptive information in the orbitofrontal cortex and amygdala. They can be stored and recalled as memorial representations in hippocampal circuits.

The Medulla is Necessary and Sufficient for Reflex Ingestive and Autonomic Control

Accepting beneficial food and rejecting potentially harmful food are fundamental behaviors for an organism to survive, and the underlying neural mechanisms have evolved throughout phylogeny. Although both behaviors are equally important in survival, this chapter focuses mainly on the mechanisms determining the acceptance (ingestion) of beneficial foods as part of energy homeostasis. The role of brainstem mechanisms in emesis and conditioned taste aversion will not be specifically discussed, and the interested reader is referred to recent reviews and reports (Grancha, Navarro, Cubero, Thiele, & Bernstein, 2002; Kinzig, D'Alessio, & Seeley, 2002; Lang, 1999; Yamamoto, Shimura, Sako, Yasoshima, & Sakai, 1994).

The anatomical organization of the caudal brainstem resembles that of the spinal cord, with clearly separated sensory and motor domains and a less clearly defined intermediate zone. Important differences from the spinal cord are the much larger intermediate zone and the additional sensory function of the area postrema. The developing medulla is faced with the problem of establishing all possible connections between myriad sensory inputs and motor outputs for sensory-motor reflex control. The solution of this problem is exemplified by the lattice-like organization within the dorsal vagal complex (Powley *et al.*, 1992). A rough rostro-caudal viscerotopy of sensory inputs is orthogonal to the longitudinal columnar organization of vagal motor outflow (Figure 2).

To understand a reflex arc, we need to know its sensory and motor limbs as well as the characteristics of its central transmission. At present, we know a lot more about the two limbs than about what connects them. Mainly dictated by easily available methodology such as anterograde and retrograde neuronal tracing and recording from nerve fibers, both the sensory and motor limbs of brainstem reflexes related to ingestion have been relatively well characterized. How sensory information is processed and leads to meaningful motor action is much less understood because it requires more challenging methodology. In this section, I introduce the numerous and diverse pathways that send a wealth of sensory information with relevance to ingestive control to the brainstem. The much more limited evidence describing how this sensory information is integrated and translated into both skeletal and autonomic motor actions is then discussed. However, first we take a brief look at the brainstem mechanisms that are the essence of ingestive behavior, getting food into the mouth and into the digestive tube.

Pathways of Mastication and Swallowing are Central to the Ingestive Response

The caudal brainstem contains the complete pathways necessary for mastication and swallowing, with all the accompanying autonomic responses such as saliva secretion (Jordan, Brownstone, & Noga, 1992). Both mastication and deglutition are complex behaviors that involve cooperation between large numbers of muscles. Protection of the airways is of critical importance, so that certain muscles cannot be activated independently. Rhythmic, temporally fixed, and sequential patterns of

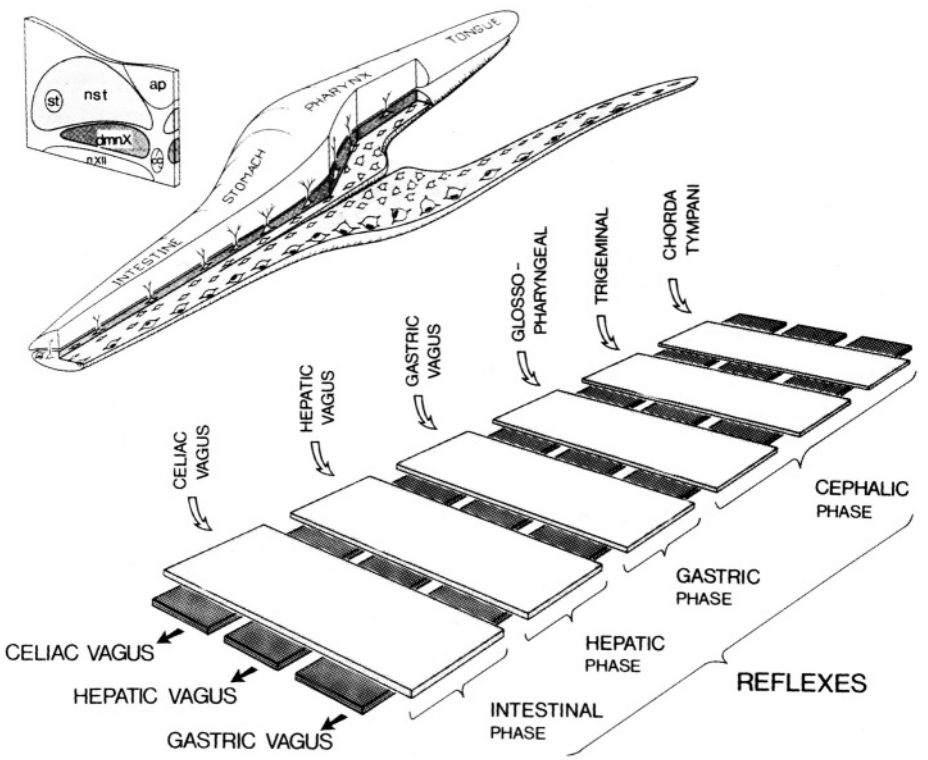

Figure 2. Sensory-motor lattice of the rat dorsal vagal complex. A three-dimensional view of the intimate anatomical relationship between the NTS and dorsal motor nucleus is shown in the upper figure, with one frontal section at the area postrema level. The viscerotopic representation of sensory input from the tongue to the more distal alimentary canal is indicated on the surface of the NTS. The bottom figure shows the formal idea of the lattice, with the rostro-caudally organized sensory inputs here represented by the corresponding cranial nerve branches, and the longitudinally organized vagal motor fibers supplying different target areas through different abdominal vagal branches. The lattice suggests an efficient way to anatomically organize all possible types of sensory-motor reflexes.

muscle action are therefore organized within specialized pattern generator circuits. Patterned activity of trigeminal motor neurons, the major innervation of jaw muscles, is coordinated by specific populations of brainstem premotor neurons (Lund, Kolta, Wertberg, & Scott, 1998; Nakamura & Katakura, 1995). The use of an *in vitro* isolated rat brainstem preparation (Kogo *et al.*, 1996) and the lamprey as models (Huard, Lund, Veilleux, & Dubuc, 1999) (Petropoulos, Lund, & Dubuc, 1999) has allowed remarkable progress in identifying the chemical anatomy of mastication circuits. Similar pattern generator circuits exist within the hypoglossal nucleus and adjacent reticular formation for the remarkably fast and precise movements of the tongue, its protection from being injured, and its coordination with respiratory control (Altschuler, Bao, & Miselis, 1994; Sawczuk & Mosier, 2001).

An equally complex task is swallowing and esophageal transport of masticated food and fluids without spillage into the airways. Several different but coordinated circuits are involved (for reviews see Altschuler, 2001; Bieger, 2001; Goyal, Padmanabhan, & Sang, 2001; Jean, 1984). For the bucco-pharyngeal phase of deglutition, viscerosensory input enters through specific subnuclei of the solitarius complex, is then relayed to the reticular formation, and finally gains access to

motor neuron pools in the hypoglossal and ambiguus nuclei. For striated muscle esophageal peristalsis, sensory input via the centralis subnucleus of the nucleus tractus solitarius (NTS) is directly relayed to parts of the nucleus ambiguus (Wank & Neuhuber, 2001). For smooth muscle esophageal peristalsis, lower esophageal sphincter function, and receptive relaxation of the gastric fundus, sensory information from vagal afferents enters another specific area of the NTS and is then relayed to the medial columns of the dorsal motor nucleus.

Organization of Sensory Input

Taste and Other Sensory Modalities from the Upper Alimentary Canal.

Gustatory input via taste receptor cells on the tongue and palate is considered most important for guiding food intake and selection. The gustatory and trigeminal systems act as "gate keepers" at the entrance to the alimentary canal (Scott & Verhagen, 2000). According to this view, the four classic taste modalities represent innate detectors for acceptable foods (sweet), dangerous or toxic foods (bitter and sour), and special needs (salt, water), providing little other information about the macro- or micronutrient composition or energy density. If this were the case, then gustatory inputs serve only as cues regarding macro-nutrient information by learned associations.

Sweet, Bitter, and Umami are Tasted by Separate Receptor Cells that Share Similar Signaling Pathways.

Taste receptor physiology has made significant progress over the last few years. Specific families of genes expressing G-protein coupled receptors have been cloned (Adler *et al.*, 2000; Hoon *et al.*, 1999; Li *et al.*, 2002; Matsunami, Montmayeur, & Buck, 2000; Nelson *et al.*, 2001; Nelson, *et al.*, 2002). The three members of the T1R family, T1R1, T1R2, and T1R3, are involved in sweet and umami taste reception. The heterodimeric combination of T1R2/T1R3 recognizes several natural and synthetic sweeteners, while T1R1/T1R3 heterodimers respond to the umami taste stimulus L-glutamate (Li *et al.*, 2002) and most other L-amino acids but not to their D-enantiomers (Nelson *et al.*, 2002). The T2R family includes as many as 80 genes in several clusters that are exclusively expressed in taste receptor cells containing the G-protein alpha subunit gustducin, known to be involved in bitter taste. Since single taste receptor cells may express a large number of T2Rs, they are likely to detect many different bitter tastants (Adler *et al.*, 2000). Most recently, a contentious issue in taste research has come to a close when it was shown that different receptor cells sharing similar signaling pathways account for coding sweet, bitter, and umami tastes, but that individual taste receptor cells are dedicated to the transduction of only one of these three taste qualities, and thus are not broadly tuned (Zhang *et al.*, 2003)

Taste-Responsive NTS Neurons are Broadly Tuned.

Taste information gathered by the taste buds is sent to the rostral NTS by primary afferent nerve fibers that travel in the greater superficial petrosal branch (roof of mouth) and chorda tympani branch (tip of tongue) of the facial nerve, the linguotonsillar branch of the glossopharyngeal nerve (back of tongue), and superior laryngeal branch of the vagus nerve (pharynx) (Hamilton & Norgren, 1984) (Figure 3). Representation of taste in the first relay station, the NTS, has been investigated anatomically by tracing central terminals of primary afferents (Contreras, Beckstead, & Norgren 1982; Hamilton & Norgren, 1984), recording electrophysiologically from NTS neurons in

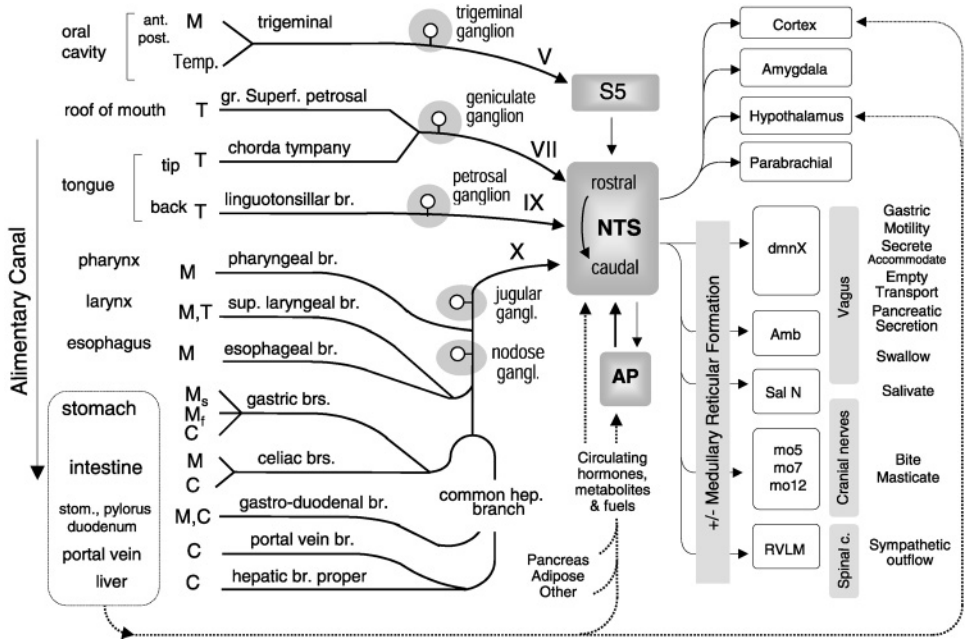

Figure 3. Food-related sensory input to the caudal brainstem. Schematic diagram showing taste and visceral input pathways that inform the brain about the arrival of nutrients. Abbreviations: Sensory modalities, T, taste; Temp., temperature; C, chemosensory; M, mechanosensory; Ms, slowly adapting; Mf, fast adapting. Amb, nucleus ambiguus; AP, area postrema; dmnX, dorsal motor nucleus of vagus; mo 5, 7, 12, motor nuclei of trigeminal, facial, and hypoglossal nerve; RVLM, rostroventrolateral medulla; S5 sensory nucleus of trigeminal nerve.

anesthetized (Smith, Van Buskirk, Travers, & Bieber, 1983; Travers & Norgren, 1995; Travers, Pfaffmann, & Norgren, 1986) and awake rats (Nakamura & Norgren, 1991, 1993), and with the c-Fos method (DiNardo & Travers, 1997; Travers & Norgren, 1995; Yamamoto & Sawa, 2000). The issue of neural coding of gustatory information by the brainstem has been discussed in several reviews (Pfaffmann, Frank, & Norgren, 1979; Scott & Giza, 2000; Smith & St John, 1999; Travers, Travers, & Norgren, 1987), and the following conclusions have been drawn. (1) Most mammalian taste neurons (primary afferent and second-order NTS neurons) respond to a range of stimuli, with only the rare cell showing specificity. In stark contrast to the taste receptor cells in the taste buds, they are broadly tuned. (2) There is only a very weak topographical organization of the different taste qualities in the NTS. (3) Since individual gustatory neurons contribute to the coding of more than one stimulus parameter, the "taste message" must be carried by a pattern of activity across many gustatory neurons, and not by specifically tuned "labeled lines." (4) Scott suggested that the dimension which unites the various taste qualities is not physical, but physiological: a dimension of well-being, bounded by nutrients on one extreme and toxins at the other (Scott & Giza, 2000). Sensitivity to physiologically significant stimuli in a given environment must have been selected over millions of years of vertebrate evolution (Finger, 1997; Glaser, 1999; Hladik, Pasquet, & Simmen, 2002).

Trigeminal Sensors Contribute to Taste Sensation. Sensory input from the trigeminal system should be considered an integral part of the gustatory

system. Comparative physiological studies suggest that the mammalian taste bud may have evolved as a conglomerate of two separate systems exemplified in two species of fish (Finger, 1997). In sea robins, taste buds are located on the pectoral fin, and they appear to function in the control of feeding behavior; however, they are connected centrally like a somatosensory system. In rocklings on the other hand, the sensors are located on the anterior dorsal fin and respond to mucous substances, and thus they may serve as predator-detectors; however, they are connected centrally like a gustatory system. The mammalian taste bud contains both types of receptors and has both kinds of central connections.

In rats, many taste-responsive NTS neurons also respond to mechanical and thermal stimulation of the oral mucosa, and many neurons respond only to mechanical or thermal stimulation (Ogawa, Hayama, & Yamashita, 1988; Travers & Norgren, 1995). Unimodal and polymodal neurons are codistributed within the rostral NTS, with only a modest topographical segregation (Halsell, Travers, & Travers, 1993; Travers & Norgren, 1995). Dual innervation of hamster taste buds has been shown using neural tracing methods (Whitehead, Ganchrow, Ganchrow, & Yao, 1999). Furthermore, a large proportion of trigeminal sensory neurons express the vanilloid (VR-1) and vanilloid-like (VRL-1) receptor (Ichikawa & Sugimoto, 2002; Matsumoto, Emori, Ninomiya, & Abe, 2001). These receptors confer sensitivity to protons, heat, and capsaicin, and lead to the sensation of tingling, stinging, warmth, burning, and pain (Liu & Simon, 2000). Involvement of trigeminal afferents in the behavioral manifestations of palatability has been demonstrated in rats (Berridge & Fentress, 1985). Peripheral transection of the sensory branches of the trigeminal nerve selectively reduced the idiosyncratic tongue protrusions and paw licking responses to sweet taste, without changing reactivity to bitter taste. Since those taste reactivity responses are unchanged in decerebrate rats (see discussion below), these findings suggest that the palatability of a sweet stimulus is markedly amplified through a mechanism within the brainstem by the presence of trigeminal orosensation. The palatability of fatty foods is also thought to be enhanced by its "mouth-feel," which is generated by somatosensory input.

Signals from Preabsorptive Sites in the Gastrointestinal Tract

A wide spectrum of mechanosensory, chemosensory, and thermosensory information from the gastrointestinal tract and other abdominal organs reaches the more caudal NTS and area postrema by way of primary vagal afferent neurons and hormones (Figure 3).

Mediation by Primary Afferent Neurons.

In addition to vagal afferents, primary afferent nerve fibers of dorsal root origin reach several brainstem targets including the NTS (Menetrey & Basbaum, 1987) and the parabrachial complex (Jasmin, Burkey, Card, & Basbaum, 1997) via the spinal cord. Thus, whereas signals conveyed by vagal afferents directly enter the NTS, signals picked up by dorsal root afferents only indirectly reach the NTS. Besides the study by Menetrey and Basbaum (Menetrey & Basbaum, 1987), there are no experiments that have specifically addressed the representation of spinal sensory input to the NTS. In contrast, there are many reports examining the anatomical and functional organization of vagal sensory input. It should be noted, however, that in experiments assuming vagal mediation, spinal pathways are usually not eliminated and therefore their participation cannot be discounted.

There is a voluminous literature on vagal sensory functions, and all of it cannot be reviewed here. The many receptive functions of vagal and spinal afferent nerve fibers innervating the gastrointestinal tract and their potential transduction mechanisms have been reviewed recently (Kirkup, Brunsden, & Grundy, 2001). The anatomical distribution of vagal afferents in the gastrointestinal tract and the liver has been investigated with tracing from the vagal sensory (nodose) ganglia (for a recent review see Berthoud & Neuhuber, 2000). The gastric distension signal detected by specialized vagal terminals in both the myenteric plexus (intraganglionic laminar endings) and external smooth muscle layers (intramuscular arrays) (Berthoud & Powley, 1992; Phillips & Powley, 2000; Zagorodnyuk, Chen, & Brookes, 2001) is thought to play a major role in satiation (Deutsch, 1985), but in itself does not seem to provide much information about the chemical composition of the ingesta (Phillips & Powley, 1996). However, when combined with signals from chemosensors in the small intestinal mucosa, it has the potential to quantitatively transmit how much of a given macronutrient was ingested.

Intraganglionic endings functioning as potential tension sensors are distributed throughout the gastrointestinal tract all the way down to the colon (Berthoud, Patterson, Neumann, & Neuhuber, 1997). In addition, vagal sensory terminals penetrate into the mucosa of the entire gastrointestinal tract, where they are likely to detect chemical, mechanical, and thermal stimuli (Berthoud, Kressel, Raybould, & Neuhuber, 1995; Patterson, Zheng, Ward, & Berthoud, 2003; Williams, Berthoud, & Stead, 1997). Compared to the gustatory system little is known about how vagal and dorsal root sensory fibers encode nutritional information from the gastrointestinal tract. Some similarities are consistent with the possibility that the two systems evolved from a common precursor. One of the specialized cell types in mammalian gastrointestinal mucosa, the brush cell (also known as tufted or caveolated cell), shares distinct features with the chemosensory taste receptor cells of the tongue. They both have an apical tuft of microvilli and express α-gustducin, the α subunit of the heterotrimeric cGMP-coupled protein involved in bitter transduction in oral taste receptor cells (Hofer, Asan, & Drenckhahn, 1999). However, other cell types are likely to be involved in signal transduction from the luminal nutrient stimuli to impulse activity of afferent nerve fibers. Peptides such as cholecystokinin, polypeptide YY, ghrelin (Date *et al.*, 2002), and the proglucagon-derived glucagon-like peptides 1 and 2, as well as serotonin released from enteroendocrine cells, may affect extrinsic nerve terminals directly or via interactions with intrinsic (enteric) primary afferent neurons (for reviews see Buchan, 1999; Hofer *et al.*, 1999; Kirkup *et al.*, 2001; Mei, 1985).

Vagal afferent neurons express receptors for the gastrointestinal hormones cholecystokinin (CCK, particularly the type A receptor) (Broberger, Holmberg, Kuhar, & Hokfelt, 1999; Patterson, Zheng, & Berthoud, 2002), ghrelin (growth hormone secretagogue receptor) (Date *et al.*, 2002), and glucagon-like peptide-1 (GLP-1) (Nishizawa, Nakabayashi, Uchida, Nakagawa, & Niijima, 1996; Nakagawa *et al.*, 2004), as well as for serotonin (5-HT, 5-HT3 receptor) (Morales & Wang, 2002) and other stimulants such as heat and proton concentration (vanilloid [capsaicin] receptor-1 [VR-1] and vanilloid-like receptor [VRL-1]) (see also review by Kirkup *et al.*, 2001; Patterson, *et al.*, 2003). As discussed above for primary gustatory afferents (Frank, Bieber, & Smith, 1988; Travers *et al.*, 1986), polymodality, convergence, or broad tuning of sensory perception is another important principle for primary vagal afferents from the gastrointestinal tract. At least in the rat, single vagal afferent fibers have been shown to respond to both mechanical and chemical stimuli

(Berthoud, Lynn *et al.*, 2001; Clarke & Davison, 1978; Davison, 1972; Schwartz, McHugh, & Moran, 1993). Thus, integration does not take place only in the brain; primary vagal afferents represent the first station exhibiting true integrative capacity.

Mediation by Circulating Hormones. Gastrointestinal hormones released into the circulation through stimulation by ingested food can affect brainstem function independent of primary afferent nerves by acting directly on neurons in the area postrema and NTS. Both of these structures have a weak or absent blood-brain barrier (Gross *et al.*, 1990, 1991). Since dendrites of vagal motor neurons penetrate deep into the NTS, they could be affected by circulating factors. Both A and B-type CCK receptors have been demonstrated in the area postrema and NTS (Hyde & Peroutka, 1989; Moran, Robinson, Goldrich, McHugh, 1986). Since CCK activation of area postrema neurons *in vitro* is blocked by a specific CCK-B receptor antagonist (Sun & Ferguson, 1997), CCK-B receptors are likely expressed by area postrema neurons. However, it is not clear whether CCK-A receptors are expressed by local neurons or exclusively by vagal afferent fibers terminating in the dorsal vagal complex.

Circulating PYY released from the distal gut in response to luminal stimuli has been shown to act in the hypothalamus to inhibit food intake (Batterham *et al.*, 2002). In contrast, PYY infused into the fourth ventricle potently increases food intake (Corp, Melville, Greenberg, Gibbs, & Smith, 1990). These discrepant effects may be due to the fact that the circulating form of the peptide, PYY(3–36), prefers the Y2 receptor, while the full-length peptide PYY has similar affinity for the Y2 and Y1 receptors; in addition, the Y2 receptor is typically in a presynaptic location, while the Y1 receptor is on the soma or dendrites (Michel *et al.*, 1998). Given that the Y2 receptor is expressed in vagal afferent neurons (Zhang *et al.*, 1997), circulating PYY(3–36) may act preferentially on presynaptic Y2 receptors to augment glutamate release from vagal afferent terminals and thereby increase satiety and decrease food intake. In contrast, fourth-ventricular injection of full-length PYY and neuropeptide Y (NPY) (Corp *et al.*, 1990) may act preferentially on Y1 receptors to increase food intake as is the case in the hypothalamus.

GLP-1 released from the ileum in response to duodenal nutrient stimulation (Anini, Hansotia, & Brubaker, 2002) acts through GLP-1 receptors expressed in the area postrema (Merchenthaler, Lane, & Shughrue, 1999; Yamamoto *et al.*, 2003) to activate catecholaminergic neurons with projections to the NTS, ventrolateral medulla, and parabrachial nucleus (Yamamoto *et al.*, 2003). Finally, ghrelin secreted from the gastric mucosa, the only orexigenic gut peptide known at present, may also act directly through receptors found in the dorsal vagal complex, since direct microinjections into this area have been shown to stimulate food intake (Faulconbridge *et al.*, 2003).

Summary. This brief discussion of the various sensors suggests that foods in the gastrointestinal tract can signal many of their properties to the caudal brainstem by a number of sequential and parallel neural and hormonal pathways. Arrival in the gastrointestinal tract, macronutrient composition, caloric density, osmolarity, and potential toxicity of foods can likely be detected by this sophisticated sensory system. However, much more research is necessary to identify the exact mechanisms and interplay between various stimuli to encode each of these parameters of ingested food.

Mediation through Primary Afferent Nerve Fibers. Except for longer-chain fatty acids reaching the general circulation through lymph vessels, absorbed nutrients are collected in the hepatic portal vein and first reach the liver, the most important metabolic factory in the body. The wall of the portal vein is innervated by vagal afferent fibers (Berthoud, Kressel, & Neuhuber, 1992) that act as glucosensors (Niijima, 1982). These portal vein glucosensors have been implicated in the hypoglycemia-induced sympathoadrenal (Hevener, Bergman, & Donovan, 1997, 2000) and feeding responses (Friedman, Tordoff, & Ramirez, 1986), and in the satiating properties of glucose in the presence of insulin (Friedman *et al.*, 1986; Stricker, Rowland, Saller, & Friedman, 1977). The liver itself provides some information about availability of fuels that are used by the brain for the control of food intake, but the role of primary afferent nerve fibers is controversial. There are studies showing that changes in fatty acid oxidation and ATP production are reflected in hepatocyte membrane potential and electrical activity of vagal afferent neurons (Horn, Tordoff, & Friedman, 2001; Lutz, Niijima, & Scharrer, 1996 for a review see Friedman, 1997). However, in a number of studies careful denervation of the liver did not result in significant changes of ingestive behavior (for a review see Bellinger, 1999) and few vagal afferent fibers are found in the hepatic parenchyma (Berthoud, Kressel, & Neuhuber, 1992).

It is interesting to note that the liver is the only major peripheral organ from which no circulating factor (except for glucose and other metabolites) informing the brain regarding metabolism relevant for energy status has been identified. Given the importance of the liver in metabolism and energy homeostasis, it seems likely that the liver communicates with the brain through a not yet identified hormone in addition to the vagal afferent nerves. Insulin-like growth factors are secreted by the liver and have actions on the brain through specific receptors, and IGF-1 administered to the brain has been shown to decrease diabetic hyperphagia (Lu, Martinez-Nieves, & Lapanowski, & Dunbar, 2001).

Mediation via Circulating Fuels and Hormones. Fluctuations in circulating glucose levels could potentially influence glucosensitive neurons in the caudal brainstem (Mizuno & Oomura, 1984). Microinjections of 2-deoxy-glucose have been used to map the sensitive sites in the dorsomedial and ventrolateral medulla (Ritter, Dinh, & Zhang, 2000). Glucoprivation selectively activates catecholaminergic neurons in the A1/C1 and A2/C2 areas (Ritter, Llewellyn-Smith & Dinh, 1998), and immunotoxic destruction of subgroups of these neurons produces selective impairment of sympathetic and behavioral glucoregulatory responses (Ritter, Bugarith, & Dinh, 2001). However, it has not yet been demonstrated whether physiological changes in blood glucose levels can influence food intake through these cell groups. Grill and Kaplan (2002) have thoroughly discussed the potential implications of medullary glucosensors for the control of food intake and energy balance. Based on findings in hypothalamic NPY, POMC, and glucosensitive neurons (for reviews see Cone *et al.*, 2001; Levin, 2002), those authors suggest that more modest perturbations of glycemia may be sufficient to drive these cells if they were sensitized by other metabolic signals (Grill & Kaplan, 2002).

Several hormones are released from the pancreas in response to neural signals, circulating glucose and other substrates, and gastrointestinal hormones (incretins). Although insulin receptors have been localized to the area postrema

(van Houten & Posner, 1981) and NTS (Unger, Moss, & Livingston, 1991), their functional implications have not yet been tested thoroughly as has been done for insulin signaling in the hypothalamus (Benoit *et al.*, 2002). Pancreatic polypeptide, which is released mainly by cephalic phase stimuli, has very potent effects on vagal motor neurons, stimulating gastric motility and acid secretion (McTigue, Hermann, & Rogers, 1997) probably via Y4 receptors that are highly expressed in neurons of the dorsal vagal complex (Larsen & Kristensen, 1997; Yang *et al.*, 2000). Although the recent observation that pancreatic polypeptide injected peripherally suppresses food intake in rats was interpreted as indicating a peripheral site of action (Asakawa *et al.*, 2003), the peptide should gain easy access to the dorsal vagal complex. However, increased gut motility and acid secretion (McTigue *et al.*, 1997) typically parallel stimulatory effects on food intake rather than the suppression observed by Asakawa *et al.* (2003). The effects on food intake following brainstem injections of this peptide have not yet been tested. Amylin, a peptide coreleased with insulin from pancreatic β-cells in response to carbohydrate absorption, appears to act through the area postrema to inhibit food intake. The anorectic effects of acute and chronic peripheral amylin administration are absent in rats with lesions of the area postrema and adjacent NTS (Lutz *et al.*, 1998; Lutz, Mollet, Rushing, Riediger, & Scharrer, 2001), and amylin, at near physiological concentrations, directly activates area postrema neurons (Riediger *et al.*, 2001; Riediger, Schmid, Lutz, & Simon, 2002).

Thus, absorbed nutrients on their way to storage, transformation, or utilization, have available a number of communication pathways to the brainstem. The strongest cases have been made for amylin, which potently suppresses food intake by direct action on the area postrema, and glucose sensors in the portal vein, which are responsible for the adrenosympathetic response to hypoglycemia. The exact pathway for signaling hepatic energy status to the brainstem has not yet been defined and may use an as yet unidentified circulating messenger rather than (or in addition to) primary afferent nerve fibers.

Signals from Stored Nutrients

Leptin. With the discovery of leptin, the existence and importance of direct signals from white adipose tissue, the major site of stored energy, to the brain has become clear. Leptin receptors (both the long and short forms) are found not only in the hypothalamus (and other forebrain areas), but also in the caudal brainstem (Grill *et al.*, 2002) and several peripheral locations such as the taste buds (Kawai, Sugimoto, Nakashima, Miura, & Ninomiya, 2000) and nodose ganglia (Buyse *et al.*, 2001b), as well as the gastrointestinal tract, liver, pancreas, and adrenal gland (Barrenetxe *et al.*, 2002; Briscoe, Hanif, Arch, & Tadayyar, 2001; Emilsson, Liu, Cawthorne, Morton, & Davenport, 1997). Leptin administration to normal mice suppresses responses of primary afferent gustatory fibers in the chorda tympani and glossopharyngeal nerve to sucrose and saccharin but not to other taste qualities (Kawai, *et al.*, 2000). Since leptin receptor mRNA was demonstrated in mouse circumvallate taste buds, and no suppression of nerve activity was found in db/db mice lacking leptin receptors, the implication is that leptin acts directly on taste receptor cells to decrease sweet taste sensation. In addition to expression in taste receptor cells, leptin receptors also may be expressed in primary afferent neurons of the geniculate and petrosal ganglia that innervate the taste buds. In contrast, it may be noted that administration of CCK did not change responses in NTS neurons to taste stimuli (Giza, Scott, & Vanderweele, 1992).

The hybridization signal for the long form leptin receptor (Ob-Rb mRNA) was also found in the dorsal vagal complex, hypoglossal, trigeminal, lateral reticular, and parabrachial nuclei. Peripherally administered leptin increased both janus kinase signal transducer and activator of transcription (STAT3) phosphorylation, and suppressor of cytokine signaling 3 (SOCS3) mRNA in both the hypothalamus and the NTS (Hosoi, Kawagishi, Okuma, Tanaka, & Nomura, 2002). In addition, leptin significantly suppressed food intake for 24 hr when injected directly into the dorsal vagal complex, in a dose subthreshold when injected into the fourth ventricle (Grill *et al.*, 2002). Leptin also may modulate sensory signals from the gastrointestinal tract mediated by vagal afferents. Leptin receptor is expressed by a subset of nodose ganglion neurons (Buyse *et al.*, 2001a), and it has been shown to modulate CCK-sensitive vagal afferent fibers innervating the gastrointestinal tract (Gaige, Abysique, & Bouvier, 2002; Wang, Tache, Sheibel, Go, & Wei, 1997).

Other Signals. Besides leptin, adipose tissue releases additional messengers such as the cytokines TNF-α and IL-1, which have potent effects on vagal primary afferent neurons (Ek, Kurosawa, Lundeberg, & Ericsson, 1998) and dorsal vagal complex neurons (Emch, Hermann, & Rogers, 2000, 2002). Adipose tissue also may send sensory information to the caudal brainstem via dorsal root spinal afferent neurons and the spino-solitary tract (Bartness & Bamshad, 1998).

Extensive work has identified the pancreatic hormone insulin, whose average plasma levels closely track the level of adiposity, as another possible adiposity signal used by the brain (Baskin *et al.*, 1999). However, its short-term signaling characteristics clearly are different from those of leptin because insulin is primarily released by ingested carbohydrates; thus, its plasma concentration has a very distinctive short-term profile. Also, a brainstem site of action for insulin's inhibitory effect on food intake and body weight has not yet been investigated. The possibility that the caudal brainstem processes such long-term feedback signals and plays a primary role in energy homeostasis is discussed below.

The other functional energy store is hepatic glycogen. Although it has long been hypothesized that hepatic glycogen generates a signal to the brain by affecting hepatocyte membrane potential and vagal sensory neurons (Russek, 1981), this hypothesis has not yet received general acceptance (see earlier). In light of the discovery of leptin, it is interesting to speculate that an analogous hormonal signal is generated by hepatic glycogen.

Thus, nutrients stored as fat in adipose tissue can affect brainstem function through leptin signaling directly in primary afferent neurons and in integrative circuits in the NTS and reticular formation. This pathway has the potential to confer long-term information about overall energy status to the brainstem. Alternatively, this brainstem system could mainly utilize information regarding meal-to-meal, short-term fluctuations of plasma leptin concentration, signaling temporary energy depletion rather than adiposity (Chin-Chance, Polonsky, & Schoeller, 2000; Speakman, Stubbs, & Mercer, 2002).

Transmission of Sensory Input to Second-Order Brainstem Neurons

There is considerable evidence that glutamate is the major neurotransmitter used by cardiovascular vagal (Andresen & Yang, 1990; Aylwin, Horowitz, & Bonham, 1997; Zhang & Mifflin, 1998) and gustatory (Li & Smith, 1997) afferents in the NTS, and that both NMDA and non-NMDA receptors are involved. There is

less evidence that this transmitter is utilized by vagal afferents from the gut, and the potential role of other classic transmitters and peptides in this transmission has not yet been investigated.

Neurotransmitters Used by Primary Vagal Afferents. Many of the vagal afferent neurons residing in the upper sensory vagal (jugular) ganglion contain peptides such as CGRP and substance P. Far fewer sensory neurons in the nodose ganglia contain peptides (Zhuo, Ichikawa, & Helke, 1997). Non-NMDA glutamatergic receptor blockers have been shown to block all taste input to the NTS (Li & Smith, 1997), but it is not clear whether glutamate plays an equally dominant role in vagal afferents from the abdominal viscera. In one study, excitatory input to NTS neurons was blocked by local microinjection of the glutamatergic blocker kynurenic acid (McCann, 1994). However, in another study, glutamate concentration in an NTS dialysate was increased by local perfusion with K^+ but not by electrical stimulation of the cervical vagus, questioning whether glutamate was the major transmitter (Sved & Curtis, 1993). Also, gastric distension-induced Fos expression in the NTS was only partially reduced by fourth-ventricular injection of the NMDA receptor antagonist MK-801 (Zheng, Kelly, Patterson, & Berthoud, 1999). There is histological and pharmacological evidence for various glutamate receptor subtypes in the dorsal vagal complex (Aicher, Sharma, & Pickel, 1999; Berthoud, Earle, Zheng, Patterson, & Phifer, 2001; Dutschmann, Guthmann, & Herbert, 1998). The ionotropic NMDA receptor subtype NMDA-R1 has been localized selectively to esophageal premotor neurons in the central subnucleus of the NTS (Broussard, Wiedner, Li, & Altschuler, 1994). About 60%–70% of NTS neurons activated by gastric balloon distension express the NMDA receptor subunit R2, and about 30%–40% express the non-NMDAR, AMPA-receptor subunit GluR1 (Berthoud, Earle *et al.*, 2001). With respect to gastrointestinal satiety signals, it has been shown that fourth-ventricular injection of the NMDA receptor antagonist MK-801 decreases gastric distension-induced Fos expression in specific areas of the NTS (Zheng *et al.*, 1999), and increases short-term sucrose intake in food deprived rats (Burns & Ritter, 1997; Treece, Covasa, Ritter, & Burns 1998; Zheng *et al.*, 1999). Both NMDA and AMPA/kainate receptors are known as ionotropic glutamate receptors that are coupled to ion channels and typically produce fast excitatory postsynaptic potentials (EPSPs) through the influx of sodium and/or calcium ions. It has been suggested that AMPA and kainate receptors may mediate different aspects of the initial depolarization (Frerking & Ohliger-Frerking, 2002), while NMDA receptors mediate the delayed component of complex EPSPs (Yen, Chan, & Chan, 1999).

Anatomical and Topological Segregation of Primary Afferent Vagal Input to the Dorsal Medulla. Central terminals of primary vagal afferents are found mainly in the NTS and area postrema, and fewer are found in the dorsal motor nucleus of the vagus. The best viscerotopic segregation is between cardiac and pulmonary afferents (lateral subnuclei of the NTS), and the alimentary canal afferents in the medial subnuclei, the area postrema, and the dorsal motor nucleus. Within the alimentary canal afferents there is a general rostro-caudal viscerotopy, with taste input from the tongue most rostral and intestinal input most caudal (Kalia & Mesulam, 1980a, 1980b; Norgren & Smith, 1988). This organization is based on experiments using retrograde tracer injections into various abdominal vagal branches or viscera and mapping of vagal afferent terminals. However, because such tracer injections cannot be limited to specific tissue compartments or

receptive sites in the periphery, and because there is no close anatomical relationship between terminal density and second-order neurons, this method can provide only crude viscerotopic maps and is not adequate to investigate sensory pathways with functional or sensory specificity.

In general, little information is available regarding the central dissemination of functionally specific sensory input from abdominal vagal mechanical, nutrient, or metabolic sensors. Besides a few electrophysiological studies that usually tested a limited number of neurons (Barber, Yuan, & Cammarata, 1990; Chambert, Kobashi, & Adachi, 1993), such information comes almost exclusively from experiments using immediate-early gene expression. Treatments that inhibit food intake such as intraperitoneal CCK, hypertonic saline, and feeding after deprivation, produced different patterns of c-Fos expression in the dorsal medulla than treatments that stimulate food intake, such as food deprivation (Fraser, Raizada, & Davison, 1995; Olson *et al.*, 1993; Rinaman, Verbalis, Stricker, & Hoffman, 1993). Furthermore, the experimentally separated cephalic, esophageal, and gastric phases of meal ingestion also generated different patterns of c-Fos expression (Emond & Weingarten, 1995; Fraser *et al.*, 1995). Finally, gastric balloon distension (Willing & Berthoud, 1997) and intestinal infusion of various nutrients (Phifer & Berthoud, 1998; Zittel, De Giorgio, Sternini, & Raybould, 1994) produced more or less distinct patterns of c-Fos expression in the dorsal medulla. However, interpretation of such studies is complicated because the c-Fos pattern induced by different stimuli has to be assessed in separate animals. In order to implicate specific neuron populations, Fos-activated neurons should be phenotyped using double-immunohistochemical or *in situ* hybridization protocols (Vrang, Phifer, Corkern, & Berthoud, 2003).

Convergence of Afferent Input and Coincidence Detection. Converging sensory inputs to neurons in the caudal medulla represent the second stage (after the primary afferent neurons) where integration takes place. Using electrical recording from NTS neurons in intact animals, convergence has been documented between various sensors lining the alimentary canal (Appia, Ewart, Pittam, & Wingate, 1986; Barber *et al.*, 1990; Chambert et al., 1993; Paton, Li, & Kasparov, 1999; Yuan, Attele, Dey, & Xie, 2000), between gustatory and visceral vagal sensors (Bereiter, Berthoud, & Jeanrenaud, 1981; Gleen & Erickson, 1976; Giza & Scott, 1983), among olfactory, visceral, and gustatory signals (Garcia-Diaz, Jimenez-Montufar, Guevara-Aguilar, Wayner, & Armstrong, 1988), and between somatic and visceral sensors (Menetrey & Basbaum, 1987). These findings suggest a large degree of sensory gating and convergence at this level. In the hippocampus, AMPA and kainate glutamatergic receptors have been shown to differ in their ability to encode temporal information embedded in afferent input signals (Frerking & Ohliger-Frerking, 2002). Since both receptor types are present in NTS neurons, afferent signals from various vagal sensors might be frequency-coded.

Organization of Motor Output

Before addressing the issue of integration, it is important to consider the organization of motor output to skeletal and autonomic effectors. The use of conventional and viral transsynaptic retrograde tracers has greatly aided identification of the motor and premotor neurons innervating various effectors.

Skeletal Muscle. On the skeletal muscle side, neuron pools innervating specific oropharyngeal and esophageal muscle systems have been described comprehensively (Bao, Wiedner, & Altschuler, 1995; Broussard, Lynn, Wiedner, & Altschuler, 1998; Cunningham and Sawchenko, 2000; Fay & Norgren, 1997a, 1997b, 1997c; Jansen, Ter Horst, Mettenleiter, & Loewy, 1992; Travers, Montgomery, & Sheriden, 1995), as mentioned earlier.

Vagal Parasympathetic Outflow. On the autonomic side, the organization of vagal motor outflow to gut, pancreas, and liver has been described in detail in many reports [e.g. (Berthoud, Fox, & Powley, 1991; Neuhuber, Kressel, Stark, & Berthoud, 1998; Streefland, Maes & Bohus, 1998). Although progress has been made in establishing the specific traits of function-specific vagal motor neurons (Browning, Renehan, & Travagli, 1999; Jarvinen & Powley, 1999), the pools of vagal motor neurons that are involved in specific vagal functions are still not completely understood.

As shown in Figure 4, vagal preganglionic motor neurons are located exclusively in the dorsal motor nucleus, and those innervating the salivary glands are located in the salivatory nucleus. First-order vagal premotor neurons are found in the hindbrain, midbrain, hypothalamus, amygdala, and cortex. The descending inputs to the caudal medulla from the hypothalamus and other forebrain sites are discussed in more detail below.

Sympathetic Outflow. Several brainstem nuclei have direct access to sympathetic preganglionic neurons in the intermediolateral column of the spinal cord that innervate target tissues relevant for energy balance, such as brown and white

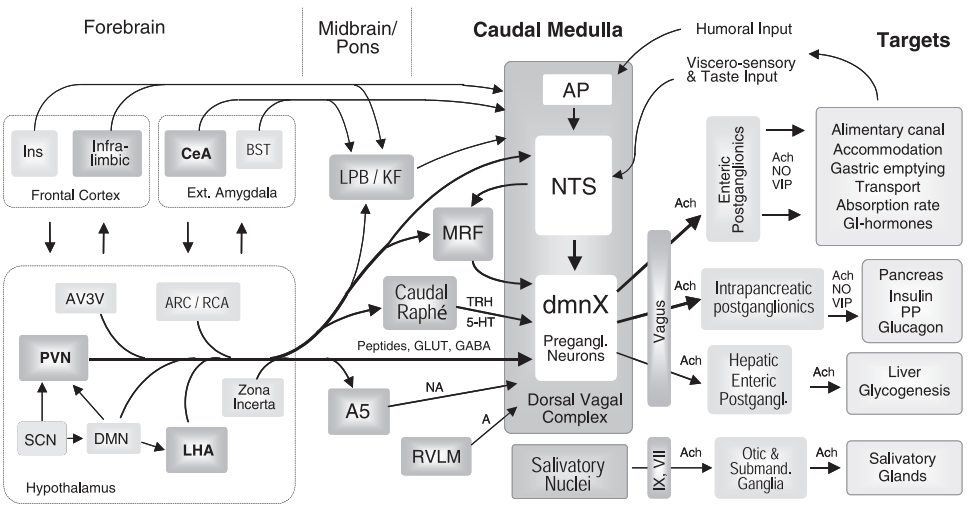

Figure 4. Organization of vagal parasympathetic outflow as related to food intake and energy balance. Arrows that terminate outside the box "dorsal vagal complex" indicate projections terminating in both the NTS and dmnX. Bifurcations do not necessarily indicate axon collaterals. Abbreviations: AP, area postrema; ARC, arcuate nucleus; AV3V, anterioventral third ventricular area; BST, bed nucleus of stria terminalis; CeA, central nucleus of amygdala; DMN, dorsomedial nucleus; Ins, insular cortex; KF, Koelliker-Fuse nucleus; LHA, lateral hypothalamic area; LPB, lateral parabrachial nucleus; MRF, medullary reticular formation; NO, nitric oxide; NTS, solitary nucleus; PVN, paraventricular nucleus; RCA, retrochiasmatic area; RVLM, rostral ventrolateral medulla; SCN, suprachiasmatic nucleus; TRH, thyrotropin-releasing peptide; VIP, vasoactive intestinal peptide.

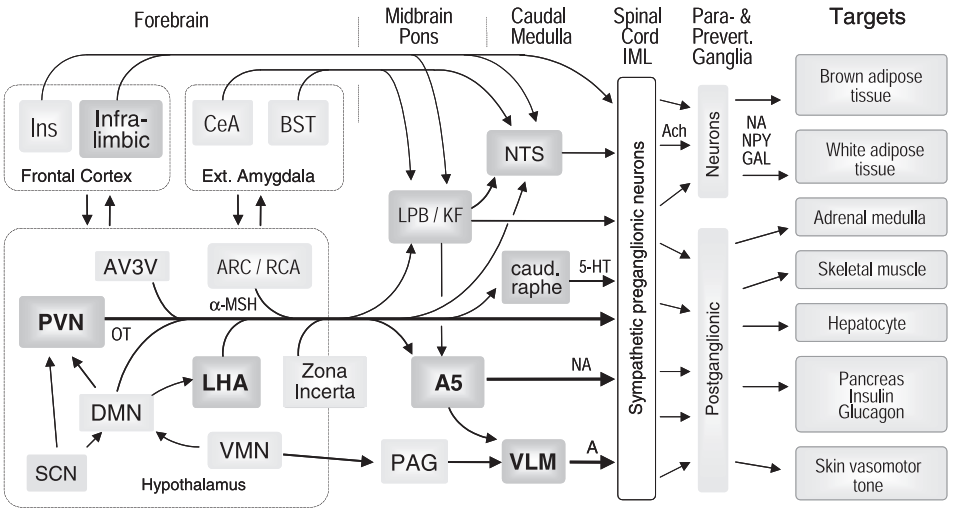

Figure 5. Sympathetic outflow as related to food intake and energy balance. Preganglionic neurons are located in the intermediolateral column (IML) throughout the spinal cord. Bifurcations do not necessarily indicate axon collaterals. Abbreviations: A, adrenaline; NA, noradrenaline; NPY, Neuropeptide Y; GAL, galanin (for other abbreviations see legend to Figure 3).

adipose tissue, skeletal muscle, liver, pancreas, and adrenal medulla (Figure 5). Catecholamine synthesizing neurons in the ventrolateral medulla, TRH and 5-HT neurons in the caudal raphé nuclei obscurus and pallidus, and neurons of unknown phenotype in the solitary nucleus, all can be retrogradely labeled by viral tracers deposited into brown and white adipose tissue, pancreas, and liver (Bamshad, Aoki, Adkison, Warren, & Bartness, 1998; Bamshad, Song, & Bartness, 1999; Bartness & Bamshad, 1998; Jansen & Ter Horst, 1992; Jansen, Hoffman, & Loewy, 1997; Loewy, 1991; Shi & Bartness, 2001; Streefland, Maes, & Bohus, 1998). In addition, first-order sympathetic premotor neurons are located in similar midbrain and forebrain areas as for the parasympathetic system.

Summary and Outlook. Transneuronal retrograde tracing with pseudorabies virus was instrumental for the comprehensive description of autonomic and skeletal muscle output pathways. The availability of genetically altered virus to express various fluorescent proteins will no doubt generate a second wave of tracing experiments for *in vitro* slice studies and more complete phenotyping of infected neurons (Davis, Williams, Xu, Glatzer, & Smith, 2003). One of the issues that needs further investigation is the function-specific organization of autonomic outflow to target tissues. There has been considerable progress on sympathetic outflow (Morrison, 1999, 2001), but relatively little information is available regarding function-specific vagal outflow (Browning *et al.*, 1999; Guo, Browning, Rogers, & Travagli, 2001).

Intramedullary Pathways of Integration

A critical task is to connect sensory input channels with appropriate motor neuron pools to achieve coordinated muscle and/or visceral action. As pointed out above, mastication and swallowing are extremely complex behaviors, with no room for error since the airways must be protected. Since certain sequences and patterns

of responses are organized in special generator units, most reflex arcs are not simply organized into linear paths containing sensory and motor neurons or sensory neurons, interneurons, and motor neurons.

Experimental identification of these paths has proven difficult, with considerable effort put into deciphering brainstem respiratory and cardiovascular control. However, the circuits linking gustatory and various visceral inputs with ingestive and autonomic responses are still ill defined. Investigation of these circuits requires multiple approaches and sophisticated methodology. Ingestive behavior can be studied only in freely moving animals and it is difficult to simultaneously "listen" to, or "challenge," the underlying neural network. Only a very limited repertoire of methods has been used in nonanesthetized animals to date, with more than 95% of all reports involving either the induction of c-Fos as an indicator of neural activity or microinjection of compounds into specific brain areas. Additional methods such as microdialysis, recording from chronically implanted electrodes, and imaging need to be adapted and applied to the brainstem. Furthermore, approaches in nonanesthetized animals need to be complemented with anesthetized whole animal and *in vitro* preparations.

It should be emphasized that integrative capacity is not only the domain of intramedullary connections. As mentioned previously, integration of sensory information that potentially influences behavioral and autonomic output takes place at the level of primary afferent neurons, and particularly in second-order neurons of the NTS.

As shown in Figure 6, there are many variants for medullary reflex pathways. Intramedullary pathways responsible for mastication, tongue movement, and swallowing have already been discussed briefly in the second section. Beckman and Whitehead (Beckman & Whitehead, 1991) and Halsell *et al.* (Halsell, Travers, & Travers, 1996) have used tracing methods to show intramedullary connections of the rostral, gustatory NTS, and Streefland *et al.* (Streefland, Farkas, Maes, & Bohus,

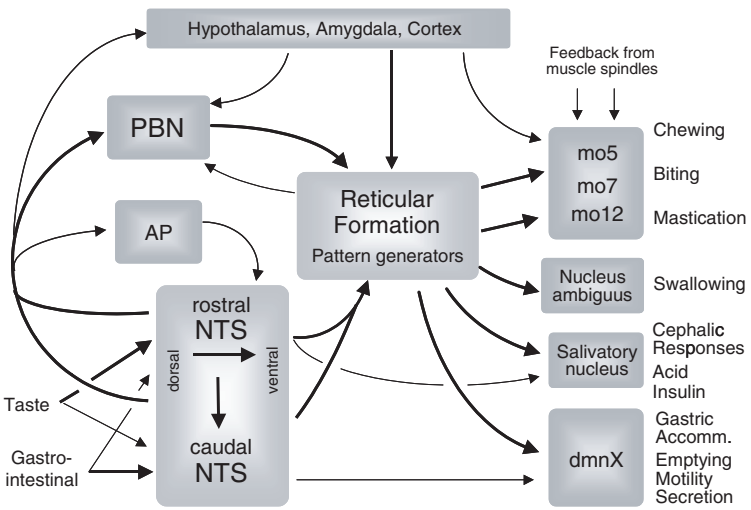

Figure 6. Intramedullary pathways of sensori-motor integration, demonstrating the prominent role of the reticular formation and the potential involvement of the parabrachial complex. For clarity, descending inputs to most of the motor nuclei, the NTS, and AP have been omitted. Note that the motor nuclei innervating skeletal, oro-lingual, and pharyngeal muscles receive constant feedback from spindle and proprioceptors.

1996; Streefland *et al.*, 1998; Streefland & Jansen, 1999) aimed to identify intramedullary pathways from the gustatory NTS to vagal preganglionic neurons innervating the endocrine pancreas that are responsible for the cephalic phase insulin secretion. Although there are direct projections from the ventral gustatory NTS to motor nuclei such as the salivatory nucleus, most reflex pathways reach motor nuclei after a relay in the medullary reticular formation. In addition, there are dense projections from the more dorsal NTS to the parabrachial nucleus in the rostral brainstem (Herbert, Moga, & Saper, 1990), which is known to project back to the caudal medullary reticular formation, and more sparse projections to the area postrema, which is known to project back to the more caudal NTS and dorsal motor nucleus. Furthermore, there also are prominent intra-NTS projections from the dorsal to the ventral gustatory NTS, and from the rostral to the caudal NTS.

Peptides and Receptors Linked to Ingestive Behavior are Expressed in the NTS and Other Brain Stem Areas. A large body of literature implicates the neuropeptides α-MSH, CART, GRP, NPY, and CRH/urocortin and their respective receptors in the control of food intake and energy balance. Besides being expressed in the hypothalamus, these peptides are expressed in neurons located in the solitary nucleus. The medullary neurons expressing these peptides may be involved in the control of food intake and energy balance or, alternatively, they may be involved in other functions of the medulla. To date not a single experiment has addressed this question. Knockout models do not distinguish between brainstem and hypothalamic expression of a peptide, and either more specific ablation strategies such as local RNA interference or immunotoxins, or local rescue strategies in knockout models will be necessary to test the functional roles of brainstem feeding peptides.

A small group of neurons in the caudal commissural NTS expresses POMC as demonstrated with an mRNA probe (Bronstein, Schafer, Watson, Akil, 1992; Grauerholz, Jacobson, Handler, & Millington, 1998), by α-MSH immunohistochemistry (Alessi, Quinlan, & Khachaturian, 1988; Joseph, Pilcher, & Knigge, 1985; Palkovits, Mezey, & Eskay, 1987), and by transgenic mice with green fluorescent protein linked to a POMC promoter (Cone *et al.*, 2001, and J. Elmquist, personal communication). In an attempt to determine the origin of α-MSH immunoreactive fibers in the medulla, monosodium glutamate (MSG)-induced lesions of the arcuate hypothalamus (Alessi *et al.*, 1988) and surgical lesions in the medulla (Palkovits *et al.*, 1987) were performed. Whereas MSG lesions significantly reduced α-MSH and β-endorphin levels in the basal hypothalamus but not in the dorsal vagal complex, the knife cuts suggested that most α-MSH fibers in the more rostral NTS originate from the hypothalamus. In the caudal commissural NTS, most fibers apparently originate from local neurons. Nothing is known about inputs and outputs of these neurons and nutritional modulation of POMC expression. Given the moderate to high expression level of MC4R receptor in the caudal medulla, particularly the dorsal motor nucleus, NTS, and certain areas of the reticular formation (Kishi *et al.*, 2003; Mountjoy, Mortrud, Low, Simerly, & Cone, 1994; Tatro & Entwistle, 1994), POMC neurons in the NTS likely provide some of the ligand to these receptors, potentially affecting autonomic reflex function and satiety. Local injection of the MC4-melanocortin receptor agonist MTII into the NTS decreased food intake and body weight, whereas the antagonist SHU9119 increased both variables (Williams, Kaplan, & Grill, 2000).

Neurons expressing CART are located in the area postrema, in parts of the NTS, and in the reticular formation of the caudal medulla as well as several

locations of the upper brainstem (Koylu, Couceyro, Lambert, & Kuhar, 1998; Zheng, Patterson, & Berthoud, 2002). Double-labeling experiments showed that CART is not coexpressed in POMC neurons of the NTS, as it is in the arcuate hypothalamus. In addition, about 30% of vagal afferent neurons in the nodose ganglia express CART (Broberger *et al.*, 1999; Zheng *et al.*, 2002). No CART receptor has yet been identified, but the very dense CART-immunoreactive fiber plexus in the dorsal vagal complex and caudal raphé nuclei suggests that CART has modulatory effects on autonomic outflow and on food intake. Injections of CART peptide into the fourth ventricle potently suppresses food intake, but also induce abnormal motor behavior and conditioned taste aversions (Aja, Robinson, Mills, Ladenheim, & Moran, 2002; Zheng, Patterson, & Berthoud, 2001; Zheng *et al.*, 2002). The critical site of action in the brainstem on food intake is not known.

Preproglucagon is processed into GLP-1 and GLP-2 in a group of neurons located in the caudal NTS, with dense projections to several hypothalamic areas involved in the control of food intake (Larsen, Tang-Christensen, Holst, & Orskov, 1997). GLP-1 receptors are widely distributed in the brain including hypothalamic areas, but also in the NTS, area postrema, and the dorsal motor nucleus of the vagus (Merchenthaler *et al.*, 1999). GLP-1 injected into the third cerebral ventricle of rats potently suppresses food intake and produces conditioned taste aversions (Tang-Christensen *et al.*, 1996; Thiele *et al.*, 1997). GLP-1 neurons are activated by gastric distension in the physiological range (Vrang *et al.*, 2003) and by gastrointestinal manipulations that cause interoceptive stress (Kinzig *et al.*, 2002; Rinaman, 1999a, 1999b; Seeley *et al.*, 2000). Because intracerebroventricular injection of the selective GLP-1 receptor antagonist exendin stimulates food intake in satiated rats (Turton *et al.*, 1996), the brain GLP-1 pathways are likely to play important roles in both satiation and nausea. GLP-1 neurons in the NTS receive input from hypothalamic oxytocin neurons, and the suppression of food intake by lateral ventricular oxytocin injection was blocked by prior injection of GLP-1 receptor antagonist (Rinaman & Rothe, 2002). Thus, GLP-1 neurons in the NTS are part of a hypothalamo-medullary-hypothalamic loop, and the contribution of GLP-1 receptors located in the dorsal vagal complex to the effects on food intake is not clear. Local blockade of medullary GLP-1 receptors or selective immunotoxic lesions of GLP-1 neurons have not yet been used as experimental tools. At least in the area postrema, circulating GLP-1 stimulates tyrosine hydroxylase expressing neurons that bear GLP-1 receptors and project to the NTS, the parabrachial nucleus, and sympathetic premotor areas in the ventral medulla (Yamamoto *et al.*, 2003).

A few neurons located in the dorsal NTS express gastrin-releasing peptide as detected by immunohistochemistry in colchicine-treated rats (Tohyama, & Takatsuji, 1998; and unpublished observations). The GRP receptor also is expressed in the caudal medulla, and its blockade with a highly selective antagonist injected into the fourth ventricle abolished suppression of feeding by peripherally administered GRP (Ladenheim, Taylor, Coy, Moore, & Moran, 1996). A small group of GRP expressing neurons with projections to the NTS has been identified in the paraventricular nucleus of the hypothalamus (Costello, Brown, & Gray, 1991), but the respective roles of hypothalamic and NTS neurons expressing this peptide are not known.

NPY is also expressed in A2 noradrenergic neurons in the NTS (Everitt *et al.*, 1984). Although these NPY/NA neurons are known mainly for their ascending projections to the hypothalamus, there is a dense NPY-immunoreactive fiber plexus in the NTS. The observation that fourth-ventricular injection of NPY increases food

intake in sated rats (Corp *et al.*, 1990) suggests that medullary Y1 receptor stimulation has similar orexigenic effects as in the hypothalamus. The distribution of Y1 receptors in the medulla and, therefore, the possible site(s) of action for the feeding stimulation are not known.

CRH, urocortin, and their respective receptors also have been implicated in food intake and energy balance (Cullen, Ling, Foster, & Pelleymounter, 2001; Heinrichs & Richard, 1999). Involvement of the caudal brainstem in these functions is indicated by the expression of both peptides and their receptors in the NTS, by the expression of c-Fos in the NTS following infusion of CRF and urocortin ICV (Benoit *et al.*, 2000) or intravenously (Wang, Martinez, Vale, & Tache, 2000), and by the potent suppression of food intake after injection of urocortin into the rat NTS (Grill, Markison, Ginsberg, & Kaplan, 2000).

In addition to these five peptide families, additional peptides involved in feeding, energy balance, and/or autonomic control, such as galanin, enkephalin, dynorphin, CCK, TRH, ANGII, and neurotensin, are expressed in the NTS (Tohyama & Takatsuji, 1998).

Pattern and Rhythm Generators: How Do They Control Multiple Oro-Lingual Response Patterns? The same oro-lingual muscle groups, including their motor neuron pools and much of their underlying pattern generator networks, are involved in the acceptance and rejection responses to nutritious and potentially dangerous foods, respectively. In addition, much of the same circuitry is involved in other physiological functions such as respiration, and autonomic outflow. What are the mechanisms that configure this common neural network to serve very different response patterns? What are the critical stimuli that reconfigure it to switch from one to another response pattern? Considerable progress has been made in the area of respiratory control, which is also organized in the caudal medulla. Using complimentary in vitro and in vivo approaches, it appears that a single medullary network underlies multiple breathing patterns, such as eupnea, sighs, and gasps, and some of the necessary conditions for switching from one mode to another have been identified (Lieske, Thoby-Brisson, Telgkamp, & Ramirez, 2000; Ramirez *et al.*, 2002).

With regard to different oro-lingual responses induced by gustatory stimuli, critical circuits have been identified electrophysiologically in awake rats (Travers, DiNardo, & Karimnamazi, 2000). Nanoliter infusions of specific amino acid agonists and antagonists have been made into the medullary reticular formation, a brain area that harbors premotor neurons to oromotor nuclei, and responses of jaw opener, tongue protruder, and tongue retractor muscles were electromyographically monitored during either intraoral infusion or voluntary drinking of small volumes of sucrose and quinine solutions (Chen, Travers, & Travers, 2001; Chen & Travers, 2003). Inactivation with GABA-agonist, or selective blockade of either NMDA or AMPA/kainate receptors in the lateral reticular formation, reversibly suppressed both licking in response to sweet tasting solutions and gaping in response to bitter tasting solutions. Remarkably, the animals still actively probed the sucrose bottle, indicating that appetitive behavior was normal and the functional lesion was very specific. This observation suggests that neither of these two receptor subtypes mediates the switch from acceptance to rejection, although some differential involvement was indicated during recovery from the chemically induced depression. In contrast, infusions of the selective GABA-A receptor blocker bicuculline increased the amplitude of jaw openings in response to intraoral delivery of

sucrose in a way that resembled the gape response to aversive stimuli. This finding suggests that the switch from acceptance to rejection responses might involve removal of tonic inhibition occurring through GABA-A receptors. Thus, a single medullary network functioning in different chemically controlled configurations might underlie different oro-lingual response patterns. This model will be very useful for analysis of medullary ingestive responses as modulated by other transmitters, including a number of feeding related peptides that are expressed in descending projections from the hypothalamus and amygdala (Berthoud, 2002).

Assessing the Intrinsic Capacity of Brainstem Circuitry: The Decerebrate Rat Model

Grill and colleagues (Grill & Kaplan, 1990, 2002) have examined the intrinsic capacity of the brainstem to orchestrate ingestive behavior. In a series of studies using intraoral liquid food delivery to the midbrain decerebrate rat, an impressive catalog of integrative mechanisms complete within the brainstem was documented. These mechanisms include discriminative responses to taste stimuli as expressed by acceptance or rejection responses (Grill & Norgren, 1978a), sympatho-adrenal counterregulatory responses to glucoprivation (DiRocco & Grill, 1979), ingestive responses to insulin-induced hypoglycemia (Flynn & Grill, 1983), sham feeding of sucrose solution (Grill & Kaplan, 1992), normal satiation (Grill & Norgren, 1978b), suppression of intraoral sucrose intake by CCK (Grill & Smith, 1988), and reduction of intake after a gastric preload (Seeley, Grill, & Kaplan, 1994). In addition, decerebrate rats confronted with the sucrose concentration contrast paradigm perform similar to intact rats, suggesting that the brainstem alone is capable of complex reward-driven behavior that depends on a comparison process involving short-term memory (Grigson, Kaplan, Roitman, Norgren, & Grill, 1997). Like intact rats, decerebrate rats always licked more for a high than for a low concentration of sucrose when they were allowed to compare the two solutions during very brief access within the same daily session. However, unlike intact rats, decerebrate rats do not respond to food deprivation by ingesting more food within a meal or over 24 hr (Grill & Kaplan, 2002; Seeley *et al.*, 1994).

Summary

The brainstem harbors an impressive array of neurons and circuits directly involved in ingestion, digestion, and absorption of food, as well as in utilization of metabolites and fuels. The neural circuits controlling most of these tasks are contained within the brainstem and do not require the forebrain for their execution. Just as for respiration and circulation, other body functions essential for survival, the regulation of nutrient supply is to a large extent autonomically organized within the brainstem. Based on experiments with the decerebrate rat, it appears that the isolated brainstem can terminate a meal and thus exhibit the basic behavior of satiety, but the specific neural pathways necessary for this response have not been identified. In particular, it is not known whether expression of satiety in the decerebrate rat requires only the basic reflex circuits (vagal afferents to oropharyngeal motor neurons) within the more caudal medulla, or the parabrachial nuclear complex in the more rostral brainstem as well. It also is not known what critical sensory events terminate further ingestion, both at the level of primary afferent and second-order brainstem neurons.

The failure of decerebrate rats to appropriately increase meal size in response to food deprivation suggests that the brainstem in isolation is not able to respond appropriately to a long-term homeostatic challenge. However, Grill and Kaplan (Grill & Kaplan, 2002) have argued that "descending projections severed by decerebration may represent, under normal conditions, a permissive factor supporting the ability of brainstem structures to respond to metabolic signals," and that "the normal physiology of important integrative substrates in the brainstem also may be altered by the loss of major ascending projections that pass through the transection plane" (p. 24).

The Caudal Brainstem Is Necessary but Not Sufficient for Control of Long-term Energy Homeostasis

Decerebrate rats show many behavioral deficits, and if anything it is surprising that they are able to perform the list of tasks mentioned above. Decerebrate rats do not thermoregulate and need a great deal of care to be able to perform these tests. Most importantly, decerebrate rats cannot initiate ingestive behavior, and food has to be placed directly into the oral cavity. They do not exhibit the compensatory increase in intraoral intake induced by food deprivation (Kaplan, Seeley, & Grill, 1993; Seeley *et al.*, 1994), and they do not display conditioned taste aversion to lithium poisoning (Grill & Norgren, 1978b). Thus, while the brainstem circuitry is capable of responding to short-term gastrointestinal signals, it cannot translate into adaptive behavior signals related to longer-term energy status and to cognitive, rewarding, emotional, and social influences. These signals (also referred to as indirect controls) originate from the hypothalamus and other forebrain sites, and they are particularly important for food intake and energy balance in humans confronted with an obesigenic environment.

Leptin may Modulate Direct Controls of Food Intake at the Brainstem Level, but there Is No Evidence of Homeostatic Regulation

When isolated from the rest of the brain, brainstem circuits clearly have the capacity to control meal size but not longer-term energy balance. Can the contribution of the brainstem go beyond control of meal size? Grill and Kaplan (2002) observed that peptide hormones thought to be acting strictly in the diencephalon had effects on energy balance when injected near or directly into the brainstem, and suggested that part of the integrative network underlying longer-term control of energy balance may reside in the brainstem.

Hybridization signal for the long-form leptin receptor (Ob-Rb mRNA) was found in the dorsal vagal complex, hypoglossal, trigeminal, lateral reticular and parabrachial nuclei, and leptin significantly suppressed food intake over 24 hr when injected directly into the dorsal vagal complex in a dose sub-threshold for fourth ventricular injection (Grill *et al.*, 2002). The observation that fourth-ventricular administration of leptin inhibits gastric emptying (Smedh, Hakansson, Meister, & Uvnas-Moberg, 1998) suggests that leptin receptors in the dorsal vagal complex also can control autonomic functions, although fourth-ventricular delivery does not completely rule out an action in the hypothalamus. In addition to leptin receptors, several peptides involved in leptin's effects on food intake and metabolism are

expressed in the caudal brainstem (as discussed in detail in the section above), and Grill and Kaplan (Grill & Kaplan, 2002) asked:

> One wonders whether the POMC neurons in the commissural NTS express leptin and insulin receptors, and the K_{ATP} channel as do POMC neurons in the arcuate hypothalamus, and whether stimulation of the respective receptors similarly depolarizes cell membranes. (p. 11)

The critical question is whether such leptin-induced activity in NTS neurons has effects beyond meal size control, that is, on longer-term energy balance. At present there is limited evidence for such effects. As some of the most convincing evidence for a role of leptin and insulin receptors in energy homeostasis comes from brain-specific receptor knockouts, selective elimination or rescue of receptor populations in the brainstem will be necessary to answer this question.

Modulation of Caudal Medullary Mechanisms by Descending Input from the Hypothalamus, Amygdala, and Cortex

Strategically located in the midst of the mammalian neuraxis, the hypothalamus receives at least three distinct types of relevant information via direct or indirect neural connections as well as via hormone receptors and substrate sensors on hypothalamic neurons. First, the medial and, to a lesser extent, the lateral hypothalamus receive a rich mix of information pertaining to the internal energy stores. Second, specific hypothalamic nuclei receive information on the behavioral status, such as diurnal clock, physical activity level, reproductive cycle, developmental stage, as well as imminent and chronic stressors, that can potentially impact short-term availability of fuels and long-term energy balance. Third, the hypothalamus, particularly its lateral aspects, receives information from areas in the forebrain involved in the acquisition, storage, and retrieval of sensory representations of particular foods, as well as from the executive forebrain involved in behavior selection and initiation. This information is then further distributed through extensive intrahypothalamic connections to various hypothalamic nuclei. The metabolic sensing mechanisms located primarily in the basomedial hypothalamus and the associated peptidergic neuron populations in the paraventricular and lateral hypothalamus have been reviewed by others (Schwartz, Woods, Porte, Seeley, & Baskin, 2000), including the Chapter 6 by Seeley in this book.

Descending Anatomical Projections. Direct projections to the dorsal vagal complex from the hypothalamus (mainly PVN and LH), extended amygdala (mainly CeA and BNST), and prefrontal cortex (mainly medial prefrontal and agranular insular) have been thoroughly investigated using various retrograde and anterograde tracing techniques (Luiten, ter Horst, & Steffens, 1987; Saper, Loewy, Swanson, & Cowan, 1976; Sawchenko, 1983; Sawchenko & Swanson, 1982; Sawchenko *et al.*, 1996; Swanson & Kuypers, 1980; Swanson & Sawchenko, 1980; ter Horst & Luiten, 1986; ter Horst, Luiten, & Kuipers, 1984; Thompson, Canteras, & Swanson, 1996; van der Kooy, McGinty, Koda, Gerfen, & Bloom, 1982; van der Kooy, Koda, McGinty, Gerfen, & Bloom, 1984; see Figures 3 and 4). With few exceptions, the majority of transmitters and peptides used by these descending projections were not identified in this first wave of tracing studies conducted in the 1970s and 1980s. Later tracing studies combined with phenotypic characterization are described below.

Jacksonian Neural Hierarchy. The concept of hierarchically organized multiple nervous system "representations" of bodily parts originated with John Hughlings Jackson in the 19th century and recently was summarized by Blessing (Blessing, 1997). In his "Principle of Evolution and Dissolution," Jackson (Jackson, 1931) suggested that the same bodily region is regulated at increasing degrees of complexity by neural circuitry in more recently evolved regions of the nervous system. According to this view, the "newer" hypothalamus modulates and influences the "older" brainstem circuits involved in the autonomic and behavioral control of homeostatic functions. Bereiter and coworkers (Bereiter, Berthoud, & Jeanrenaud, 1980) conducted the first direct electrophysiological test of this principle in the rat. They demonstrated that NTS neurons receiving *oral* input through the chorda tympani nerve are modulated by lateral hypothalamic stimulation. Such facilitation of *gustatory* responses in NTS neurons by LH stimulation was recently confirmed in the hamster (Cho, Li, & Smith, 2002). Matsuo and Kusano (Matsuo & Kusano, 1984) demonstrated that LH stimulation enhances the activity of the gustatory-salivary reflex in the caudal brainstem. Rogers and Herman then demonstrated that stimulation of the paraventricular nucleus (PVN) was able to activate some of the same NTS neurons that could be activated through stimulation of *gastrointestinal* vagal afferents, and that neurons in the PVN can control gastric function by altering the sensitivity of neurons that form the sensory limb of gastric vago-vagal reflexes (Rogers & Hermann, 1985).

Descending modulation is not limited to the hypothalamus. Neurons in the gustatory NTS are modulated by electrical and chemical stimulation of the CeA and insular taste cortex (Cho, Li, & Smith, 2003; Smith & Li, 2000). A series of comprehensive studies using simultaneous stimulation of the hypothalamic defense area and vagal baro- and chemoreceptor nerves demonstrated that hypothalamic stimulation also affects cardiovascular and respiratory reflex functions (for a review see Spyer, Lambert, & Thomas, 1997). Collectively these studies clearly support Jackson's hypothesis and demonstrate that stimulation of certain hypothalamic areas can strongly influence activity of neurons in the dorsal vagal complex and in turn modulate various homeostatic reflex functions. A schematic diagram depicting the potential organization of this descending modulation is shown in Figure 7.

Smith's Direct and Indirect Controls of Meal Size. Jackson's principle can be applied also to the control of ingestive behavior. Smith's proposal (Smith, 1996) for a new classification of the factors controlling meal size has stimulated considerable research. He defined food stimuli contacting preabsorptive receptors along the surface of the gut from the tip of the tongue to the end of the small intestine as "direct controls" and everything else as "indirect controls." Indirect controls (e.g., metabolic, ecologic, and rhythmic) that require the forebrain change meal size by modulating the potency of direct controls. Recently, Schwartz and Moran (Schwartz & Moran, 2002) demonstrated opposing modulatory effects of intracerebroventricularly injected leptin and NPY on the responsiveness of NTS neurons to gastric distension. Leptin increased distension-induced spike activity, whereas NPY decreased this activity. Although this study suggests that these peptides act at the level of the hypothalamus and via descending projections, a direct action on the medulla cannot be excluded, emphasizing the need for more comprehensive studies.

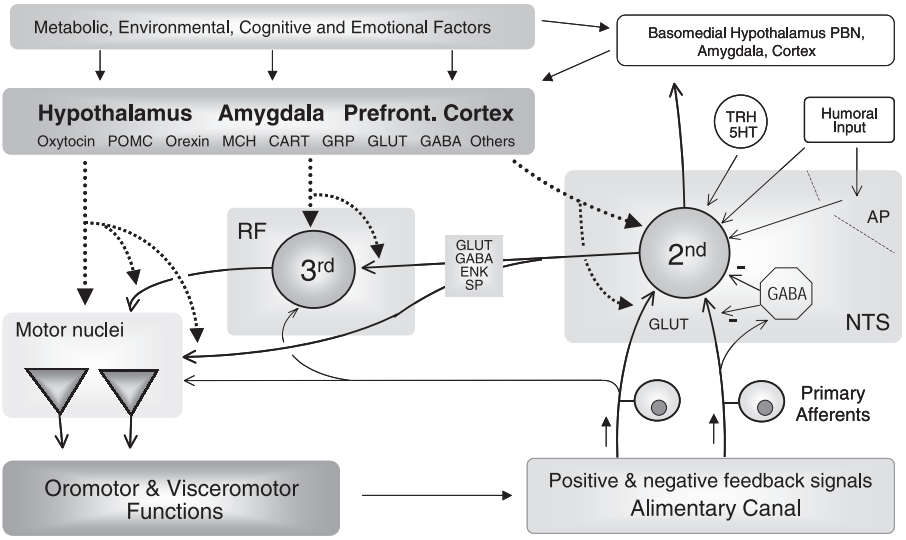

Figure 7. Forebrain modulation of caudal brainstem reflexes relevant for ingestive behavior and energy balance. This is a schematic flow diagram depicting the basic reflex path from alimentary canal afferents to oropharyngeal and visceral-autonomic motor response including second-order neurons in the NTS and third-order neurons in the medullary reticular formation. Descending projections from hypothalamus, amygdala, and prefrontal cortex containing various peptides and classical transmitters are in a position to modulate neurons at every level of the reflex loop either through presynaptic or direct effects. Potential presynaptic modulation of direct inputs from primary afferents to third-order neurons and monosynaptic inputs to vagal motor neurons, as well as inputs from caudal raphé TRH neurons, have been omitted.

Proopiomelanocortin-Derived Peptides

α-MSH and β-Endorphin. POMC-expressing neurons are restricted to a small area in the medio-basal hypothalamus, within and lateral to the arcuate (infundibular) nucleus. A small percentage of these neurons projects to the dorsal vagal complex (unpublished observations) and to the spinal cord (Elias *et al.*, 1998), as determined by combination retrograde tracing and immunohistochemistry. The fact that there is a second small population in the commissural NTS providing up to half of the α-MSH immunoreactive axon terminals in the dorsal vagal complex has already been discussed. Together with strong MC4R melanocortin receptor expression in the dorsal motor nucleus and moderate expression in the NTS, dorsal and ventral medullary reticular nuclei, and raphé pallidus (Kishi *et al.*, 2003; Mountjoy *et al.*, 1994), hypothalamic POMC neurons are well situated to influence brainstem reflexes.

Regarding autonomic and feeding effects of melanocortin receptor manipulation in the caudal medulla, direct NTS injection of melanocortin agonist reduced and infusion of an antagonist increased food intake (Williams *et al.*, 2000). Unilateral microinjection of low doses of α-MSH (0.3–12 pmol) into the NTS of urethane-anesthetized rats did not modify blood pressure or heart rate, but prior injection of 0.3 pmol of α-MSH attenuated the pressor effect of subsequent dynorphin injection (Carter & Lightman, 1987). Higher doses of α-MSH caused dose-dependent hypotension and bradycardia (Li *et al.*, 1996). Effects of α-MSH on electrical and transcriptional activity have been studied in the hypothalamus but not in the caudal brainstem.

Agouti-Related Protein and NPY. Agouti-related protein (AgRP) is expressed exclusively in a group of neurons located in the more medial aspects of the arcuate nucleus (Bagnol *et al.*, 1999). All AgRP neurons coexpress NPY, but NPY is also expressed in many other brain areas, including a small number of neurons in the NTS (unpublished observations). A moderate number of AgRP-immunoreactive axons has been detected in the NTS (Bagnol *et al.*, 1999; and unpublished personal observations), and because of coexpression with NPY, these axons are likely to contain also NPY. Thus, while AgRP in the NTS is exclusively of hypothalamic origin, NPY can be of hypothalamic or local medullary origin. Both peptides have strong orexigenic effects if injected into the rostral cerebral ventricles or into specific areas of the hypothalamus (Hagan *et al.*, 2000; Stanley, Magdalin, Seirafi, Thomas, & Leibowitz, 1993). NPY also strongly stimulates food intake when injected into the fourth ventricle (Corp *et al.*, 1990), but this site has not been tested for AgRP. As the dorsal vagal complex harbors a very high density of MC4R receptors, AgRP released from hypothalamic projections is likely to act as an endogenous antagonist. The synthetic MC4R receptor antagonist SHU9119 injected into the dorsal vagal complex increased food intake (Williams *et al.*, 2000).

Oxytocin and Vasopressin. The modulatory role of the "older" hypothalamic peptides oxytocin and vasopressin has been investigated over the last 15 years and can serve as a model. Projections from oxytocinergic and vasopressinergic neurons in the paraventricular nucleus of the hypothalamus to the dorsal vagal complex were first demonstrated by Sawchenko & Swanson by means of retrograde tracing (Sawchenko & Swanson, 1982). Oxytocin neurons project profusely to the medial but sparsely to the lateral subnuclei of the NTS, and mainly to the lateral column of the dorsal motor nucleus (Rinaman, 1998; Siaud, Denoroy, Assenmacher, & Alonso, 1989). Oxytocin's excitatory action, coupling to second messengers, has been described, as has the presence of oxytocin receptors on vagal motor neurons (Raggenbass, Alberi, Zaninetti, Pierson, & Dreifurs, 1998; Yoshimura *et al.*, 1993). Furthermore, oxytocin microinjected into the dorsal vagal complex mimicked the excitatory effect of paraventricular hypothalamic stimulation on gastric acid secretion and the inhibitory effect on gastric motility. These PVN stimulation-induced effects were blocked by microinfusion of an oxytocin antagonist into the NTS (Rogers & Hermann, 1986, 1987). More recently, Rinaman showed that oxytocin's inhibitory effect on food intake may be mediated by oxytocinergic projections abutting on glucagon-like peptide (GLP-1) neurons in the caudal NTS (Rinaman, 1998; Rinaman & Rothe, 2002).

Melanin-Concentrating Hormone. Melanin-concentrating hormone (MCH) neurons located in the hypothalamus project widely and diffusely to forebrain, hindbrain, and spinal cord (Bittencourt *et al.*, 1992; Bittencourt & Elias, 1998; Elias & Bittencourt, 1997; Skofitsch, Jacobowitz, & Zamir, 1985; Zamir, Skofitsch, & Jacobowitz, 1986). With regard to descending projections, certain areas of the NTS are very densely innervated, and individual MCH neurons in the LHA can project both to the vagal dorsal motor nucleus and sympathetic preganglionic neurons (Buijs, Chun, Niijima, Romijn, & Nagai, 2001). MCH receptors have been shown in the NTS and dorsal motor nucleus (Kokkotou, Tritos, Mastaitis, Slieker, & Maratos-Flier, 2001; Saito, Cheng, Leslie, & Civelli, 2001). MCH-binding sites also have been shown in the human medulla oblongata (Sone, *et al.*, 2000). However, the location and phenotype of dorsal medullary neurons receiving synaptic inputs from MCH

projections have not been explored, nor have the effects of MCH on neuronal activity and on ingestive behavior and autonomic functions.

Orexin. Orexin (hypocretin) neurons located in the hypothalamus also project to the caudal medulla including the NTS, certain reticular areas, the spinal trigeminal and principal trigeminal motor nuclei, and to the caudal raphé nuclei (Date *et al.*, 1999; Fung *et al.*, 2001; Krowicki *et al.*, 2002; Peyron *et al.*, 1998; Zhang & Luo, 2002). About 10% of retrogradely labeled neurons in the hypothalamus were orexin-A immunoreactive after very large Fluorogold injections into the dorsal medulla (Harrison, Chen, Dun, & Chang, 1999). Both OX1R-receptor immunoreactivity (Hervieu, Cluderay, Harrison, Roberts, & Leslie, 2001; Krowicki *et al.*, 2002) and OX2R-receptor mRNA (Sunter *et al.*, 2001) are expressed in the NTS and dorsal motor nucleus of the vagus.

Regarding effects on autonomic functions and food intake, orexigenic doses of ICV orexin (Edwards *et al.*, 1999) stimulated vagus nerve dependent gastric acid secretion (Takahashi, Okumura, Yamada, & Kohgo, 1999), and orexin microinjected directly into the dorsal motor nucleus (dmnX) potently stimulated gastric motor function (Krowicki *et al.*, 2002). This excitatory action on preganglionic vagal motor neurons is likely the result of direct receptor-mediated depolarization via a nonselective cationic conductance (Hwang, Chen, & Dun, 2001). Orexin's effects on neurons in the NTS and adjacent reticular areas have only started to be investigated. Orexin-B (hypocretin 2) depolarized about half of the recorded neurons under basal conditions, and increased the amplitude of solitary tract-evoked excitatory postsynaptic currents in the caudal NTS in medulla slices of neonatal rats (Smith, Davis, van den Pol, & Xu, 2002). Since vagal primary afferent neurons also express orexin receptors (Kirchgessner, 2002), it is possible that orexin released from hypothalamic projections acts both pre- and postsynaptically to modulate glutamate transmission. This effect was shown in lateral tegmental (Burlet, Tyler, & Leonard, 2002) and lateral hypothalamic neurons (Gao & van den Pol, 2001), where orexin produced an increase in frequency and amplitude of spontaneous EPSPs, triggering action potentials and enhancing spike-evoked synaptic transmission in glutamatergic afferents. Thus, part of orexin's effect on food intake might be mediated by its reciprocal actions on the sensory and motor limbs of vago-vagal reflexes and by resulting stimulatory actions on gastrointestinal functions.

Gastrin-Releasing Peptide. As mentioned above, hindbrain GRP receptors are involved in control of meal size (Ladenheim *et al.*, 1996; Ladenheim *et al.*, 2002). As GRP is expressed both in NTS neurons (Tohyama & Takatsuji, 1998) and in NTS projecting PVN neurons (Costello *et al.*, 1991), it is not clear which cell group provides the ligand for natural feeding suppression. Zhang *et al.* (Zhang, Sun, Renehan, & Fogel, 2002) recently have shown that inhibition of gut-related vagal motor neurons by electrical stimulation of the PVN is abolished after injection of a specific GRP receptor antagonist near the recording site. Thus, it is possible that this pathway can also influence meal termination.

Neurotensin. Intracerebroventricular injection of neurotensin decreases food intake, and targeted inactivation of the type 1 neurotensin receptor in mice significantly increased body weight and disrupted temperature control (Remaury *et al.*, 2002). In addition, neurotensin appears to mediate the central inhibitory effects of leptin on food intake (Sahu, 1998; Sahu, Carraway, & Wang, 2001). Many neurons

in the paraventricular and lateral hypothalamus exhibit neurotensin-immunoreactivity after colchicine-treatment (Tohyama & Takatsuji, 1998), and some of these neurons may project to the NTS, where neurotensin binding sites are highly expressed (Tohyama & Takatsuji, 1998).

Other Peptides and Classic Transmitters. Small populations of cells in the PVN that cross-react with antisera against somatostatin and enkephalin also project directly to the dorsal vagal complex (Sawchenko & Swanson, 1982). However, as noted by Sawchenko and Swanson (Sawchenko & Swanson, 1982), many of the hypothalamic neurons identified as projecting to the dorsal vagal complex by retrograde tracing techniques do not express recognized transmitters (unpublished observations). The participation of the classical excitatory (glutamate) and inhibitory (GABA) transmitters is indicated by the observation that PVN-stimulation-induced excitation of gut-related vagal motor neurons can be partially blocked by the AMPA receptor antagonist CNQX applied to the recording site (Zhang & Fogel, 2002).

Modulation by Cortico-Limbic Structures. As indicated in Figures 3 and 4, direct descending projections to the dorsal vagal complex have been shown to originate from the amygdala, bed nucleus of the stria terminalis, insular cortex, and the infralimbic, limbic, and anterior cingulate areas of the medial prefrontal cortex (Bagaev and Panteleev, 1994; Hayama & Ogawa, 2001; Holstege, Meiners, & Tan, 1985; Neafsey, Hurley-Gius, & Arvanitis, 1986; Owens & Verberne, 1996; Shipley, 1982; Terreberry & Neafsey, 1983, 1987; Torrealba & Muller, 1996; van der Kooy *et al.*, 1984; Whitehead, Bergula, & Holliday, 2000). Amygdala projections to the NTS use GABA as an inhibitory transmitter (Saha, Batten, & Henderson, 2000). Some of these GABA-ergic projections colocalize somatostatin (Saha, Henderson, & Batten, 2002), and neurons expressing immunoreactivity to neurotensin, vasoactive intestinal polypeptide, nitric oxide synthase, and alpha adrenergic receptors, have also been shown to project to the NTS (Batten, Gamboa-Esteves, & Saha, 2002; Glass, Colago, & Pickel, 2002). On the receiving side in the NTS, several studies have identified catecholamine-synthesizing neurons among the recipients of amygdala input (Petrov, Jhamandas, & Krukoff, 1996; Pickel, van Bockstaele, Chan, & Cestari, 1995). Furthermore, the presence of mu-opioid receptors in dendrites of NTS neurons that are targets of amygdala input (Pickel & Colago, 1999), and the finding that amygdala stimulation-induced food intake was blocked by NTS injection of the opioid antagonist naltrexone (Giraudo, Kotz, Billington, & Levine, 1998), suggest that projections from the amygdala may also express the opioid peptides enkephalin and/or dynorphin, and that they are involved in some aspect of food intake control.

Prefrontal cortex projections are composed of both glutamate excitatory (Torrealba & Muller, 1996) and GABA inhibitory (Smith & Li, 2000) inputs to the NTS, but no peptidergic inputs have yet been identified. Only a few studies examined inputs to feeding-related neurons, all of which were taste-responsive neurons in the NTS (Cho *et al.*, 2003; Di Lorenzo & Monroe, 1995; Smith & Li, 2000).

Summary

The discovery of leptin promised to end the search for the perfect long-term negative feedback signal that prevents excessive adiposity through inhibition of food intake and stimulation of energy expenditure (adipostat). The presence of the

long-form leptin receptor on neurons that comprise important medullary reflex paths clearly suggests that this hormone directly affects brainstem mechanisms of food intake. The question remains, however, whether leptin's actions in the brainstem are concerned only with control of meal size or whether they contribute to energy homeostasis. The observation of a decrease in 24-hr food intake and weight loss following injection of leptin into the dorsal vagal complex may be taken as support for its contribution to energy homeostasis, but additional approaches will be necessary for confirmation. Monitoring energy balance after targeted disruption of leptin or insulin signaling in the caudal brainstem, or selective rescue of such signaling in leptin or insulin receptor deficient mice, would be among the critical tests.

Recent observations have led to an alternative view of the biological importance of leptin. Rather than limiting excess adiposity, leptin may act as a starvation signal to limit loss of body fat. This view is based on observations that almost all obese patients exhibit high leptin levels, and that the first clinical trials with leptin therapy had very disappointing outcomes (Heymsfield *et al.*, 1999; Hukshorn *et al.*, 2000). It appears that the dose-effect curve of circulating leptin with respect to food intake and energy expenditure, while very steep at low leptin levels, is relatively flat at higher leptin levels (Hofbauer, 2002). At low leptin levels associated with low levels of body fat, small changes in circulating leptin are very powerful in modulating food intake and energy expenditure, while at higher leptin levels the effects are only modest. Therefore, it has been suggested that the major pressure to evolve this hormonal signal may have come from the need to guarantee high levels of energy intake and conservation, allowing early reproduction in a low-abundance food environment, and not to curb appetite or increase energy expenditure when food is abundant (Friedman, 1999; Speakman *et al.*, 2002). Applied to leptin's capacity to modulate short-term, direct controls of food intake at the medulla level, this view suggests that very low leptin levels powerfully reduce the potency of satiety signals, but that above a certain circulating leptin concentration additional leptin only poorly enhances the potency of satiety signals.

The other source of modulation of brainstem reflexes comes by way of prominent neuronal projections from the forebrain. There is general support for this descending modulation, but many important questions have not yet been answered. The neurochemical phenotype of the majority of lateral hypothalamic neurons and almost all cortico-limbic neurons projecting to the dorsal medulla has not yet been identified. Neurons receiving descending input have not been systematically mapped and neurochemically identified. The intracellular mechanisms by which integration between visceral signals and forebrain inputs is achieved have not been studied. It is not known how the second messengers activated by glutamatergic visceral inputs and peptidergic hypothalamic inputs converge and interact in NTS neurons, nor how the various transmitters (glutamate, GABA, peptides) interact in other medullary neurons.

Conclusions

Enormous progress has been made during the last 12 years in demonstrating the role of the caudal brainstem in ingestive behavior and energy balance. The caudal brainstem is no longer ignored by leaders in the field and is now considered an

integral part of the overall feeding network. Progress was made on the sensory and motor side as well as on the intramedullary pathways of integration and descending modulation.

We have now a quite complete picture of what kind of information the caudal brainstem receives, where this information is coming from, and how it is transmitted. Taste transduction mechanisms, so important for ingestive behavior, are just about deciphered. Significant advances have also been made on sensory input from the gastrointestinal tract, pancreas, the portal hepatic axis, and adipose tissue. This impressive system supplies minute-to-minute mechano- and chemosensory information directly to the caudal brainstem about the food ingested and the state of digestion, absorption, metabolism, and storage of nutrients. On the output side, the use of transneuronal retrograde tracing techniques has allowed an almost complete description of autonomic and skeletal motor pathways originating in the brainstem and further up in the neuraxis. A lot has been learned about intramedullary pathways and pattern generators responsible for integrating the vast sensory information into specific behavioral and autonomic responses. A number of hypothalamic "feeding peptides" and feeding-related hormone receptors have been shown to be also expressed in neurons of the caudal brainstem. Finally, the decerebrate rat model continues to provide insights into the basic capacities of the brainstem.

From this impressive array of observations, we can conclude that the brainstem contains the complete basic neural circuitry to (1) orchestrate ingestion of food and fluid placed into the oral cavity, (2) generate most of the parasympathetic support accompanying the ingestive and digestive processes through the vagus nerve, (3) stop ingestion when the taste is aversive, when gastrointestinal feedback signals reach "satiety" levels, or when visceral sensors detect noxious or toxic stimuli, and (4) generate sympathetic responses related to severe energy depletion. Clearly, ingestive behavior and regulation of energy balance cannot occur without this circuitry. However, many questions remain. Although accumulating evidence suggests that there is a single pattern generator circuit underlying multiple oro-lingual motor responses, the exact components of this circuit and the neurochemical triggers for the switch in its mode of action are not known. It is not clear which gastrointestinal satiety signals stop ingestion, and how they are propagated from the NTS and area postrema to other medullary areas. The potential role in energy balance for the many feeding-related peptides expressed in brainstem neurons has not been addressed. The role of the parabrachial nucleus in relation to brainstem control remains obscure. Finally, the relationship between the dorsal vagal complex (major site of sensory input and parasympathetic output) and the caudal raphé/ventrolateral medulla (sympathetic output) is poorly understood. The increasing availability of powerful, new investigative tools such as recording and imaging of medulla slices, and targeted manipulations of gene expression, intracellular protein signaling, and neural transmission, will go a long way in answering some of these questions in the coming decade.

Arguably the most interesting aspect of medullary control of ingestive behavior and energy homeostasis is provided by the modulation it receives from the hypothalamus, amygdala, and cortex. The hypothalamus integrates many more bits of information provided by neural inputs, hormones, and circulating fuels than the brainstem, and is able to generate more sophisticated and coordinated changes in energy input and output. In addition, the hypothalamus synchronizes ingestion and metabolism with other behaviors and conditions such as the diurnal cycle,

pregnancy and lactation, as well as fight and flight (stress). Hypothalamo-medullary projections were discovered years ago, and many of the participating peptides have recently been identified. Oxytocin, CRH, and GRP-expressing neurons located in the PVN orexin/dynorphin and MCH-expressing neurons in the lateral hypothalamus, as well as POMC/CART and AGRP/NPY-expressing neurons in the arcuate nucleus, all have direct projections to specific brainstem areas. Similarly, there are prominent direct brainstem projections originating in the amygdala and the prefrontal cortex. The neurochemical phenotype of many of these direct projections awaits identification.

There is increasing consensus that most human obesity is caused by a changing environment from conditions of relative famine to an abundance of easily available and inexpensive calories from fat and sugar and a sedentary lifestyle (Drewnowski, 2000; Hill & Peters, 1998; Ravussin & Bogardus, 2000). An important component of this obesigenic environment is the constant exposure to a flood of powerful food cues starting in childhood. The nervous system is the main interface by which this environment influences regulatory processes. Thus, mechanisms of perception and cognition of food-related external stimuli in cortico-limbic structures and their connections with the hypothalamus and brainstem may be just as important as the homeostatic regulatory circuits themselves for solving the obesity problem.

Acknowledgments

The author's research is supported by National Institutes of Health grants DK47348 and DK57242.

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Signals

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10

Metabolic Signals in the Control of Food Intake

Stephen C. Woods

Introduction

The fundamental goal of food intake is to provide the body with nutrients that provide the building blocks and energy necessary for life. The energy-rich substrates that are consumed by animals include carbohydrates, lipids, and proteins, with each being necessary in mammals. Key questions relate to what correlates of substrates cause animals to seek and ingest them, and what correlates cause them to cease ingestion when unlimited supplies are at hand and more food could be ingested. Correlates of substrate availability and utilization that influence the brain to alter food intake or energy expenditure are referred to as signals in this chapter. In considering signals related to energy homeostasis, it is important to remember that eating is but one of many necessary behaviors such as food intake, water intake, sleeping, securing warmth and shelter from the elements, and mating and rearing of the young. In social species such as humans there are of course other behaviors that must be factored into the equation. An important point is that the same signal(s) often influence multiple behavioral systems simultaneously.

There are several ways to view the control of food intake. A common perspective in the scientific literature is that the consumption of food is controlled by a series of interacting negative feedback loops. Perhaps the best known of these is based on the factors that act before and during meals, with a gradual decline of substrates generating a signal to seek and ingest food, and the gradual accumulation of substrates during a meal creating a feeling of fullness or satiety. A second feedback

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Neurobiology of Food and Fluid Intake, 2nd Ed., Volume 14 of *Handbook of Behavioral Neurobiology,* edited by Edward Stricker and Stephen Woods, Kluwer Academic / Plenum Publishers, New York, 2004.

loop includes the factors that control the amount of fat and other energy stores within the body, the principle being that because body fat tends to remain relatively constant over time, signals related to energy stores must be linked to the control of food intake. Both of these negative feedback systems rely upon multiple overlapping or redundant signals and coordinated activity in multiple areas of the brain, and numerous other control systems are thought to interact with them. For example, food intake in individual bouts or meals, rather than being based upon total energy available, could be based upon specific substrates. Hence, eating could occur in order to satisfy a deficit of carbohydrate, fat, protein, or some combination of these substrates. Alternatively, eating a meal could function to raise body temperature in a cold environment. Eating in response to deficits of energy or their correlates implies a kind of automatic or reflexive control over eating.

At the other end of the spectrum, food intake can be considered to be a psychological event divorced from constraints such as energy needs. In such a schema, individuals might initiate meals because of learned, or social, or hedonic reasons, and the amount consumed would be driven by factors such as how much food is present or readily available, how much effort is required to obtain it, how palatable it is, and other environmental factors. In the extreme, these models would state that there are few if any internal controls, and that the amount consumed varies with environmental conditions and situations. Analogously, the amount of stored energy also would vary, individuals becoming obese in some environments and lean in others. Eating would occur when food was available and/or when eating was convenient or expected rather than being dictated by dwindling energy supplies. This model best accounts for what has been called the "epidemic of obesity" as people are getting fatter in an environment where highly palatable and calorically dense foods are prevalent and where average daily physical exercise is low (Hill, Wyatt, Reed, & Peters, 2003).

This chapter focuses on the metabolic signals that collectively interact with the brain to influence food intake; there are several ways to consider them. Energy-rich substrates themselves, including glucose and fatty acids, could comprise the actual signal that controls eating. Specialized neurons would monitor glucose or fatty acid levels or availability and generate a proportional signal that influences circuits that control food intake and energy expenditure (Figure 1a). These neurons could theoretically be located wherever key fluxes of available energy occur. In point of fact, most early hypotheses on the factors that determine food intake posited that substrates or their metabolites reflecting their utilization comprised the actual signals controlling food intake. As an example, blood glucose level, or else energy derived directly from glucose oxidation, was hypothesized to be continuously monitored by brain cells that were connected synaptically with circuits controlling food intake (Carlson, 1916; Mayer, 1955; Mayer & Thomas, 1967).

Rather than substrates themselves, the key signals monitored by the nervous system could be reliable correlates of the availability of substrates (Figure 1b). For example, the peptide cholecystokinin (CCK) is thought to provide a signal indicating the presence of newly ingested substrates in the intestine, and the hormones insulin and leptin are thought to provide a signal to the brain indicating the amount of stored fat in the body. CCK, insulin, and leptin each in turn have a major influence on feeding behavior because specialized neurons have receptors that monitor their levels. Hypotheses based upon this model have dominated research on food intake for the past 30 years, and new potential signals, most of them peptides related to the ingestion or metabolism of food, are being discovered at a rapid pace.

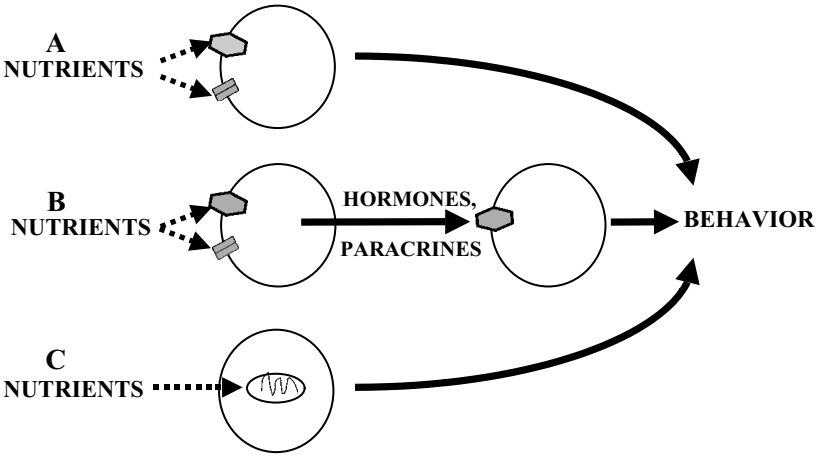

Figure 1. Ways in which nutrients generate neural signals. A, Nutrients (glucose, fatty acids) stimulate receptors on neurons, altering neural activity and neurotransmitter release; B, Nutrients stimulate receptors on endocrine cells eliciting the secretion of hormones or paracrines that in turn stimulate receptors on neurons; C, Nutrients enter neurons and are metabolized, generating signals that alter neural activity and neurotransmitter release.

In recent years there has been a resurgence of substrates-as-signals hypotheses as brain areas important in the control of food intake have been found to be sensitive to intracellular signals directly related to the oxidation of substrates (Figure 1c). This schema posits that a signal is generated by enzyme systems associated with the release of chemical energy within cells, whether from the metabolism of carbohydrates, fatty acids, or other energy-rich molecules. These neurons then are classed as generalized energy sensors, and are the topic of considerable contemporary research. The three theoretical positions concerning the signals that influence food intake, substrates themselves (i.e., glucose and/or fatty acids), hormonal or other correlates of substrates, and intracellular correlates of energy metabolism, are considered in turn.

Substrates as Signals: The Concept of Homeostasis

In the 19th century, Claude Bernard noted that in order to survive in a world that has a frequently changing external environment, organisms developed the ability to create and maintain a relatively constant internal environment. The physical properties of the internal environment allow optimal functioning of the various organ and cellular systems. Hence, the successful species or individual is able to conduct its obligatory processes with reasonable efficiency because its fragile internal components are buffered from the external world. The term "homeostasis" was coined more than half a century later by Walter Cannon (Cannon, 1929), and it generally refers to the ongoing processes that collectively function to maintain the properties of the internal world as near to ideal as possible. Many physiological parameters are recognized as being vitally important for survival and hence these parameters are under the strictest level of homeostatic control. Obvious examples are the levels of oxygen and of immediately available energy, for when either becomes too low, key metabolic activities of cells cease and the cells consequently

enter a state of suspended activity or die. In large, multicellular organisms such as mammals another level of complexity of the homeostatic process is based upon distribution of critical resources. Cells located deep within the interior of the organism have no easy access to environmental oxygen or to energy substrates or other nutrients, and they rely upon an efficient circulatory system to provide these commodities as needed; the same is true for the ability to eliminate wastes. Hence, parameters such as blood volume and pressure, and the precise chemical constituents of the blood, are all under homeostatic control.

The concept of homeostatic regulation, in its strictest form, includes several necessary criteria (Ramsay & Woods, 1997). First, there must be a means to monitor critical parameters and to convey the information to integrative circuits and ultimately to motor circuits whose activity can influence the value of the parameter. Besides the sensory and motor components that are necessary for any reflexive behavior, the concept of homeostasis implies the existence of a set point or predetermined ideal level of the critical parameter. When the value of the parameter deviates away from this ideal level in either direction, corrective responses are initiated. Hence, the homeostatic system operates by comparing the actual value of the parameter with the ideal value, and the magnitude of the difference (called the error signal) dictates the appropriate corrective responses to be made. These responses are terminated when the error signal declines below some threshold level. The value of the set point is thought to be genetically determined and/or shaped by ontogenetic modifications early in life. See Berridge for an excellent review of these principles (Berridge, 2004).

There is an important caveat when considering the application of homeostasis to the control of food intake and body weight. Many hypotheses invoke the principles of homeostasis without advocating a strict set point (Pinel, Assanand, & Lehman, 2000; Wirtshafter & Davis, 1977). One could presume, for example, a flexible set point whose value fluctuates with environmental conditions. An obvious example concerns the homeostatic regulation of body temperature. Whereas temperature is normally maintained around 37°C, it rises but is still regulated when animals are exposed to heat (Hainsworth, 1967). Analogously, the amount of fat maintained by the body may vary with such environmental factors as exercise level, stress, or food palatability, but still be regulated. A more extreme position is that there is no set point for parameters that appear to be regulated homeostatically. Advocates of this position state that body fat, for example, is the byproduct of numerous reflexes, each of which individually influences body fat content, and which collectively cause body weight to settle at a level that appears to be regulated but isn't (Bolles, 1980; Pinel *et al.*, 2000; Wirtshafter & Davis, 1977). The salient points in this chapter do not rely upon one or another position with regard to the presence or absence of a set point.

Viewed against the background of the concept of homeostasis, food intake can be considered a necessary behavior that provides the body with needed energy, critical amounts of fluid, vitamins, minerals, and other micronutrients necessary for many enzymatic reactions. Numerous organ systems then act upon ingested food to convert it into usable substrates and distribute it to tissues, or store it until needed. And so, the problem of considering the metabolic controls over food intake must take into account the numerous reflexively controlled parameters that have to be satisfied. As stated above, the longest-standing hypotheses as to the controls over food intake have been based upon one or more homeostatically controlled metabolic parameter.

Carbohydrate Substrates and the Control of Food Intake. The best-known and most influential metabolic hypothesis of the control of food intake is the glucostatic theory of Jean Mayer (Mayer, 1955; Mayer & Thomas, 1967). It was based upon observations that although the brain requires a continuous supply of readily available energy in the form of glucose, it has a very limited capacity to store glucose; thus, a steady stream of glucose must be supplied by the circulatory system. The original version of the hypothesis posited that blood glucose *per se* was the regulated parameter; it was based on observations that blood glucose is relatively low when individuals fast, that it rises during meals, and that it is relatively high when eating stops (Mayer, 1953). Hence, low blood glucose was thought to trigger eating whereas high blood glucose was thought to terminate a meal. However, it soon became apparent that the correlation between behavior and blood glucose does not always hold. Individuals with insulin-deficiency diabetes mellitus, for example, are characterized by very high blood glucose, yet they eat greater than normal amounts of food. Mayer therefore amended the hypothesis, basing meal initiation and cessation on glucose utilization rather than on blood glucose levels (Mayer, 1955). He further postulated that the key sensory cells were in the hypothalamus and were sensitive to the hormone, insulin, unlike most brain cells but analogous to most tissues throughout the body (Mayer, 1955; Mayer & Thomas, 1967). This change aptly accounted for what occurs in diabetics since the lack of sufficient insulin precluded adequate glucose utilization by these sensor cells and since glucose utilization in nondiabetics is directly correlated with circulating glucose.

Mayer's hypothesis has enjoyed tremendous popularity, especially in the popular press and literature, in part because of its simplicity and in part because of its face validity. That is, a single parameter related to blood glucose accounted for both meal initiation and cessation, under normal and pathological states. The hypothesis was further bolstered when Mayer and others discovered that glucose uptake by certain areas of the hypothalamus is in fact sensitive to insulin (Baile, McLaughlin, Zinn, & Mayer, 1971; Debons, Krimsky, & From, 1970; Debons, Krimsky, From, & Cloutier, 1969; Debons, Krimsky, Likuski, From, & Cloutier, 1968; McLaughlin, Baile, Trueheart, & Mayer, 1973). It is now recognized that there are glucose-sensitive neurons in many areas of the brain, including the hypothalamus, many of which are sensitive to insulin (Levin, Dunn-Meynell, & Routh, 1999), and the brain also receives information about glucose availability from other sites in the body such as the taste buds and the hepatic portal vein (Hevener, Bergman, & Donovan, 2000).

Research during the ensuing decades has chipped away at the tenets of the hypothesis (see reviews in Epstein, Nicolaidis, & Miselis, 1975; Grossman, 1986; Langhans, 1996a). For example, although it is true that eating occurs when blood glucose, or glucose utilization, is experimentally reduced in animals or humans, most spontaneous meals are initiated when blood glucose is much higher and not decreasing. Analogously, meals are still initiated when glucose is infused intravenously into animals or humans. A general conclusion that has been reached is that whereas extreme fluctuations of glucose can drive behavior, normal eating occurs in the absence of major fluctuations. Further, numerous alternative signals were found to have major influences over meals.

That said, the fact remains that animals and humans do initiate eating when the ability to utilize glucose is acutely compromised. This effect was initially demonstrated by administering insulin (MacKay, Calloway, & Barnes, 1940), and the consequent eating was found to be secondary to reduced glucose availability from the blood (Lotter & Woods, 1977; Lovett & Booth, 1970). In fact immediately after

insulin is administered, most cells are, if anything, taking up and burning more glucose than they otherwise would, and eating is delayed until glucose reaches a nadir and starts returning toward baseline. Eating is initiated also after administration of glucose analogues that can enter cellular metabolic pathways but cannot generate energy. This so-called glucoprivic eating can be elicited by 2-deoxy-glucose (Smith & Epstein, 1969) or 5-thioglucose (Ritter & Slusser, 1980), or their analogues. The sensors that respond to glucoprivic challenges are located in the brainstem (Ritter & Dinh, 1994; Ritter, Llewellyn-Smith, & Dinh, 1998; Ritter, Scheurink, & Singer, 1995), and their activation sends signals anteriorly to the hypothalamus to initiate a meal while simultaneously stimulating sympathetic activity to increase epinephrine secretion from the adrenal medulla. Hence, stimulation of the sensors triggers both behavioral and neuroendocrine responses. As discussed below, this dual control over behavior and the neuroendocrine control of systemic substrates such as glucose has become an area of considerable research activity in recent years.

Carbohydrate can be stored in limited amounts in some tissues, and in larger amounts in the liver as glycogen. Novin and his colleagues hypothesized that hepatic glycogen stores are monitored and that eating is triggered when the stores become low (VanderWeele, Skoog, & Novin, 1976). He and his colleagues manipulated and monitored carbohydrate parameters in the liver relative to the rest of the body and provided considerable evidence supporting their point of view. In particular, infusion of glucose or glucagon directly into the hepatic portal vein increases neural activity in afferent fibers of the vagus nerve, the major direct connection between the liver and the brain (Martin, Novin, & Vanderweele, 1978; Vanderweele, Geiselman, & Novin, 1979). Hepatic portal glucose infusion also decreases food intake and increases gastric motility (Rezek & Novin, 1977; Tordoff & Friedman, 1986; Vanderweele, Novin, Rezek, & Sanderson, 1974), effects that are no longer present when the vagus nerve is transected (Rezek & Novin, 1976). The liver also is sensitive to fluctuations of the carbohydrate, fructose, and animals eat less when fructose is infused into the hepatic portal vein (Tordoff & Friedman, 1988); this response is eliminated following vagotomy (Tordoff & Friedman, 1994). The observation that intravenous fructose inhibits insulin-elicited eating even though the fructose does not enter the brain (Stricker, Rowland, Saller, & Friedman, 1977) indicates that the ability of sensors in the hepatic portal circulation to detect carbohydrates can provide an important contribution to meals and is probably most prominent when food is being actively absorbed from the intestines. That said, and as reviewed in Chapter 9 by Berthoud, there is little evidence that cutting the vagus nerves has much effect on total daily food intake or body weight.

In recent years, the possibility of a glucostatic regulation of food intake has been reconsidered because of technological advances. Specifically, the ability to measure blood glucose continuously in animals by means of an indwelling intravenous glucose sensor revealed that blood glucose declines in rats prior to "spontaneous" meals (Campfield, Brandon, & Smith, 1985; Louis-Sylvestre & Le Magnen, 1980). The decline begins 10 to 15 min prior to the onset of eating, is small in magnitude, and reverses just before eating begins (Campfield & Smith, 1986b, 1990b, 2003) (see Figure 2). Analogous findings have been made in humans (Campfield, Smith, Rosenbaum, & Hirsch, 1996). Campfield and Smith have hypothesized that when the decline is perceived and meets certain criteria, it provides a signal to the brain that eating can proceed (Campfield & Smith, 1990b, 2003). In their model, the brain periodically sends messages to organ systems that will be impacted by eating a meal, probing them in order to determine whether conditions are safe and/or

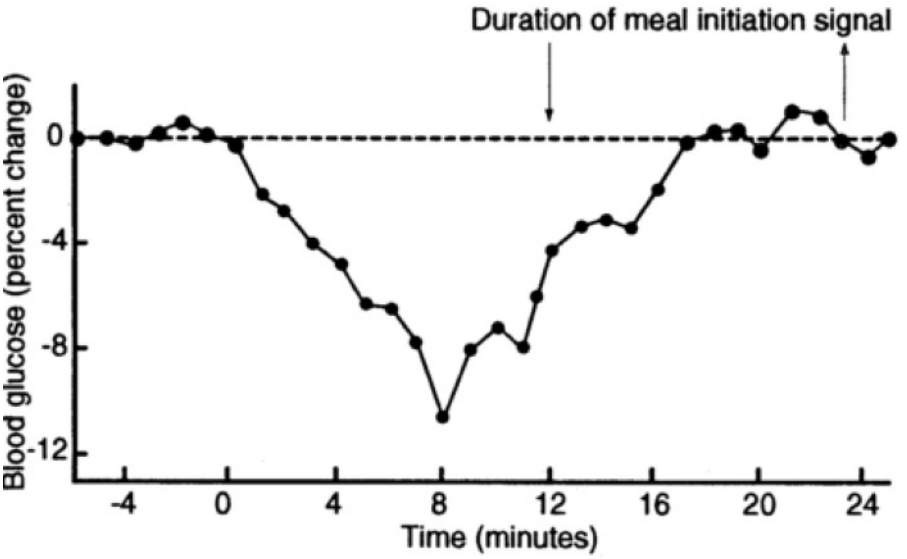

Figure 2. Premeal glucose decline in rats. Beginning, on average, 12 min prior to spontaneous meals, blood glucose of rats begins decreasing, reaching a nadir of −10% in an average of 8 min and then increasing toward baseline. Meals begin 4–5 min after the nadir. If no food is available, glucose continues to increase and returns to baseline in an average of 17 min after the start of the decline. (From Campfield & Smith, 1986b.)

appropriate for a meal to occur. In this process, the brain stimulates the pancreas via the vagus nerves, eliciting a small burst of insulin secretion that results in a small decline of glucose (Campfield & Smith, 1986a, 1990a). Because the brain continuously monitors blood glucose, it can evaluate the parameters of the insulin-elicited glucose decline and decide whether or not a meal should be initiated; that is, when the pattern (magnitude and timing) of the glucose decline does not match an idealized pattern, it indicates that metabolic activity is not appropriate for a meal to be initiated at that time. While the premeal decline of glucose can be interpreted as being consistent with the glucostatic theory of eating, it cannot be said to reflect a decrease of available energy since tissues receive ample glucose throughout the entire process. An alternative explanation is that it is a correlate of the individual's anticipation of an impending meal (Woods, 1991; Woods & Strubbe, 1994).

The Thermogenic Control of Food Intake. Because the consumption and processing of food generate heat, eating has been considered to be a behavior aimed at maintaining body temperature. The thermogenic hypothesis postulated that a reduction of body temperature stimulated eating and that the postprandial rise of temperature elicited satiety (Brobeck, 1947–1948). Hence, temperature (or the generation of heat) in some sensor was postulated to be an important metabolic signal influencing food intake. Consistent with this hypothesis, animals eat more food when maintained in cold environments and less food in warm environments (Cabanac, 1975). The thermogenic hypothesis has never enjoyed widespread support, however, because so many other factors have been found to be important. Further, minute-by-minute analysis of body temperature in rats reveals that body temperature is increasing, not decreasing, at the time that meals are initiated (de Vries, Strubbe, Wildering, Gorter, & Prins, 1993).

Lipid Substrates and the Control of Food Intake. Homeostatic hypotheses of food intake based on lipids have presumed either that fatty acids or other lipids (or their utilization) constitute signals influencing ingestion, and/or that the amount of fat stored in adipocytes generates a signal that influences ingestion. As depicted in Figure 3, different compartments of the body oxidize different substrates to generate energy. Most tissues (e.g., muscle, heart, most viscera, skin, and bone) can derive energy from either glucose or fatty acids depending upon their relative availability and how much insulin is present. When blood glucose is high (typically during and immediately after meals), it stimulates the secretion of insulin, enabling insulin-sensitive tissues to remove glucose from the blood and use it as energy. When blood glucose and insulin are low, in contrast, insulin-sensitive tissues cannot take up sufficient glucose to meet their energy needs and they take up and oxidize fatty acids instead. The brain differs in that it requires a steady supply of glucose from the blood to meet its energy needs, and brain cells are able to take up and derive energy from glucose even during a fast because they do not require insulin for this activity. Additionally, the brain has a limited capacity to derive energy from ketones, molecules that are not available except during extreme fasts. These properties ensure that when blood glucose is relatively low (as during a fast, or between meals), the paucity of insulin mandates that tissues such as muscle do not take up glucose, thereby sparing blood glucose to be preferentially utilized by the brain. Insulin therefore has a pivotal role in the use of substrates by the body (Figure 3). When plasma insulin is elevated, it enables most tissues to use glucose for energy or to store it, and it simultaneously provides a signal to the brain that glucose is abundant. Low insulin, on the other hand, provides a signal that the body is relying mainly on lipids for energy, and that glucose present in the blood is being reserved for use by the brain. Low insulin coupled with low blood glucose also stimulates the liver to generate new glucose from amino acids, a process called gluconeogenesis, ensuring an uninterrupted glucose supply for the brain. Low glucose levels reaching the brain (or, as discussed above, administration of drugs that compromise glucose utilization within the brain) also stimulate eating.

The liver has a contrasting profile for substrate utilization. The liver oxidizes little or no glucose for energy, and instead has an obligatory need to oxidize fatty acids (Figure 3). The liver is insulin-sensitive, however, with liver cells taking up more glucose as plasma insulin becomes elevated. However, rather than using it for energy, liver cells convert the glucose to glycogen and store it for later release and

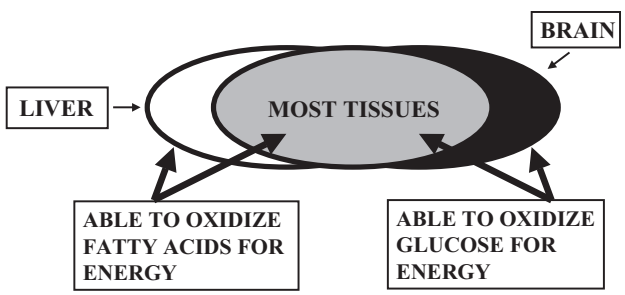

Figure 3. Most tissues are able to oxidize either glucose or fatty acids for energy. When plasma insulin is high, such as during meals, these tissues utilize mainly glucose; when plasma insulin is low, these tissues utilize mainly fatty acids. The liver is unique, with a limited capacity to utilize glucose. When insulin is relatively high, the liver converts glucose to fatty acids. The brain, on the other hand, utilizes glucose almost exclusively, and this is independent of insulin levels.

use by other tissues, or else they convert it to fatty acids that are released into the blood to be used by other tissues or stored in fat cells. Homeostatic theories of food intake based upon energy derived from lipids have therefore identified the liver as the critical receptor organ (Friedman, 1990; Langhans, 1996b; Langhans & Scharrer, 1992; Russek, 1981).

Consistent with this perspective, animals eat more food when given drugs that compromise the oxidation of fatty acids for energy such as methyl palmoxirate or mercaptoacetate (Friedman & Tordoff, 1986; Langhans & Scharrer, 1987b; Scharrer & Langhans, 1986). Importantly, the orexigenic effect is larger when these drugs are administered directly into the hepatic portal vein, implying that reduced metabolism of fatty acids in the liver generates a signal that is translated into increased food intake (see reviews in Friedman, 1997; Langhans, 1996a). Several aspects of this phenomenon are important. The first is that lipoprivic eating is most evident when animals are maintained on a high-fat diet and most tissues are therefore adapted to deriving the majority of their energy from fatty acids (Friedman & Tordoff, 1986; Langhans & Scharrer, 1987b; Scharrer & Langhans, 1986). Another is that infusion of mercaptoacetate into the hepatic portal vein increases activity in vagal afferent fibers (Lutz, Diener, & Scharrer, 1997), and that lipoprivic eating is prevented when the vagus nerve is cut (Langhans & Scharrer, 1987a). That said, a possible role of neural information from the liver controlling normal food intake is controversial because food intake, meal patterns, and the maintenance of body weight appear normal when all nerves innervating the liver have been cut (Bellinger, 1999; Bellinger, Dula, & Williams, 1994; Bellinger & Williams, 1983; Louis-Sylvestre, Servant, Molimard, & Le Magnen, 1980) or after a denervated liver has been transplanted (Bellinger, Fabia, & Husberg, 1997). The implication is that lipoprivic (or other) signals from the liver have a nonessential impact on normal feeding, at least on a chronic basis. When the vagus nerves to the liver are acutely severed, there is a transient increase of food intake (Chavez, Kelly, York, & Berthoud, 1997; Kelly, Chavez, & Berthoud, 1999), suggesting that vagal signals may set an overall inhibitory tone on food intake.

Within the brainstem, the neural circuitry mediating lipoprivic eating is distinct from but parallel to the circuitry that mediates glucoprivic eating (Ritter *et al.*, 1998; Singer & Ritter, 1996). Whereas the liver normally derives most of its energy from lipids, it also can derive energy from one carbohydrate, fructose. Furthermore, increased eating can be caused by the administration of drugs that compromise fructose utilization by the liver, such as 2,5-anhydro-mannitol (Park *et al.*, 1996; Park, Seeley, Benthem, Friedman, & Woods, 1995; Tordoff, Rafka, DiNovi, & Friedman, 1988), an effect that is more robust when animals are maintained on a high-fat diet instead of a high-carbohydrate diet and which is signaled by the vagus nerve (Tordoff, Rawson, & Friedman, 1991). Given the normal reliance of the liver upon fatty acids for energy (Figure 3), it is reasonable that neural signals passing to the brain from the liver would be interpreted as insufficient lipid. The point is that neural signals to the brain from the liver seem to indicate a reduced availability of lipid-derived energy, and eating is one way to reverse this condition. Analogously, signals arising within the brain from glucose sensors indicate a deficit of glucose-derived energy, a need that also could be met by increased food intake.

In point of fact, eating is not an efficient way to provide needed energy rapidly. When food is ingested, it must be chewed, swallowed, processed in the stomach, passed to the intestine, processed again, and ultimately absorbed into the body in usable units. These processes take considerable time and are especially slow for

lipids since most ingested fats are packaged into chylomicrons (small vesicles containing ingested fat) by intestinal cells and then released into the lymphatic system rather than absorbed directly into the blood. Significant energy from the ingestion of a fat-rich meal might not enter the blood and get to the liver for several hours. On the other hand, there are highly efficient ways for the body to protect its supply of lipids and glucose to critical organs. When the brain's supply of glucose is compromised, numerous reflexes are activated that cause an immediate increase of glucose in the blood, including the activation of the sympathetic nerves to liver cells to release stored glucose, to the pancreas to inhibit insulin secretion and increase glucagon secretion, and to the adrenal medulla to secrete epinephrine (Virally & Guillausseau, 1999). Glucagon and epinephrine both potently increase blood glucose. Hence, eating-induced increases of glucose might be considered to be more of a hedge against future declines of glucose than a corrective response to a current need. Analogously, when lipoprivic (or fructose utilization-blocking) drugs are administered to the liver, the immediate response is an increased release of fatty acids into the blood from fat cells triggered by sympathetic reflexes. The point is that neuroendocrine reflexes are rapidly activated whenever brain or liver metabolism is compromised. This system efficiently restores whichever substrate is in short supply. Eating is simultaneously stimulated, but its utility to address the immediate need is less significant than the concomitant physiological responses (Ramsay, Seeley, Bolles, & Woods, 1996; Seeley, Ramsay, & Woods, 1997).

To summarize, energy-rich substrates (mainly glucose and fatty acids) have long been postulated to constitute the key signals that determine meal onset and offset. The brain, having an obligate need for a continuous stream of glucose, monitors its levels closely, whereas the liver monitors parameters related to fatty acids. While these signals are important for coordinating metabolic activity throughout the body, their contribution to the control of most meals in freely feeding individuals has not been firmly established.

Metabolic Peptides as Surrogate Signals to the Nervous System

When food is consumed, substrates enter the body in the form of carbohydrates and lipids, as well as other macro- and micronutrients. The ingested food or chyme is processed in the stomach and once its density, acidity, and degree of initial digestion are appropriate, it is delivered through the pyloric sphincter into the duodenum at a rate dependent upon the rate of eating and the caloric density of the chyme. In the duodenum, the chyme is further processed by enzymes and cofactors from the intestinal wall, exocrine pancreas, and liver. As the chyme progresses along the length of the intestine, digested nutrients are passed through the cells (absorption) and into the blood or lymph and ultimately made available for energy or storage. As discussed above, this process can be lengthy, requiring hours for some nutrients. As a consequence, most animals stop eating long before significant amounts of new energy become available. In other words, by monitoring signals that are correlated with ingested calories, the brain is able to terminate a meal when ample calories have been ingested but well before they have entered the blood in significant amounts. This ability is important for preventing the body from becoming overwhelmed by a flood of newly ingested glucose and lipids (Woods, 1991; Woods & Strubbe, 1994).

In principle, an individual could gauge the caloric content of food it is eating in many ways. It could use vision, smell, and taste to provide a rough estimate of actual calories that are being consumed, or it could rely upon more precise analyses of the energy and macronutrient content of the food after it has entered the body but before it is absorbed into the blood. In actuality, the latter seems to be the case. Chyme is continuously monitored for numerous physicochemical properties as it traverses the gastrointestinal tract in order to ensure that the appropriate mix of digestive enzymes and cofactors are added as needed, that it is adequately mixed, that the proper amount of water is added, and that it proceeds along the length of the intestine in a timely manner.

Digestive System Signals that Influence Individual Meals

The digestive system includes the gastrointestinal tract (i.e., oral cavity, esophagus, stomach, small and large intestine, and rectum) and associated organs that synthesize enzymes and cofactors to aid the processing of ingested food (i.e., liver, pancreas). The control of digestion is complex and requires the coordinated activity of multiple neural and hormonal signals. Many of these same signals provide information to the brain that influence energy homeostasis, and these signals can be direct (by penetrating the blood-brain barrier) or indirect (by stimulating neurons that in turn forward signals to the brain). Table 1 lists the peptides secreted from the digestive system during meals that have been found to alter meal size. Several points are important. First, most of the signals generated in the gastrointestinal tract also are synthesized in the brain. Understanding the communication and/or coordination between peripheral and central signals that interact with the same receptor type to influence food intake is an ongoing goal in many labs, but to date no generalized conclusions can be made. A second point is that these peripheral signals normally are neither secreted nor active in isolation. Rather, both prior to and during meals complex "cocktails" of signals are generated that reflect the physicochemical properties of the food, and the various individual signals combine in myriad ways to influence intake.

Another general point is that all but one of these signals causes meals to terminate sooner than they otherwise would. Hence, they are said to elicit satiation or satiety. The lone exception is the stomach hormone, ghrelin. Ghrelin is a peptide normally secreted prior to meals (Cummings *et al.*, 2001), and its levels decrease during eating or when nutrients are infused into the stomach (Shiiya *et al.*, 2002). When exogenous ghrelin is administered, animals and humans eat larger

Table 1. Gut Peptides that Influence Food Intake

Reduce meal size	Increase meal size
Cholecystokinin	Ghrelin
Bombesin Family (BBS, GRP, NMB)	
Glucagon	
Glucagon-like peptide-1, Glucagon-like peptide-2	
Amylin	
Somatostatin	
Enterostatin	
Apolipoprotein A-IV	
Peptide YY	

than normal meals (Wren, Seal *et al.*, 2001; Wren *et al.*, 2000) and obesity develops when its administration is chronic in rats (Tschöp, Smiley, & Heiman, 2000; Wren, Small *et al.*, 2001). Most obese humans have low levels of circulating ghrelin (Tschöp *et al.*, 2001), a noteworthy exception being individuals with the Prader-Willi syndrome. Those individuals are characterized by uncontrollable appetite and extreme obesity (Dimitropoulos *et al.*, 2000), and they have considerably elevated plasma ghrelin (Cummings *et al.*, 2002). For all of these reasons ghrelin has been hypothesized to be a normal stimulant of eating (Cummings & Schwartz, 2003). The signals that elicit ghrelin secretion from the stomach are as yet unknown.

Ghrelin also is synthesized in small amounts in the ventral hypothalamus (Cowley *et al.*, 2003), and ghrelin receptors are found on neurons in the arcuate nucleus that secrete neuropeptide Y and agouti-related protein (NPY/AgRP neurons) (Cone *et al.*, 2001; Cowley *et al.*, 2003). Ghrelin administration stimulates NPY/AgRP neurons electrically and elicits c-Fos expression in them (Kamegai *et al.*, 2001; Kohno, Gao, Muroya, Kikuyama, & Yada, 2003; Wang, Saint-Pierre, & Tache, 2002). Although circulating ghrelin can enter the brain from the blood (Banks, Tschop, Robinson, & Heiman, 2002), there is evidence that the signal generated by systemic ghrelin requires an intact vagus nerve to influence the brain (Date *et al.*, 2002), as also is true of CCK (Smith, Jerome, Cushin, Eterno, & Simansky, 1981; Smith, Jerome, & Norgren, 1985). The role of ghrelin in the control of normal food intake is an area of active investigation.

The most-studied of the GI peptides that reduce food intake is CCK. CCK is secreted from duodenal cells in response to nutrients in the chyme, with different specific nutrients being most effective in different species. Some of the secreted CCK enters the blood and stimulates the exocrine pancreas and liver/gall bladder to secrete appropriate enzymes into the duodenum to facilitate the digestive process. In 1973, Gibbs and Smith and their colleagues observed that exogenous CCK, when administered just prior to a meal, reduces food intake in rats (Gibbs, Young, & Smith, 1973). This phenomenon has been replicated and extended in numerous labs over the ensuing 30 years, and the following conclusions can be made. Virtually all species investigated, including humans (Kissileff, Pi-Sunyer, Thornton, & Smith, 1981; Muurahainenn, Kissileff, Derogatis, & Pi-Sunyer, 1988; Muurahainenn, Kissileff, Lachaussee, & Pi-Sunyer, 1991), eat smaller meals when CCK is administered (Gibbs *et al.*, 1973; Kulkosky, Breckenridge, Krinsky, & Woods, 1976; Smith & Gibbs, 1992, 1998). Although intravenous administration is efficacious in humans and nonhuman primates (Figlewicz, Sipols, Green, Porte, & Woods, 1989; Gibbs & Smith, 1977; Muurahainenn *et al.*, 1988), CCK is most potent when administered either intraperitoneally in rats or into arteries supplying the region of the upper duodenum (Cox, Perdue, & Tyler, 1995). This observation is consistent with the finding that the key receptors for the reduction of meal size, the CCK-1 receptors (formerly called CCK-A receptors), are expressed on sensory fibers of the vagus nerves innervating, among other areas, the tissue around the pyloric sphincter and the proximal duodenum (Corp, McQuade, Moran, & Smith, 1993; Mercer & Lawrence, 1992).

The model that has evolved is that when nutrients enter the duodenum and stimulate CCK secretion, some CCK acts in a local paracrine manner to stimulate CCK-1 receptors on the sensory fibers of the vagus nerves (Moran, Baldessarini, Salorio, Lowery, & Schwartz, 1997; Moran, Shnayder, Hostetler, & McHugh, 1988). Other branches of the same vagal sensory nerves emanate from the stomach wall and elsewhere such that the same neurons are sensitive to both CCK and other stimuli such as gastric distension (Berthoud & Powley, 1992). Consistent with the

concept that vagal neurons integrate different kinds of signals relevant to ingestion, electrophysiological recording studies and behavioral studies have found that the effect of a given dose of CCK is increased in the presence of stomach stretch (Schwartz, McHugh, & Moran, 1993; Schwartz & Moran, 1996; Schwartz, Tougas, & Moran, 1995). The CCK signal carried in the vagus nerve enters the hindbrain, where it initiates local reflexes and also is relayed to the forebrain (Moran & Schwartz, 1994; Rinaman *et al.*, 1995). Disruption of the path at any point, whether by cutting the vagus nerves or lesioning the nucleus of the solitary tract, renders CCK ineffective at reducing meal size (Edwards, Ladenheim, & Ritter, 1986; Moran *et al.*, 1988; Smith *et al.*, 1981, 1985).

A role for CCK in limiting the size of normal meals was demonstrated by the observation that antagonists to the CCK-1 receptor, when administered just prior to a meal, cause a larger meal to be consumed in animals (Hewson, Leighton, Hill, & Hughes, 1988; Moran, Ameglio, Peyton, Schwartz, & McHugh, 1993; Reidelberger & O'Rourke, 1989) and humans (Beglinger, Degen, Matzinger, D'Amato, & Drewe, 2001). Consistent with this finding, administration of CCK-1 antagonists also blocks vagal afferent activity elicited by the presence of protein (Eastwood, Maubach, Kirkup, & Grundy, 1998) or long-chain fatty acids in the duodenum (Lal, Kirkup, Brunsden, Thompson, & Grundy, 2001).

CCK is considered an acutely acting signal since it has a very short half-life (1–2 min) and since administering it more than 15 min prior to the start of a meal is ineffective at reducing meal size (Gibbs *et al.*, 1973). An important question concerns the possibility that drugs that interact with CCK receptors and/or the receptors of other signal-generating peptides might have therapeutic potential to treat obesity or eating disorders. Several types of experiments have addressed this possibility with CCK. When CCK is administered continuously, it rapidly becomes ineffective (Crawley & Beinfeld, 1983). When short-acting CCK is administered intermittently, prior to the start of every spontaneous meal in rats, it continues to be effective at reducing meal size, but the animals compensate by eating more meals over days and there is little impact on total daily food intake or body weight (West, Fey, & Woods, 1984; West, Greenwood, Marshall, & Woods, 1987). The implication is that humans given CCK therapeutically to reduce food intake also would compensate by increasing their snacking between meals. That said, rats with spontaneous mutations of the CCK-1 receptor (Otsuka Long Evans Tokushima Fatty or OLETF rats) eat larger than normal meals (Moran, Katz, Plata-Salaman, & Schwartz, 1998) and gradually gain weight and body fat over their lifetime (Funakoshi *et al.*, 1994), implying that a chronic absence of CCK signaling has a small but cumulative effect over time in rats (Bi & Moran, 2002). In apparent contrast, mice with a targeted deletion of the CCK-1 receptor that do not reduce meal size in response to exogenous CCK have a normal body weight (Kopin *et al.*, 1999), implying either that compensation for lack of CCK signaling occurred differentially in the two models or that CCK is not involved in long-term body weight maintenance in mice. Therefore, no general conclusion can be reached as yet regarding the possible therapeutic potential in humans of CCK analogs, especially long-acting analogs. Another implication of this area of research is that as more transgenic and knockout mouse models become available, and as more pharmacological tools become available to apply in *in vivo* situations, the number of discrepancies in the conclusions reached concerning the normal role of metabolic signals and their receptors are likely to increase, rendering strict conclusions as to the role of one or another signal tenuous (Seeley & Moran, 2002).

In Volume 13 of this *Handbook*, Deutsch reviewed the signals that terminate meals, emphasizing what was known about CCK. Since then there has been an explosion of new information on other gut-derived signals that control food intake. Since 1991, leptin, melanin concentrating hormone, the orexins, ghrelin, cocaine-amphetamine related transcript (CART), AgRP, and many other important signals that influence food intake have all been discovered; in addition, at the time of the previous volume, compounds such as peptide YY, glucagon-like peptide-1, apolipoprotein A-IV, and amylin were relatively obscure and thought to have little relevance to behavior, and the importance of the arcuate nucleus and melanocortin receptors was not known; and finally, the application of transgenic technology to the study of behavior was in its infancy at that time. Hence, the dozen years since the previous edition of this *Handbook* was published have been very productive in this scientific field. While many of these exciting developments are covered in other chapters in this volume, many are directly relevant to this chapter.

Exogenous administration of peptides in the bombesin family reduces meal size (Gibbs, Fauser, Rowe, Rolls, & Maddison, 1979; Gibbs & Guss, 1995). Bombesin itself is an amphibian peptide, but gastrin releasing peptide (GRP) and neuromedin B (NMB) are mammalian analogs that reduce food intake when administered systemically to animals (Ladenheim, Wirth, & Moran, 1996) and humans (Muurahainen, Kissileff, & Pi-Sunyer, 1993). Both GRP and NMB and their respective receptors are synthesized in the mammalian brain (Ladenheim, Jensen, Mantey, & Moran, 1992; Minamino, Kangawa, & Matsuo, 1983), and central administration of either peptide reduces food intake (Ladenheim *et al.*, 1997). Consistent with the possibility that endogenous GRP and NMB reduce food intake, mice deficient for the GRP receptor do not suppress their food intake when administered either GRP or NMB, eat significantly large meals, and develop late-onset obesity (Ladenheim *et al.*, 2002).

Glucagon is a peptide hormone cleaved from a larger precursor molecule called preproglucagon. It is secreted from both the endocrine pancreas and the intestine, and its best-known action is to increase glucose secretion from the liver. Glucagon also reduces meal size when administered systemically (Geary, 1998; Salter, 1960) but not centrally (Woods, Lotter, McKay, & Porte, 1979), the signal being detected in the liver and relayed to the brain (Geary, Le Sauter, & Noh, 1993). A role for glucagon in the normal control of meal size was demonstrated by the observation that blocking the action of endogenous glucagon increases food intake (Langhans, Zieger, Scharrer, & Geary, 1982; Le Sauter, Noh, & Geary, 1991).

Glucagon-like peptide-1 (GLP-1), which also is cleaved from preproglucagon, is synthesized in the distal small intestine as well as in the brainstem and helps increase insulin secretion during meals. Intraventricular administration of GLP-1 reduces food intake in rats (Tang-Christensen *et al.*, 1996; Turton *et al.*, 1996) although the effect is nonspecific since it also elicits symptoms of malaise (Thiele *et al.*, 1997; van Dijk *et al.*, 1996; van Dijk, Thiele, Seeley, Woods, & Bernstein, 1997). It has recently been found that the receptors that trigger the satiety action of GLP-1 are located in the hypothalamus whereas those that mediate malaise are in the amygdala (Kinzig, D'Alessio, & Seeley, 2002). That same group further observed that GLP-1 mediates both the endocrine and the behavioral responses to stress in rats (Kinzig *et al.*, 2003), implying that the GLP-1 signal has complex actions only some of which are directly relevant to the control of food intake. Systemic administration of GLP-1 also causes hypophagia and weight loss in animals and humans (Larsen, Fledelius, Knudsen, & Tang-Christensen, 2001; Naslund *et al.*, 1999), and

administration of a selective antagonist to GLP-1 increases food intake and body weight (Meeran *et al.*, 1999), implying that endogenous GLP-1 normally helps reduce food intake. Glucagon-like peptide-2 (GLP-2), which is another cleavage product of preproglucagon in both the intestine and the brain, also decreases food intake (Tang-Christensen, Larsen, Thulesen, Romer, & Vrang, 2000).

Apolipoprotein A-IV (apo A-IV) is a large peptide synthesized by intestinal cells during the packaging of digested lipids into chylomicrons that enter the blood via the lymphatic system (Tso, Liu, Kalogeris, & Thomson, 2001). Apo A-IV also is synthesized in the arcuate nucleus (Liu *et al.*, 2001). Systemic or central administration of apo A-IV reduces food intake and body weight of rats (Fujimoto, Fukagawa, Sakata, & Tso, 1993; Fujimoto, Machidori *et al.*, 1993), and administration of apo A-IV antibodies has the opposite effect (Fujimoto, Fukagawa *et al.*, 1993). Because both intestinal and hypothalamic apo A-IV are regulated by absorption of lipid but not carbohydrate (Liu *et al.*, 2003), this peptide may be an important link between short- and long-term regulators of body fat (see review by Tso *et al.*, 2001). A second digestion-related peptide, enterostatin, also is closely tied to lipid digestion. When ingested fat enters the intestine, the exocrine pancreas secretes lipase and colipase to aid in the digestive process; enterostatin is a cleavage byproduct of the formation of colipase from pro-colipase. Administration of enterostatin either systemically (Okada, York, Bray, & Erlanson-Albertsson, 1991; Shargill, Tsuji, Bray, & Erlanson-Albertsson, 1991) or directly into the brain (Mei & Erlanson-Albertsson, 1992) reduces food intake, and when rats are given a choice, the reduction is specific for fats; that is, enterostatin does not decrease the intake of carbohydrates or proteins (Okada, York, Bray, Mei, & Erlanson-Albertsson, 1992). Therefore, two peptides that are secreted from the gut during the digestion and absorption of lipids, apo A-IV and enterostatin, act as signals that decrease food intake, and at least one of them selectively reduces the intake of fat. Macronutrient specificity has not been assessed with apo A-IV.

Peptide YY (PYY) is secreted from the same cells in the distal small intestine that secrete GLP-1. When administered into the brain of rats, PYY increases food intake (Corp, Melville, Greenberg, Gibbs, & Smith, 1990; Morley, Levine, Grace, & Kneip, 1985). There is one report that systemic PYY has the opposite effect and decreases food intake in rats and monkeys (Batterham *et al.*, 2002). The slight decrease of food intake has been replicated in monkeys but not in rats (Moran, Knipp, Smedh, & Ladenheim, 2003; Thöne-Reineke, Ortman, Castaneda, Birringer, & Tschöp, 2003). Systemic PYY passes through the blood-brain barrier (Nonaka, Shioda, Niehoff, & Banks, 2003) and reportedly elicits activation of neurons in the arcuate nucleus that synthesize proopiomelanocortin (POMC) (Batterham *et al.*, 2002).

Amylin, a peptide hormone secreted by pancreatic B cells in tandem with insulin secretion during meals, inhibits gastric emptying and gastric acid secretion (Ludvik, Kautzky-Willer, Prager, Thomaseth, & Pacini, 1997). Amylin causes a dose-dependent reduction of meal size when administered systemically (Chance, Balasubramaniam, Zhang, Wimalawansa, & Fischer, 1991; Lutz, Del Prete, & Scharrer, 1994; Lutz, Geary, Szabady, Del Prete, & Scharrer, 1995) or directly into the brain (Rushing, Hagan, Seeley, Lutz, & Woods, 2000). In contrast to the effects of several other satiety peptides that reduce food intake by sending a signal to the brain via the vagus nerves, amylin appears to stimulate neurons in the area postrema of the brain directly (Lutz, Althaus, Rossi, & Scharrer, 1998; Lutz, Del Prete, & Scharrer, 1995; Lutz, Senn *et al.*, 1998).

CCK, bombesin-related peptides, glucagon, GLP-1, apo A-IV, amylin, and other gastrointestinal signal-generating peptides are called satiety factors because their major action is to decrease meal size. An important question is whether the relative absence of these satiety factors has a role in meal initiation. If so, it would suggest that there is strong endogenous activity compelling animals to eat, and that signals generated by food consumption and processing provide multiple brakes that are activated during meals. As the strength of the braking signals wane during the intermeal interval, the omnipresent drive to eat gains predominance and meals are again initiated. If administration of satiety signals during an intermeal interval prolonged the time until the start of a subsequent meal, it would support this hypothesis. When administered to rats during the intermeal interval, CCK does not have this effect (Gibbs *et al.*, 1973; Miesner, Smith, Gibbs, & Tyrka, 1992). However, when administered after an initial meal has ended, both bombesin and GRP increase the latency until a second meal is initiated (Rushing & Gibbs, 1998; Rushing, Henderson, & Gibbs, 1998; Stuckey, Gibbs, & Smith, 1985; Thaw, Smith, & Gibbs, 1998). Since GRP increases during meals and decreases thereafter in mammals, it is possible that meal onset and offset are causally linked to its normal fluctuations.

An important question concerns how "satiety" signals generated during meals are integrated with other signals that control food intake. As implied from the list of gastrointestinal peptides that reduce food intake, and as discussed in detail below in the concluding section, the general model that is emerging is that different types of signals converge to affect meal size. That is, information related to body adiposity, rather than initiating or terminating meals directly, appears to act by changing the sensitivity of the brain to other signals that in turn terminate meals. Thus, when body fat becomes low, associated signals cause the brain to be less sensitive to CCK and other satiety signals, resulting in larger meals being consumed to help restore fat reserves; the opposite occurs when body fat becomes excessive (see Schwartz, Woods, Porte, Seeley, & Baskin, 2000; Woods, Schwartz, Baskin, & Seeley, 2000; Woods, Seeley, Porte, & Schwartz, 1998).

Signals Generated by the Metabolism of Substrates

Cells oxidize glucose and/or fatty acids to release and capture the chemical energy they contain. As oxygen combines with the substrates in the mitochondria (Figure 4), water and carbon dioxide are produced, and the substrate's energy is transferred into molecules such as adenosine triphosphate (ATP) that can be used by cellular processes. Any treatment that compromises the formation of ATP disables cells. When this occurs in either the brain or the liver, it might be expected to generate signals that lead to increased eating. In fact, this occurs. Most cells have complex means of maintaining adequate ATP generation because they are able to oxidize either glucose or fatty acids. Hence, when one or the other substrate becomes low, enzymatic changes occur to increase the ability of the cell to take up and oxidize the alternate fuel more rapidly.

Historically, several hypotheses have posited that cells in either the brain or the liver function as sensors of their own oxidation of substrates and thereby generate a signal that interacts with other controllers of energy homeostasis. Hence, prominent hypotheses have focused on the liver (Friedman, 1997, 1998; Langhans, 1996a; Russek, 1981), where there is compelling evidence that a close correlate of

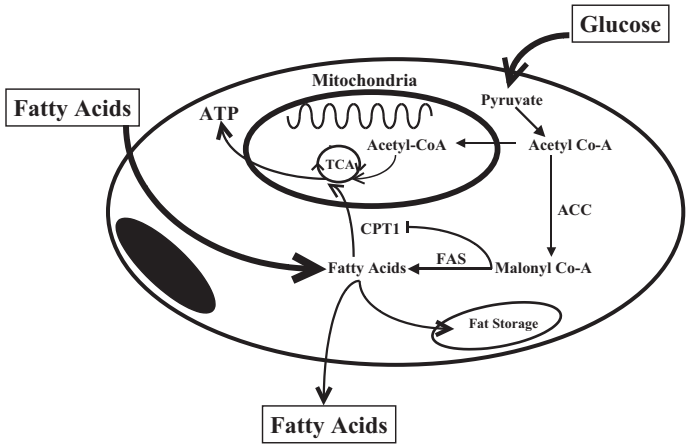

Figure 4. Model of generalized cell metabolism. As described in the text, most cells oxidize glucose when insulin is high and fatty acids when insulin is low. In both instances, energy is derived as the glucose or fatty acid molecule is converted to acetyl-co-A and enters the tricarboxylic acid (TCA) or Kreb's cycle where it is oxidized and ATP is generated. When glucose and insulin are abundant, many cells (e.g., liver cells) can convert glucose to fatty acids via acetyl-co-A and malonyl-co-A with the aid of the enzymes acetyl-co-carboxylase (ACC) and fatty acid synthase (FAS). The newly generated lipid can then be stored or released into the blood. Fatty acids are not oxidized because the elevated malonyl-co-A inhibits the enzyme CPT-1, which enables fatty acids to enter the mitochondria. When glucose and insulin are low, little glucose enters the cell and malonyl-co-A levels are low. Fatty acids consequently enter the TCA to provide energy. As described in the text, there is strong evidence that some brain cells function in the same way, and that food intake is reduced if either glucose or fatty acid levels are increased locally, or if FAS or CPT-1 are inhibited in the brain.

ATP production is signaled to the brain via the vagus nerves (Friedman, Koch, Graczyk-Milbrandt, Ulrich, & Osbakken, 2002; Ji, Graczyk-Milbrandt, & Friedman, 2000), or the brain (Nicolaidis, 1978; Nicolaidis & Even, 1984), where local stimulation causes coordinated changes in metabolism and feeding (Ruffin & Nicolaidis, 1999). It has generally been assumed that the brain is sensitive mainly to changes of carbohydrate oxidation. However, several recent observations have challenged this long-held belief and suggest that a population of brain cells is sensitive to local lipid metabolism as well as to carbohydrate metabolism and can generate powerful signals that integrate the two and influence food intake.

When energy is abundant, most cells in the body have the ability to synthesize fatty acids via the cellular enzyme, fatty acid synthase (FAS, see Figure 4). In recent years, this lipid-metabolizing pathway also has been described in hypothalamus (Kim *et al.*, 2002), and animals eat less food and lose weight when FAS activity is inhibited locally in the brain by the drug C75 (Clegg, Wortman, Benoit, McOsker, & Seeley, 2002; Kumar, Shimokawa, Nagy, & Lane, 2002; Loftus *et al.*, 2000). Over the course of a few days, they selectively lose body fat. A similar phenomenon occurs when C75 is administered systemically (Loftus *et al.*, 2000), but the reduction of food intake in that instance is confounded by a generalized malaise that is not apparent when the drug is given into the brain (Clegg *et al.*, 2002). An important implication is that at least some brain cells are able to oxidize fatty acids (Obici, Feng, Arduini, Conti, & Rossetti, 2003), and that a signal related to this metabolic process is tightly linked to the controls over energy homeostasis. Whereas C75 does cause changes in lipid metabolism, there is evidence that it elicits compensatory changes in local carbohydrate

metabolism as well (Wortman, Clegg, D'Alessio, Woods, & Seeley, 2003). Consistent with the principle that generalized energy sensors exist in the hypothalamus, increases of either carbohydrate or long-chain fatty acid availability locally in the arcuate nucleus reduce food intake and send signals to the liver to reduce the secretion of energy-rich fuels into the blood (Obici, Feng, Morgan *et al.*, 2002).

The concept that some brain neurons can utilize either glucose or lipids for energy and hence function as overall energy sensors is not new (e.g., see Langhans, 1996a; Nicolaidis, 1978). What is new is the application of highly selective molecular probes to assess the role of individual enzymatic steps in the metabolic cascade of brain cells that influence physiology and behavior, and the concept that a population of cells in the brain is sensitive to local manipulations of fatty acids and/or glucose. As originally reported by Oomura (Oomura, 1983; Oomura, Ono, Ooyama, & Wayner, 1969; Oomura, Ooyama, Sugimori, Nakamura, & Yamada, 1974), and consistent with glucose-sensing cells in other parts of the body, the electrical activity of these cells can be changed by local fluctuations of either glucose or insulin (Levin, 2002; Levin *et al.*, 1999). These cells also contain receptors and enzymes that are characteristic of glucose-sensing pancreatic B cells. Hence, like B cells, these cells can detect changes of glucose and generate signals that influence metabolism and behavior (Levin, 2002; Levin *et al.*, 1999). There also is evidence that the same or proximally close neurons contain receptors for leptin and insulin, signals that provide important information about ongoing metabolism (see below). The picture that is emerging is of a population of neurons that collectively sample different classes of energy-rich molecules (i.e., glucose and fatty acids) as well as hormones whose levels reflect metabolism throughout the body (i.e., insulin and leptin). The same neurons are also sensitive to the numerous neuropeptides known to be important regulators of energy homeostasis (see Chapter 6 by Seeley, in this volume).

Signals Regulating Body Fat. The above discussion has focused on metabolic signals important in the regulation of food intake. At a different level, food intake can be considered as one of several behaviors that maintain a precise amount of fat in the body. This section considers body fat as a homeostatically regulated parameter, with signals informing the brain as to the level of stored fat. The underlying premise is that the brain must have a means of detecting exactly how much fat is stored at any point of time. If all of the body fat was localized in a single discrete depot, then receptors attached to sensory nerves could simply assess the volume of that depot much like stomach or bladder volume is sensed by stretch receptors, and the information could be relayed to the nervous system. In fact, sensory nerves that originate in some fat depots have been described (Fishman & Dark, 1987), but their function is unknown. Body fat is dispersed throughout the body, and when total fat becomes greatly increased, fat becomes stored in other cell types such as liver and skeletal muscle (Shulman, 1999). While each fat deposit might in principle have its own volume receptors and attached axons to the CNS, such a network has not been described. Rather, a different signaling system has evolved in which the secretion of specific hormones is directly correlated with total stored fat. Hence, the brain need only have receptors for those hormonal signals to obtain a reliable estimate of body fat, a hypothesis initially proposed by Kennedy (Kennedy, 1953).

At least two hormones meet the criteria to be adiposity signals to the brain, insulin, and leptin. Insulin is secreted from B cells of the islets of Langerhans of the pancreas in response to circulating glucose, fatty acids, and amino acids. However, the amount of insulin secreted both in basal conditions and in response to

increased glucose is directly proportional to body fat, such that obese individuals have elevated basal and stimulated insulin compared to lean individuals (Bagdade, Bierman, & Porte, 1967; Polonsky, Given, & Carter, 1988). Leptin is secreted directly from white adipose cells in response to ongoing metabolic activity of the cell, the level of secretion being directly proportional to the amount of stored fat (Havel, 1999, 2001). Hence, any tissue with receptors for insulin and leptin will receive information as to the size of the fat reserves in the body.

Insulin and leptin share several features in common as adiposity signals (see reviews in Baskin, Figlewicz-Lattemann *et al.*, 1999; Niswender & Schwartz, 2003; Porte, Baskin, & Schwartz, 2002; Schwartz *et al.*, 2000; Woods *et al.*, 1998). Both are large peptides that cannot easily diffuse through the blood-brain barrier. However, both peptides are passed from the blood into the interstitial fluid of the brain by means of receptor-mediated transport through brain capillary endothelial cells (Banks & Kastin, 1998; Banks, Kastin, & Huang, 1996; Baura *et al.*, 1993, 1996; Schwartz *et al.*, 1991; Schwartz, Peskind *et al.*, 1996; Woods, Seeley, Baskin, & Schwartz, 2003). Receptors for both insulin and leptin are located on the luminal surface of brain capillaries, and when they interact with insulin or leptin, the molecules are taken into the cell, transported to the brain side of the cell, and released biologically intact. Receptors for both insulin and leptin also are located on neurons in the hypothalamus and elsewhere (Baskin, Hahn, & Schwartz, 1999; Baskin *et al.*, 1990; Baskin, Schwartz *et al.*, 1999; Corp *et al.*, 1986; Havrankova, Roth, & Browstein, 1978; Schwartz, Seeley, Campfield, Burn, & Baskin, 1996; Tartaglia, 1997), including the key neuronal populations in the arcuate nucleus that synthesize proopiomelanocortin (POMC) and NPY/AgRP (Baskin, Breininger, & Schwartz, 1999; Baskin, Sipols, Schwartz, & White, 1994; Benoit *et al.*, 2002; Schwartz, Seeley *et al.*, 1996). Finally, changes in the levels of either insulin or leptin within the vicinity of the ventral hypothalamus cause predictable changes of food intake and body weight. Increased insulin or leptin activity results in stimulation of POMC and inhibition of NPY/AgRP, decreased food intake, and increased energy expenditure and weight loss. Conversely, decreased insulin or leptin activity results in inhibition of POMC and stimulation of NPY/AgRP, increased food intake, and decreased energy expenditure and weight gain (see reviews in Baskin, Figlewicz-Lattemann *et al.*, 1999; Niswender & Schwartz, 2003; Schwartz *et al.*, 2000; Woods *et al.*, 1998). Details of this system are found in Chapter 6 by Seeley, in the volume.

The normal reliance of the control system for energy homeostasis on insulin and leptin is best demonstrated when their influence is removed. Decades before leptin was discovered, its existence was inferred from the hyperphagia and obesity that exist in animals unable to synthesize leptin or its receptor (Coleman, 1973, 1978). Similarly, a hallmark symptom of insulin deficiency is hyperphagia. Insulin-deficient individuals are lean rather than obese because a critical action of insulin is to enable the storage of lipid in fat cells. The hyperphagia associated with insulin deficiency is attenuated by the local administration of insulin (Sipols, Baskin, & Schwartz, 1995) or leptin (Sindelar *et al.*, 1999) in the vicinity of the arcuate nucleus, just as the hyperphagia of leptin-deficient animals is attenuated by the central administration of leptin (Campfield, Smith, Gulsez, Devos, & Burn, 1995). Conversely, the intracerebral administration of antibodies to insulin in normal rats causes increased food intake and body weight (McGowan, Andrews, & Grossman, 1992; Strubbe & Mein, 1977). Consistent with these observations, hyperphagia and obesity result from the selective knockout of either the leptin receptor (Cohen *et al.*, 2001) or the insulin receptor (Brüning *et al.*, 2000; Obici, Feng, Karkanias, Baskin, & Rossetti, 2002) in the brain.

While insulin and leptin share many properties as adiposity signals to the brain, there are important differences between them. Perhaps most importantly, their levels do not reflect the same adipose depots. Most leptin comes from subcutaneous fat, and its levels in the blood reflect subcutaneous fat better than total body fat (Dua *et al.*, 1996; Wajchenberg, 2000). In contrast, insulin is secreted in proportion to visceral fat, and plasma insulin is a better correlate of visceral than total body fat (Cigolini *et al.*, 1995; Wajchenberg, 2000). Hence, the two signals combined give a better picture of total fat than either signal alone. Because females have relatively more fat distributed in subcutaneous depots whereas males have more fat distributed in visceral depots (Bjorntorp, 1997; Despres, 1998; Wajchenberg, 2000), leptin is a better predictor of total body fat in females and insulin is a better predictor in males. Moreover, the brains of female rats are relatively more sensitive to low doses of leptin whereas the brains of male rats are relatively more sensitive to low doses of insulin (Clegg, Riedy, Smith, Benoit, & Woods, 2003).

While both insulin and leptin stimulate arcuate POMC neurons and inhibit arcuate NPY/AgRP neurons, they are not synergistic in this regard. Rather, when combinations of low doses of insulin and leptin are coinfused into the third ventricle of rats, the acute response is interference, with the net catabolic response being less than the sum of the two individual effects. Only after 4–6 hr does the combination become additive (Air, Benoit, Clegg, Seeley, & Woods, 2002). The interaction is interesting in light of the fact that leptin has a half-life of around 45 min in plasma (Ahren, Baldwin, & Havel, 2000) whereas insulin has a half-life of only 2–3 min (Bagdade *et al.*, 1967; Polonsky *et al.*, 1988). Hence, leptin represents a relatively stable indicator of activity in subcutaneous fat whereas insulin is a rapidly changing signal reflecting changes in recent carbohydrate metabolism.

Leptin and insulin, when they interact with their respective receptors, initiate unique chains of intracellular events that ultimately mediate their actions, and these pathways are the subject of intense investigation as potential therapeutic applications of insulin and leptin signaling are considered (Bates *et al.*, 2003; Kloek *et al.*, 2002; Niswender *et al.*, 2001; Niswender & Schwartz, 2003). An important recent observation is that the intracellular signaling cascades of leptin and insulin overlap, such that drugs that block the activity of phosphatidylinositol-3 kinase prevent either leptin or insulin from exerting its catabolic actions (Niswender *et al.*, 2003; Niswender *et al.*, 2001) and both leptin and insulin stimulate neuronal ATP-sensitive potassium channels (Spanswick, Smith, Groppi, Logan, & Ashford, 1997; Spanswick, Smith, Mirshamsi, Routh, & Ashford, 2000). Hence, there is a convergence of the signals for different adipose tissue stores within the brain. Consistent with a high degree of interaction of the insulin and leptin signals, rats with deficient leptin receptors (fatty Zucker rats) do not become hypophagic when insulin is administered into the brain (Ikeda *et al.*, 1986).

Conclusions

Adiposity signals such as insulin and leptin are integrated with satiety signals and with signals related to myriad other factors including learning, the social situation, and stress. The nature of these interactions is not well understood, but several generalizations can be made. The first is that the negative feedback circuits related to body fat, meal ingestion, and ongoing cellular metabolism can be overridden by situational events. For example, even though signals might indicate that

no more food should be eaten at some moment, the sight, smell, and perceived palatability of an offered dessert can stimulate further intake. Similarly, even though an individual is severely underweight and food is available, the influence of stressors can preclude significant ingestion. Because of such interactions, attempts to relate intake of an individual meal to recent energy expenditure or energy stores are futile, at least in the short term. Rather, the influence of homeostatic signals becomes apparent only when intake is considered over long intervals. For example, in rabbits, energy expenditure correlated better with weekly food intake than with intake after 1 or 3 days (Gasnier & Mayer, 1939). Likewise, if homeostatic signals predominated, a relatively large intake in one meal should be compensated by reduced intake in the subsequent meal. However, detailed analyses have revealed that such compensation, if it occurs at all, is apparent only when intervals of one or more days are considered, especially in humans with no constraints on their eating (Birch, Johnson, Andresen, Peters, & Schulte, 1991; de Castro, 1998).

A second generalization is that homeostatic regulation is most apparent on meal size rather than meal initiation or patterning. A large literature has documented that the specific meal pattern adopted by individuals is idiosyncratic and related more to environmental constraints than to metabolism or energy stores (Collier, 1986; Collier, Johnson, & Mitchell, 1999; Strubbe & Woods, 2004; Woods, 2002; Woods & Strubbe, 1994). Hence, individuals can be flexible, scheduling meals at times that food is available and obtainable while simultaneously

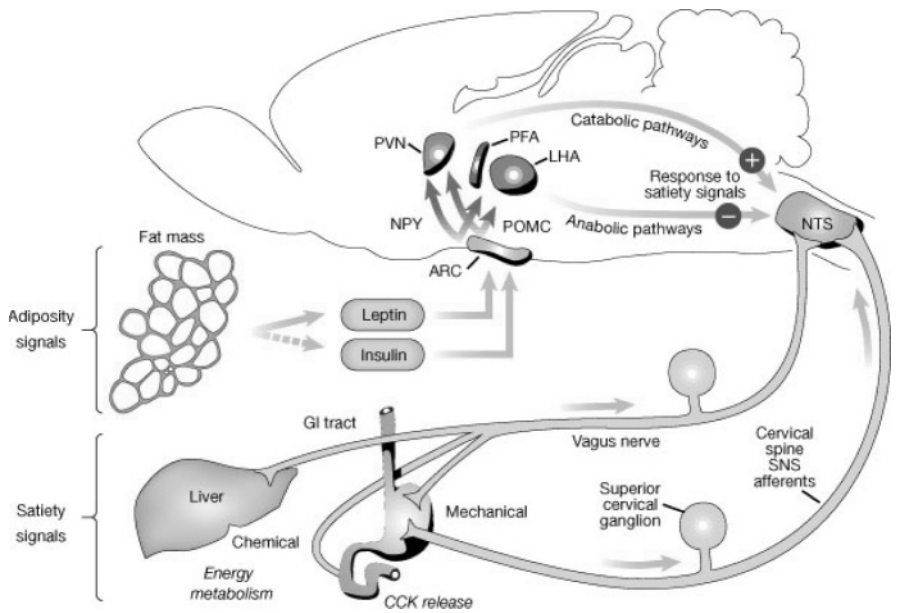

Figure 5. Model depicting several types of signals that influence food intake. Hormones whose levels are directly proportional to body fat (leptin and insulin) are called adiposity signals and interact with the brain at the hypothalamic arcuate nucleus (ARC). Satiety signals are generated in the gastrointestinal (GI) tract during meals and provide information about mechanical (e.g., stomach stretch, volume) and chemical properties of the food (as indicated by CCK release). The signals are conveyed via sensory axons in the vagus and sympathetic (SNS) nerves into the nucleus of the solitary tract (NTS) in the brainstem. Within the brain, neural circuits integrate information from the NTS and several hypothalamic nuclei (ARC, paraventricular [PVN], lateral hypothalamic [LHA], and perifornical [PFA]) to determine food intake and energy expenditure. (From Schwartz *et al.*, 2000.)

coordinating ingestion with other behaviors. Regulation occurs in the determination of how much food is eaten once a meal is underway.

A final generalization is that biological controls over ingestion act by changing the sensitivity to satiety signals. For example, the adiposity signals insulin and leptin change the sensitivity to CCK. Hence, when an animal has gained excess weight, more insulin and leptin stimulate the brain, and this in turn renders CCK more effective at reducing meal size (Barrachina, Martinez, Wang, Wei, & Tache, 1997; Figlewicz *et al.*, 1995; Matson, Reid, Cannon, & Ritter, 2000; Matson, Wiater, Kuijper, & Weigle, 1997; Riedy, Chavez, Figlewicz, & Woods, 1995). An increased insulin signal in the brain also renders rats more sensitive to the meal size-reducing action of amylin (Rushing, Lutz, Seeley, & Woods, 2000) and corticotropin releasing hormone (Richardson, Omachi, Kermani, & Woods, 2002). The site of integration of satiety peptides with other signals that influence meal size begins in vagal afferent fibers with gastric stretch and continues into the brainstem (Moran, Ladenheim, & Schwartz, 2001; Schwartz & Moran, 1996; Schwartz, Moran, White, & Ladenheim, 1997) where meal size is ultimately determined (Grill & Kaplan, 2002). At the same time, the arcuate nucleus receives signals related to adiposity as well as information about an ongoing meal from the brainstem, and it is able to monitor ongoing metabolism directly, providing it with the capacity to integrate multiple signals that determine ingestion (Ahima, Saper, Flier, & Elmquist, 2000; Cone *et al.*, 2001; Elmquist, Elias, & Saper, 1999; Schwartz *et al.*, 2000). Figure 5 depicts this overall control system schematically.

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11

Gastrointestinal Signaling in the Control of Food Intake

Joel M. Kaplan and Timothy H. Moran

Introduction

Gastrointestinal (GI) signals play a variety of roles in the control of ingestive behavior. In this chapter, we focus primarily on their role in feeding over the short term—mostly with respect to the impact of postingestive feedback on the progress and termination of an ongoing meal. The individual meal provides the clearest behavioral referents for the physiological significance of a variety of neural and hormonal signals that arise directly from the ingestion and digestion of food, and it probably is fair to say that the majority of research concerning gut-brain behavior linkages addresses the controls of intake within meals. We also discuss, to a lesser extent in keeping with the quantity of available evidence, the probable contributions of GI signals to feeding control over the intermediate and longer term. Ingested nutrients remain within the GI tract well after a meal has ended and may give rise to signals that influence the timing and/or size of the next meal. There now is evidence that signals arising from the gut not only serve to provide inhibitory feedback but also may provide a stimulus for meal initiation. Finally, GI signals interact with metabolic signals at multiple levels providing a role for gut in the control of long-term energy balance. In this chapter, we outline the basis for considering a role for various GI signals in the control of feeding, identify specific candidate signals and the mechanisms underlying their actions, and attempt to provide a framework for assessing their likely physiological significance.

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Neurobiology of Food and Fluid Intake, 2nd Ed., Volume 14 of *Handbook of Behavioral Neurobiology,* edited by Edward Stricker and Stephen Woods, Kluwer Academic / Plenum Publishers, New York, 2004.

The physiology of satiation is largely a story about the inhibition of behavior. This idea almost falls from the definition of the term, "satiation," as the process leading to the termination of a meal once it has begun. Nearly all of the GI signals recognized or proposed as physiological contributors to the short-term controls of food intake exert an inhibitory influence on ingestive behavior. The preponderance of inhibitory controls is evident in the anorexic responses observed when a neural or hormonal influence is stimulated (e.g., by gastric or intestinal nutrient infusion) or simulated (e.g., by exogenous administration of the agent in question). Although there has been broad consensus that most GI signals involved in feeding control are inhibitory, few issues about how such signals exert their effects and their relative contributions have escaped controversy. The broader field of GI physiology offers a wide range of signals stimulated by the ingestion of food, but many are not likely to affect feeding behavior, except under pathological conditions. More than a few signals, however, *are* no doubt important for satiation, but a considerable amount of experimental work is required for a satisfactory demonstration of a physiological role for even the most promising individual candidate.

In due course, we identify several GI signals believed to be important for intake control and discuss what is known about their overall contribution to satiety and their mechanisms of action. To provide appropriate functional guideposts for our treatment, we first devote considerable attention to behavioral analysis. Results from focused behavioral experiments offer salient clues about where to look for physiological relevant signals (i.e., the relative importance of feedback arising from the gastric and postgastric compartments) and about what kind of signals to look for (e.g., those driven by volume versus chemical properties of ingested food). More generally, we consider which aspects of the meal (its overall size, duration, or internal structure) are most directly under feedback regulatory control.

The Control of Meal Size

The case for the relevance of a given physiological parameter for ingestion control often begins with the demonstration that a targeted treatment (e.g., nutrient delivery to a given site within the GI tract, or exogenous administration of a putative satiety hormone or hormone receptor antagonist) systematically affects the amount consumed during short-term tests or during an individual meal. An effect on meal size has been proposed by Smith (1996) to be an obligate signature of a true satiety signal. But meal size is a summary parameter, offering no insight into the behavioral evolution of the meal (Davis, 1998). So it may be best if the behavior can be measured within the meal at high temporal resolution. Indeed, important insights into the controls of intake have come from detailed analysis, in the rat, of the sequence of ingestive movements during nutritive fluid intake (Stellar & Hill, 1952; Davis & Levine, 1977). At the most fine-grain levels of analysis are the relatively fixed inter-lick interval (Halpern, 1977), and the more variable but informative distributions of licking bursts and inter-burst intervals (Davis, 1996; Davis & Perez, 1993; Spector, Klumpp, & Kaplan, 1998). The most common representations, however, are minute-by-minute records of the rate of ingestion (in licks or milliliters per minute). The shape of the curve illustrating changes in lick rates over time reflects two basic processes that codetermine how much will be consumed during the meal. The animal's evaluation of taste input is reflected most clearly in the lick rate at the beginning of the meal, before significant postingestive

accumulation. Responses to treatments that alter GI signals or their neural interpretation are expressed as changes in the rate of decline of licking rate over the course of the meal. In general, analysis of the pattern of behavior within a meal provides clues about the operating characteristics of the neural mechanism controlling the production of the ingestive movement pattern, and a framework for evaluating the selectivity of treatment effects on postingestive feedback inhibition. Such analysis also conveys the sense that the ultimate size of the meal can be anticipated by following the trajectory of rate curves collected under controlled laboratory conditions. However, ingestion rate analysis does not predict the point at which intake will terminate and thus does not provide information about the final meal-size.

Clinicians interested in therapeutic approaches to obesity also have focused on the microstructure of ingestive behavior, in a way that raises basic questions about what signals bring about the end of the meal. The guiding idea is that with a lowering of the rate of ingestion—by taking smaller bites, chewing more, and pausing more often during the meal—people are more likely to leave the table having ingested smaller meals. This recommended strategy, formally proposed by Ferster, Nurnberger, and Levitt (1962), was based on two assertions: that overweight individuals ingest at a higher rate than do their leaner counterparts, and that the prospects for a "normalization" of meal size would be improved if ingestion rate were treated as the target behavior in behavior-modification approaches to the obesity problem. It is fair to say that the eat-slowly practice is widely recommended, both in the popular press and by clinical specialists trying to help their patients lose weight (Jordan & Levitz, 1975; Stuart & Davis, 1972; see, e.g., Brownell, 1990; Brody 2003 [*New York Times* 8/19, 2003 Section F, p. 7]). Upon critical evaluation, however, the validity of the assumptions involved, as well as the clinical utility of the approach, are open to question. At one level, it is an arithmetic fact that meal size will be lowered in direct proportion to a reduction in ingestion rate—provided that the duration of the meal stays the same. The question is whether one is fully sated after the smaller meal, or whether in order to eat less, one must leave the table still hungry but "on time," as an act of will. At issue, then, is whether meal termination is indeed precipitated largely by the passage of time from meal onset, such that the same sense of fullness can be reached with less food ingested more slowly, or alternatively, whether in fact satiety depends primarily on just how much of a given food is consumed.

Fully apart from its clinical interest, the question of whether meal termination depends critically on the duration of feeding, or on an accurate tracking of the amount cumulatively ingested, is of more than passing interest for our discussion of basic physiological mechanisms underlying satiation and meal-size control. If the duration of feeding were the critical variable, it would establish a set of considerations and constraints for bench scientists concerned with the behavioral relevance of their physiological parameters. For one thing, static correlates of cumulative intake (e.g., a report of the volume-based distention of the stomach) would not be sufficient to explain systematic variations in meal size related to ingestion rate adjustments. Perhaps an additional, integrative process would be required to modulate the impact of such signals in relation to some representation of time elapsed during the meal. A simpler solution might be found by ignoring many candidate signals (e.g., again, feedback about stomach distention), and looking for those that first appear in appreciable magnitude only during later phases of the meal. Attention would be focused, then, on the leading edge of the digestive progression or, at least, on postgastric mechanisms engaged once food leaves the passive gastric

reservoir. The competing perspective—that meals end under normal conditions simply as a function of the amount consumed—also presents challenges for physiological analysis. This is because there is a temporal dynamic to virtually all biological measurements; from the shape of the curve describing the meal-related increase in blood levels of a given gut hormone, to the firing properties of even slowly adapting afferent nerves sensitive to gastric distention. How, then, would the intake control system derive an accurate representation of cumulative intake? For an indication of which set of problems is most deserving of attention, we require an empirical resolution of the fundamental tension between time- and intake-volume-based models of satiation.

We review studies with human subjects and with rats that aimed to explicitly test the therapists' hypothesis linking ingestion rate and meal size. The judgment in both cases was negative. In the human-subject experiment of Terry Spiegel and colleagues (Spiegel, Kaplan, Tomassini, & Stellar, 1993), lean and obese women came for a "laboratory lunch" on several occasions, during which they ate as much or as little as they liked while the experimenter in another room monitored cumulative intake and the pattern of biting and chewing movements. The food in the experiment was always the subject's preference between tuna and turkey spread, on white bread rolled sushi-style and cut into pieces. The independent variable of the experiment was simply the size of the sandwich piece (5, 10, or 15 g), varied across test-meals, with the only other constraint being an instruction to eat whole pieces, one at a time. The study focused on ingestion rate, meal size, and meal duration, but the subjects were told that the experiment was about details of the chewing pattern (measured by EMG recording from the masseter muscle). At one level of analysis, the behavior therapists' advice to take smaller bites proved quite effective, in this situation, at lowering the rate of ingestion. Ingestion rate (g/min) was markedly lower early in the meal with the smaller pieces, and although the ingestion rate curves for the respective conditions tended toward convergence as meals progressed, meal-average ingestion rates varied directly and significantly with bite size. The main question, of course, was the effect of bite size on meal duration and the amount consumed. The result was clear: meal size was defended across conditions. Subjects ingested three times as many of the 5-g than 15-g pieces, compensating for the lower ingestion rates with measured increases in meal duration. Incidentally, there were no differences between lean and obese groups with respect to basal rates of ingestion, to the effect of bite size on ingestion rate, and to the extent and accuracy of their compensatory responses.

Physiological interpretations of human feeding behavior, particularly when the observations are laboratory-based as in the previous example, must often be qualified in light of potent cognitive, experiential, and social influences that may be in play. Such factors may be less important in animal studies. Environmental contingencies and the animal's history with the testing situation and test food generally are well controlled and the rat is not likely to be wondering about who is watching it eat or what the experiment is *really* about. The case for the general behavioral and physiological relevance of human feeding results, then, is strongest when similar effects are observed with analogous treatments delivered to animals. In this way, the conclusions from Spiegel's study are reinforced by recent observations of licking behavior in the rat (Kaplan, Baird, & Grill, 2001; Kaplan, Donahey, Baird, Simansky, & Grill, 1996). Where Spiegel *et al.* (1993) varied bite size across test-meals in humans, varied in the rat experiments was the size of the aliquot of sugar solution the rat acquired with each spout-lick. The rules of the respective studies were the

same, and the same ideas were at risk. Here, both meal size and the number of licks could vary, but it was not possible for the rat to defend both parameters simultaneously; and if lowering the drop size caused reductions in the average rate of ingestion (ml/min) during the meal, then meal size would have been proportionally lower if there were no compensatory increases in meal duration. The rats responded in the same way as the humans: meal size remained stable across conditions, with the exception of a modest decrease at the lowest drop size tested (i.e., 1 μl, which is about an eighth of the typical lick volume during unconstrained licking [Halpern, 1977; Stellar & Hill, 1952]). The conclusion that meal size was actively defended, as a goal-directed behavioral process, is supported by the systematic increase in the number of licks emitted during the meal (e.g., a doubling of licks, compensating for a halving of drop size). In some cases, rats managed to fit their extra licks into meals that were not particularly extended in duration. In others, a reduction in drop size induced a very prominent decrease in the average rate of ingestion during the meal (Figure 1).

In these experiments, accurate monitoring of cumulative intake apparently held primacy over behavioral parameters relating to the amount of behavior during the meal (i.e., number of bites or licks, and, we can infer [Kaplan & Grill, 1988; Weijnen, Wouters, & van Hest, 1984], swallows), and to how long the feeding bout was sustained. Meal termination also could not be anticipated by following the trajectory of the ingestion rate curve (Figure 1). Even though in both the rat and human experiments, ingestion rate did decline gradually (cf. Johnson, 1996; Rushing, Houpt, Henderson, & Gibbs, 1997), with a slope negatively correlated with the rate at which the cumulative intake grew, there was no "stopping rule" that could be inferred on the basis of the behavior itself. Terminal rates of ingestion were distinctly non-zero (see also McCleer, 1977; Skinner, 1932), and there was no characteristic terminal ingestion rate (in volume or movement terms), or percent decline in ingestion rate from the beginning of the meal, which reliably signaled the imminent ending of the meal. Despite the utility of ingestive microstructure analysis, the results described favor a top-down view of the relationship between meal size and the behavioral pattern that brings it about. The behavior demonstrates flexibility, in measured adaptation to the constraints imposed in these experiments, and in apparent service of what can be portrayed as a meal-size goal.

Other studies, in which different behavioral challenges were employed (e.g., fixed-ratio bar-press requirements [Collier, Johnson, Hill, & Kaufman, 1986] and imposed pauses varying in number, temporal patterning and duration [Mook & Dreiffuss, 1986; Seeley, Kaplan, & Grill, 1993]) have yielded results largely consistent with the principle of meal-size defense. There are, to be sure, conditions across which meal-size defense breaks down. We noted, for example, the 1-μl/lick condition of the rat experiment, where intake was lower than that observed in tests with higher lick volumes. Lucas and Timberlake (1988) observed stable intakes for pelleted meals presented with inter-pellet delays between 16 and 128 s but larger meals were taken in the unconstrained condition. Additional work is required to both expand and to qualify our sense of the range of testing conditions under which the principle of meal-size defense applies. The important point here is that it does apply, systematically, for different species, different paradigms, and different nutritive stimuli commonly tested in laboratory studies of the behavioral and physiological underpinnings of satiation. The principle does not specify the process by which the meal-size "target" is set. This involves the integration of such disparate influences as gustatory evaluation and the satiating potency of the given test food

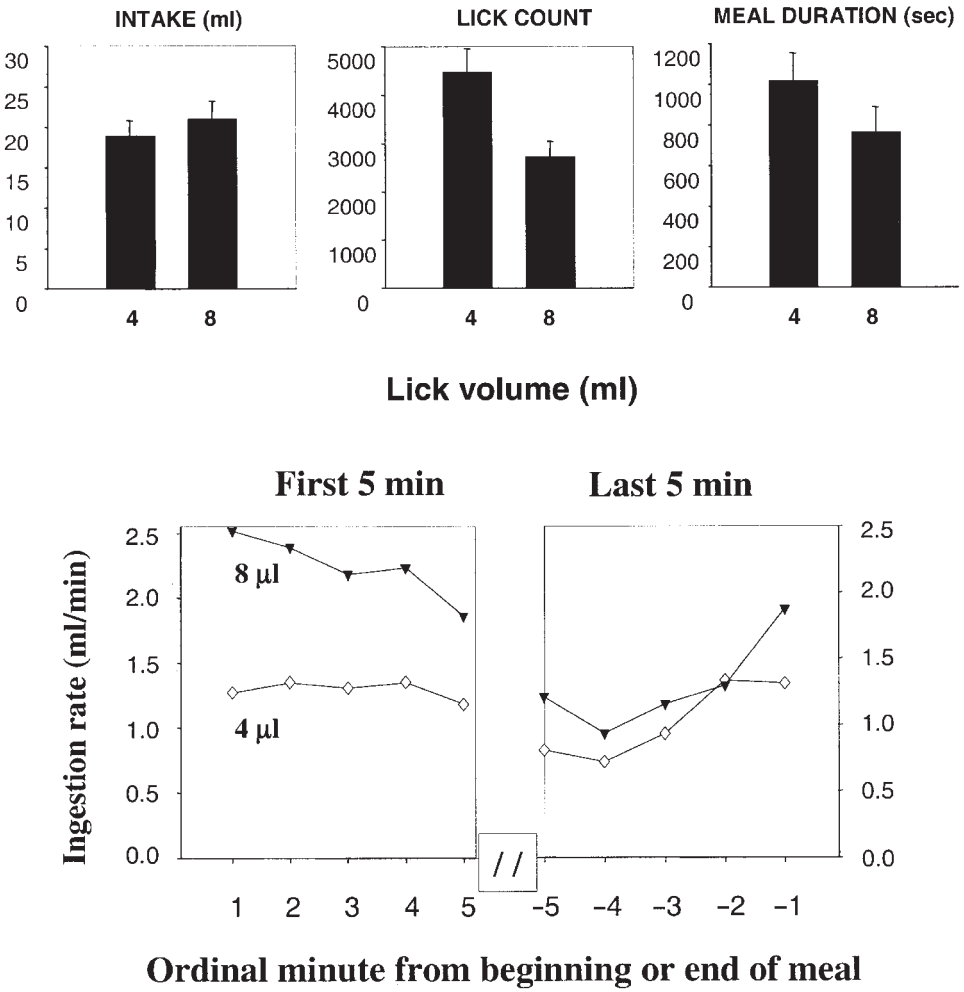

Figure 1. Behavioral compensation for lick-volume adjustment in rats ingesting 12.5% maltodextrin (adapted from Kaplan, Baird, & Grill, 2001). Rats defended meal size (top left) against a halving of lick volume by approximately doubling the number of licks they emitted (top middle) during meals that were significantly prolonged in duration (top right). The left portion of the bottom graph shows the effect of lick volume on ingestion rate over the first 5 min of the test meals. Simple group curves of ingestion rate over time (not shown) give the impression that the rate gradually descends to 0. Alignment of the curves retrospectively from the end of the meals (bottom right), however, reveals substantial terminal ingestion rates, and little to support the idea that meal termination can be anticipated by following the "behavioral trajectory" of the meal. Rather, intake defense, and meal-size control more generally, must reflect accurate GI feedback monitoring of the cumulative postingestive load.

(i.e., the "direct controls" of meal size), and the full range of "indirect controls" (Smith, 1996) that are largely beyond the scope of this chapter. It is the process by which the target is met that refocuses attention on sources of postingestive feedback. The material just reviewed takes our discussion beyond GI signals as generalized inhibitory influences on ingestive behavior and meal-size, to the question of how feedback signals from disparate sources are integrated to provide information correlated precisely with the amount of food consumed. That part of our review begins now with a characterization of the postingestive stimulus distribution.

During a meal, ingested nutrients come into contact with multiple sites of potential signal generation—signals that can serve as feedback mediators influencing the size of the meal. Ingested substances contact taste receptors in the mouth allowing an assessment of the appropriateness or the acceptability of the food. Ingested nutrients accumulate within the stomach, activating gastric mechanoreceptors and resulting in gastric relaxation. During a meal, some ingested nutrients pass from the stomach and contact intestinal mechanoreceptive and chemoreceptive elements, which results in local peptide release and the activation of neural fibers producing reflex alterations in GI motor and secretory activity. Since meals end when the majority of the ingested nutrients still are in the stomach and upper intestine and not yet available to the body as metabolic fuels, oral, gastric, and intestinal sources of feedback during ingestion may be critical in determining meal size.

The GI distribution of ingested nutrients during a meal specifies the range of potential signaling sites that could be important in controlling meal termination. A major factor in determining this distribution is the rate at which the stomach delivers its contents to the upper intestine. Traditionally, controls of liquid gastric emptying have been studied in situations in which gastric loads have been administered as boluses (Hunt & Stubbs, 1975; McHugh & Moran, 1979). Emptying of a nutrient bolus is characterized by an initial rapid phase followed by a slower linear phase that delivers gastric contents to the duodenum at a constant caloric rate. Emptying during the initial phase is dependent primarily on the initial gastric volume (Moran, Wirth, Schwartz, & McHugh, 1999). In contrast, emptying during the slower linear phase is dependent on intestinal feedback and relatively independent of gastric volume (McHugh, Moran, & Wirth, 1982; Moran *et al.*, 1999).

Although the linear phase described above may characterize nutrient gastric emptying during the post-meal period, examinations of the dynamics of bolus emptying do not adequately capture the controls of gastric emptying, or the GI nutrient distribution, during the ongoing meal. In more recent studies in both rat and nonhuman primate, the controls of fluid emptying have been examined in situations in which nutrient loads are delivered to the stomach at rates similar to those at which these same fluids normally would be consumed. In the rat, emptying during the period of gastric fill is characterized by much more rapid rates of gastric emptying than those evident following the filling period. The rapid rate of emptying remains constant throughout the duration of the filling period and, for glucose, the rate of emptying is relatively independent of the caloric concentration and of duodenal feedback. Thus, a constant proportion of the infused volume is continually being emptied throughout the filling period. In the rat, this amounts to approximately one third of the infused gastric volume (Kaplan, Siemers, Smedh, Schwartz, & Grill, 1997; Kaplan, Spector, & Grill, 1992). For glucose, this constant ratio of gastric emptying to gastric filling is obtained whether the volume is gastrically infused or voluntarily consumed (Kaplan, Siemers, & Grill, 1997). Although the dynamics of the gastric emptying of corn oil are similar to those of glucose during intragastric infusions, the emptying patterns obtained during voluntary consumption reflect different controls. In this case, significantly less emptying occurred and the amount emptied was independent of changes in consumption duration or consumption rate (Kaplan *et al.*, 1997). The basis for this nutrient-related difference in the controls of gastric emptying during ingestion has not yet been identified.

This pattern of more rapid emptying during a meal than in the period following a meal also was found in a situation in which emptying was monitored across multiple meals in free-feeding rats (van der Velde, Koslowsky, & Koopmans, 1999). In rats consuming technesium-labeled, Ensure liquid diet, emptying rate was fivefold faster during a meal than in the post-meal period, and accounted for more than 25% of the ingested kilocalories. The same rapid rate of emptying was found across successive meals even though the stomach was not empty when later meals were initiated.

A number of the controls underlying rates of emptying during fill in the rat also hold in the rhesus monkey (Moran, Knipp, & Schwartz, 1999). Nutrient emptying is more rapid during than after fill, the rapid rate of emptying during fill remains constant for the duration of the filling period and the rapid rate of emptying ceases abruptly upon infusion offset (Figure 2). There do appear to be some difference between rat and primate in the relative sensitivity to duodenal feedback during fill and the concentration range over which such influence is most strongly expressed. The important common point is that during ingestion a significant proportion of the consumed calories passes from the stomach to contact intestinal sites. Thus, during meals there is substantial concurrent stimulation of both gastric and postgastric sites.

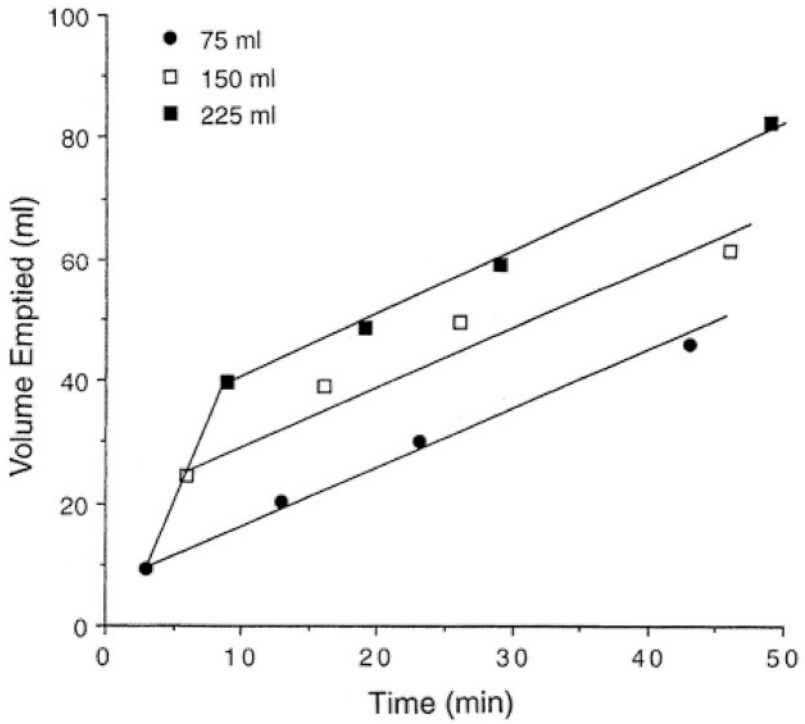

Figure 2. Gastric emptying of glucose test meals in the rhesus monkey during and following the intragastric infusion of glucose delivered at a rate of 25 ml/min (from Moran, Knipp, & Schwartz, 1999). Emptying during fill is more rapid than in the postfill period as indicated by the volumes emptied at infusion offset for volumes of 75, 150, and 225 ml. The linear slope between these points indicates that the same rapid rate of emptying continues for as long as the infusion is sustained. Emptying slows in the postfill period and the rate of emptying during that period is independent of gastric volume, as indicated by the parallel slopes.

The analysis of gastric emptying provides a characterization of the gastric and postgastric distribution of the nutrient load, but does not speak directly to the relative contribution of signals arising from the respective compartments to the termination of an ongoing meal. Although gastric and intestinal sites are stimulated concurrently during feeding, a cogent argument for the primacy of gastric feedback in the satiation process was developed by Deutsch and colleagues (Deutsch, 1983; Deutsch, 1990; Deutsch & Gonzalez, 1980; Deutsch, Young, & Kalogeris 1978). The two general conclusions derived from their studies—that gastric feedback alone determines how much will be ingested and that the critical signals are chemosensory in nature—were both provocative insofar as postgastric mechanisms were taken entirely out of the equation. By disallowing any direct postgastric contribution to the termination of the ongoing meal, Deutsch (1990) discounted as "non-physiological" and/or aversive a large number of results showing intake suppression in response to intestinal nutrient infusions [or to systemic delivery of putative intestinal hormonal mediators such as cholecystokinin (CCK)]. Along with experiments in which large amounts of nutrient had been delivered, the criticism also blankets a number of other studies (e.g., Reidelberger, Kalogeris, Leung, & Mendel, 1983; Yox & Ritter, 1988) in which graded intake suppression was obtained with variation of infusion parameters arguably within or very near the physiological range. The gastric model stimulated much constructive debate over a two-decade period before yielding to studies challenging each of its principal contentions. A consideration of Deutsch's original evidence along with the more recent studies will lead to our specific conclusions about the kinds of feedback derived from gastric and postgastric sources and the manner by which the respective influences are integrated in the satiation process.

Deutsch's group replicated and elaborated upon an earlier study reported by Campbell and Davis (1974) showing that when the gastric contents are removed after the rat finishes a meal of liquid diet, the rat promptly returns to the drinking spout and consumes an additional amount that matches, with impressive precision, the amount withdrawn (Deutsch & Gonzalez, 1980; see also Figure 3). The same result was obtained when the pylorus was occluded during testing, indicating that the compensatory response could be supported exclusively by feedback from the stomach. It was further observed that when an amount withdrawn from the stomach was replaced with an equal volume of water, rats still went back to the spout and compensated accurately for the evacuated nutrient. (The exception to this general result was the under-compensation for withdrawn nutrient in rats with the largest intakes. Their "early satiety" was taken to be a protective response to an excessive gastric volume [Deutsch, Gonzalez, & Young, 1980; see also Davis, Smith, & Sayler, 1997, 1998 for a more comprehensive analysis].) With accurate compensation, net nutrient intake was the same under the "simple" withdrawal condition as under the withdrawal + water replacement condition, despite the fact that the gastric volume at the end of the respective test meals would have been different (i.e., much larger for the water replacement condition). The finding suggested that the signals that allow accurate compensation for withdrawn nutrient are derived specifically from the chemosensory properties of gastric chyme.

Phillips and Powley (1996) were concerned that the pyloric cuff in these earlier experiments may have been positioned too caudally so that feedback from the upper duodenal, rather than gastric, sensors may have governed the behavioral response. This was apparently the case as Philips and Powley (1996) failed to

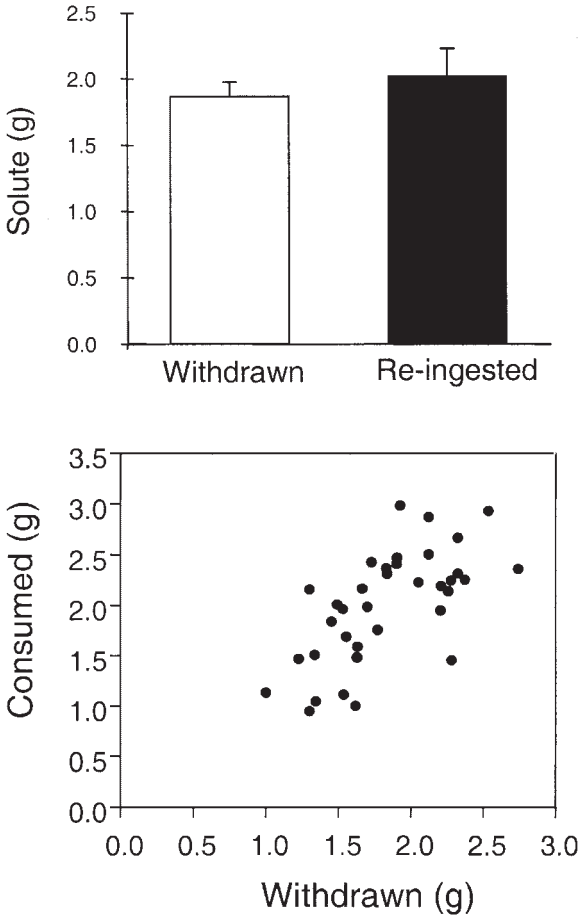

Figure 3. Representative behavioral outcome of the gastric withdrawal paradigm, where after an initial intake test, the gastric contents are removed and the rat is given an additional opportunity to ingest (from Kaplan , Siemers, & Grill, 1994). Rats ingested an amount (12.5% glucose, in this case) that accurately matched that withdrawn from the stomach. The top graph shows mean withdrawal and "reingestion" values (grams solute); the bottom graph shows individual-subject values for nine rats each tested on four separate occasions. It is argued (see text) that, because of significant gastric emptying during the reingestion period, the compensatory response reflects accurate tracking of nutrient within and beyond the stomach.

replicate the original result. With true pyloric occlusion, they found no difference in the rat's ingestive behavior between water and nutrient load conditions; intake was suppressed in each case as a graded function of the volume, not the concentration, of the fluid delivered to the stomach.

These results of Philips and Powley reinforce the consensus that the postgastric contribution to meal-size control under normal conditions is derived from the chemical properties of food that leaves the stomach. There was room, however, for different ideas about how this information is reckoned by the satiation mechanism. According to one view (McHugh & Moran, 1985), the role of postgastric feedback may be limited to the identification of what is being ingested and of the nutrient concentration of intestinal chyme. This information, then, could be used to set a gastric volume threshold that when reached would cause the animal to stop

eating. This view would permit a modified version of Deutsch's gastric model, where the stomach remains the primary source of feedback about how much (now in volume terms) has been ingested. A competing idea holds that the intestine does more than just "taste" its luminal contents; that postgastric feedback also provides accurate information about how much nutrient has emptied from the stomach. Here, both gastric and postgastric feedback signals would be seen as essential for accurate monitoring of the total postingestive load under normal conditions.

Almost ironically, we arrive at the latter view by returning to the basic results reported by Deutsch, Young, and Kalogeris (1978) and Deutsch *et al.* (1980) (see also Kraly & Smith, 1978; Seeley, Kaplan, & Grill, 1995). Pyloric occlusion yields a result that might seem surprising. Restriction of gastric emptying, whether achieved by a tightening of a pyloric noose or inflation of a pyloric cuff, yields intake values that are no different from those obtained with gastric emptying proceeding normally. The "no-effect" of pyloric occlusion is an important result that has been replicated with rats ingesting a variety of nutritive fluids (e.g., amino acids, oils, and carbohydrate solutions; Rauhofer, Smith, & Gibbs, 1993). It is easy to see how this general finding would be taken as strong support of the primacy of gastric feedback. The outcome of the pyloric occlusion experiment, however, is ultimately damaging even to modified forms of the gastric model.

On logical grounds, the claim that gastric feedback can account for the termination of a normal meal depends critically on how much of the amount ingested remains within the stomach and how much empties. The pyloric occlusion experiment supports the gastric model for normal meal-size control if, and only if, gastric emptying during ingestion is quite slow. If little nutrient empties during the meal, then amounts remaining in the stomach at the end of the meal in the normal and pylorus-occluded cases would hardly differ. We reviewed evidence, however, showing that the emptying rate throughout the meal is considerably higher than had been previously estimated. This cannot be reconciled with the gastric model as applied to the interpretation of the pyloric occlusion experiments. With equivalent intakes but rapid gastric emptying in the pylorus-open condition (i.e., the normal condition of greatest interest), considerably less should be found in the stomach at meal's end than when the entire load is retained in the stomach. This expectation was confirmed by Seeley *et al.* (1995) in rats bearing inflatable pyloric cuffs, and gastric fistula to permit evaluation of the stomach contents after the intake test. Thus, even though the same amounts were consumed with the pylorus occluded or open, the gastric volumes at which ingestion stopped were quite different.

The gastric withdrawal paradigm, specifically the version in which gastric emptying is not restrained by the experimenter (i.e., no pyloric occlusion), provides an assessment of the ingestive effects of the postgastric biasing of the postingestive distribution (Kaplan, Siemers, & Grill, 1994). When glucose solution was ingested, a portion (about 25% in this study) of the amount ingested emptied from the stomach during the initial meal. A significant additional amount emptied when the rat took the opportunity to ingest again after the gastric contents had been removed. This finding becomes interesting when viewed in relation to the principal behavioral result: as in earlier experiments, the rats "reingested" an amount equivalent to that withdrawn after the initial test (Figure 3). Therefore, the rat appears to defend (net) intake against a postgastric biasing of the nutrient distribution.

These reevaluations of the pyloric occlusion and gastric withdrawal paradigms, in which explicit measurements of the gastric/postgastric nutrient distributions

were taken at the end of the normal baseline and respective treatment conditions, contradict any version of the gastric model. If there were a gastric volume threshold that precipitates meal termination, then it should be reached with lower than normal meal sizes when the pylorus is occluded, and greater than normal intakes would be required to reach threshold under the gastric withdrawal condition. These predictions were countered by the classic demonstrations that net intake does not vary across the conditions of interest.

Two general conclusions can now be drawn. First, it is clear that the amount that empties during ingestion is indeed reckoned in the animal's decision to terminate its meal. As discussed above, intestinal infusion studies supporting postgastric contributions to normal meal-size control have been criticized because of the treatment's potential aversiveness. The studies just reviewed, however, are not subject to this criticism because in them, intestinal nutrient was delivered via the normal gastric emptying process. Even for the withdrawal experiment, where more than the normal amount is emptied before intake is terminated, the aversion argument cannot be invoked because intake was not at all suppressed relative to baseline values. The second conclusion goes further in specifying the manner by which gastric and postgastric signals are combined as the animal accurately tracks its cumulative intake during the meal. That intake is "defended" despite substantial biases in the postingestive stimulus distribution—that is, with the postgastric bias of the withdrawal/reingestion condition and the extreme gastric bias with pyloric occlusion—is consistent with the hypothesis that intake-inhibitory feedback signals derived from respective gastric and postgastric sources are summated in the satiation process. For the occlusion condition, the absence of the normal degree of intake inhibition arising from postgastric sources is precisely compensated for by the increased inhibition driven by the overfilled stomach. For the withdrawal condition, conversely, the less than normal gastric contribution is offset by the greater postgastric inhibition corresponding to the increased portion of the meal delivered to the intestine. The cumulative intake appears to be tracked via the simple addition of respective signals that accurately correspond to the amount of food within and beyond the stomach.

In summary, meal termination reflects the summation of feedback signals that provide accurate information about the amount of food within and beyond the stomach. The anatomically separated sensors that concurrently contribute to postingestive inhibition of feeding behavior present a spatial integration problem for neural analysis. In addition, insofar as the gastric feedback is volume/distention-based and the postgastric contribution is stimulated by both the volume and the chemical properties of food leaving the stomach, a comprehensive neural model of meal-size control must also address the interesting problem of multimodal signal integration. The physiological mediation of gastric and postgastric intake-inhibitory feedback, as well as possible sites and mechanisms of signal convergence and integration, are discussed in the following sections.

Within-Meal Gut Peptide Signaling

Peptides released from GI sites in response to the intraluminal presence or absorption of nutrients can act either as blood-borne signals or locally to stimulate GI neural elements that provide feedback information relevant to meal termination. Gibbs, Young, and Smith (1973a), in their initial work demonstrating a feeding

inhibitory action of the gut peptide CCK, suggested the following criteria for assessing whether a gut peptide was acting as a satiety signal.

1. The peptide must be released during the ingestion of food.
2. Administration of the peptide prior to the meal should decrease meal size, and the inhibitory effect should be dose related.
3. The peptide should have a relatively rapid onset and brief duration of action to account for the termination of one meal but allow the occurrence of the subsequent meal.
4. The inhibitory effect of the peptide should not be due to illness.
5. The inhibitory effect of the peptide should be obtained with doses of the peptide that produce circulating concentrations within the range of those observed during or following a meal.

These criteria provide a framework for evaluating the physiological relevance of candidate gut peptides as satiety signals. We would like to modify this list, at least for the purposes of this discussion, in three ways. First, we would eliminate criterion #5. Behind this criterion is the assumption that the candidate peptide must have a hormonal mode of action according to the classical definition. It is now clear, however, that satiety relevant peptides can interact with local neural elements within the GI tract, through what are more appropriately described as neurocrine or paracrine mechanisms. This distinction is relevant here because plasma concentration may not reflect the local concentration at the critical site of action.

Second, we would add one criterion: "Administration of a receptor antagonist (or a specific antibody in the case of a peptide with a hormonal mode of action) should result in increased meal size." This criterion speaks to the issue of receptor specificity and, most importantly, to the functional relevance of the endogenous peptide's action for limiting meal size. That is, does meal size increase when the actions of the endogenous peptide are blocked?

Finally, there should be some sense of how the peptide or the peptide-induced signal reaches the brain. We would not elevate the latter to a formal criterion but such information is useful in considering the diversity of direct and indirect mediating mechanisms, and for establishing strategies for further evaluating the physiological relevance of a given peptide's action.

We begin our discussion with CCK, the gut peptide for which a physiological role in limiting meal size has been demonstrated. As noted, the exogenous administration of CCK was first shown to inhibit food intake in rats by Gibbs, Young, and Smith (1973a). The effect was shown to be dose related, of relatively short duration, and behaviorally specific in that CCK inhibited food intake in food-deprived rats but not water-intake in water-deprived rats. Subsequent experiments demonstrated that CCK administration inhibited both real and sham feeding (Gibbs, Young, & Smith, 1973b), elicited a behavioral satiety sequence (Antin, Gibbs, Holt, Young, & Smith, 1975), and was effective across a range of species, including man (Moran & McHugh, 1982; Kissileff, Pi-Sunyer, Thornton, & Smith, 1981). The dynamics of CCK release and metabolism are consistent with a role for the peptide in meal termination. Plasma CCK levels rapidly peak following the initiation of feeding, and CCK has a brief half-life in circulation. Meal contingent CCK administration results in repeated reductions in meal size with a compensatory increase in meal number, indicating a primary role of the peptide in satiation (West, Fey, & Woods, 1984).

Although exogenous CCK inhibits food intake in a variety of situations, its physiological role in satiety has been controversial. Assessments of the ability of

CCK to produce a conditioned taste aversion produced mixed results (Deutsch & Hardy, 1977; Holt, Antin, Gibbs, Young, & Smith, 1974), and CCK administration resulted in different patterns of GI activity and hormone secretion than those observed during a meal (Verbalis, McCann, McHale, & Stricker, 1986). Although some of these findings could be attributed to the use of high CCK doses (Smith & Gibbs, 1992), such results questioned whether the inhibition of food intake produced by exogenous CCK was indeed mimicking a physiological action of the endogenous peptide. The development of potent and specific CCK antagonists finally allowed a direct assessment of this issue. Data from multiple species and a variety of testing paradigms have demonstrated that pharmacological blockade of the actions of exogenous CCK results in increases in food intake, and more specifically, increased meal size (Dourish, Rycroft, & Iversen, 1989; Moran, Ameglio, Schwartz, Peyton, & McHugh, 1993; Reidelberger & O'Rourke, 1989; Silver, Flood, Song, & Morley, 1989). These results have been interpreted to signify a physiological role for endogenous CCK in meal termination (Smith & Gibbs, 1992). Such interpretations are further supported by data from the Otsuka Long Evans Tokushima Fatty (OLETF) rat that has a 64-kb deletion in the gene for CCK-A receptors (Funakoshi *et al.*, 1995), the receptor subtype that mediates the satiety actions of both administered and endogenously released CCK. In the absence of this functional CCK signaling pathway, the ability of OLETF rats to limit the size of both spontaneous and scheduled meals is significantly impaired (Moran, Katz, Plata-Salaman, & Schwartz, 1998).

The satiety actions of CCK depend upon vagal afferent signaling. Total subdiaphragmatic vagotomy essentially eliminates the ability of exogenous CCK to inhibit food intake (Smith, Jerome, Cushin, Eterno, & Simansky, 1981), and the satiating effect of low doses of CCK is blocked by specific afferent vagotomy (Moran, Baldessarini, Salorio, Lowery, & Schwartz, 1997; Smith, Jerome, & Norgren, 1985). A second action of CCK, depending on pyloric CCK receptors and the inhibition of gastric emptying, also has been shown to contribute to the overall inhibition of food intake produced by larger doses of CCK (Moran, Shnayder, Hostetler, & McHugh, 1988).

Consistent with the ability of dietary fats with chain lengths of 12 or greater to release CCK (McLaughlin, Grazia Luca, Jones, D'Amato, Dockray, & Thompson, 1999), the peptide seems to play a major role in mediating fat-induced inhibition of feeding. Thus, although the physiological relevance of direct intestinal fat infusions has been questioned (Ramirez, Tordoff, & Friedman, 1997), the ability of these infusions to inhibit food intake can be blocked by pretreatment with a specific CCK-A antagonist (Greenberg, Torres, Smith, & Gibbs, 1989; Matzinger *et al.*, 1999).

Apolipoprotein A-IV (Apo A-IV) also is involved in mediating the satiety actions of fats. Apo A-IV is a protein secreted by enterocytes in the small intestine in response to active lipid absorption (Kalogeris, Monroe, Demichele, & Tso, 1996; Kalogeris, Rodriquez, & Tso, 1997). Apo A-IV synthesis occurs primarily in the jejunum (Abumrad, Coburn, & Ibrahimi, 1999), and apo A-IV is secreted into the lymph in response to fat absorption and the formation of chylomicrons. Apo A-IV plasma levels are significantly elevated within 15 min of meal initiation and remain elevated for an additional 30 min, timing consistent with a role in meal termination (Rodriguez, Kalogeris, Wang, Wolf, & Tso, 1997). When administered intravenously, apo A-IV inhibits food intake in a dose-dependent manner (Fujimoto, Cardelli, & Tso, 1992).

The site of action for the inhibition of food intake by intestinal apo A-IV is not clear. Although centrally administered apo A-IV also inhibits food intake, and central apo A-IV antibody administration stimulates intake (Fujimoto, Fukagawa, Sakata, & Tso, 1993), the relative importance of the actions of peripheral and central apo A-IV actions remains to be determined.

Roles for other GI peptides in meal termination have been proposed. Both glucagon and amylin are pancreatic peptides that are thought to affect meal size through hormonal actions. Plasma glucagon levels transiently increase in response to meal ingestion (de Jong, Strubbe, & Steffens, 1997) and a similar pattern of release is likely for amylin since it is obligatorily coreleased with insulin (Cooper, 1994). Both peptides produce rapid, dose-related decreases in meal size following their systemic administration (Le Sauter & Geary, 1991; Lutz, Del Prete, & Scharrer, 1995), and administration of glucagon monoclonal antibodies or amylin antagonists produce hyperphagia supporting feeding-inhibitory roles of the endogenous peptides (Le Sauter, Noh, & Geary, 1991; Lutz, Del Prete, Szabady, & Scharrer, 1996). Either total or selective hepatic vagotomy blocks glucagon's satiety action (Geary & Smith, 1983) indicating a peripheral site of action and vagal afferent transmission for the effect. In contrast, the critical site of action for amylin appears to be central. Amylin-induced inhibition of food intake is not affected by subdiaphragmatic vagotomy (Lutz, Del Prete, & Schrarrer, 1995), and lesions of the area postrema/nucleus of the solitary tract significantly attenuate the actions of amylin on feeding (Lutz, Althaus, Rossi, & Scharrer, 1998; Lutz *et al.*, 1998).

Data supporting a role for gastrointestinal mammalian bombesin-like peptides in meal termination are not as clear. Although both gastrin releasing peptide (GRP) and neuromedin B (NMB) are found within the GI tract (McDonald *et al.*, 1979) and both inhibit food intake following their peripheral administration (Ladenheim, Wirth, & Moran, 1996), both peptides and their respective receptors also are found in the brain (Ladenheim, Jensen, Mantey, & Moran, 1992; Minamino, Kangawa, & Matsuo, 1983). Thus, the sites of release and action for the endogenous forms of these peptides in reducing food intake remain to be identified. Data demonstrating that GRP given at the end of a meal can extend the intermeal interval have led to the hypothesis that bombesin-like peptides affect food intake primarily by prolonging satiety rather than by an action on the termination of individual meals (Thaw, Smith, & Gibbs, 1998).

The GI tract also is a source for leptin (Badon *et al.*, 1998), the protein product of the ob gene that has been shown to play an important role in signaling the brain about the degree of adiposity and thus the availability of nutrient stores. Although the primary source of leptin is the adipose mass, leptin has been localized to "chief cells" within the gastric mucosa. Gastric leptin release is stimulated by pepsinogen secretagogues such as CCK, gastrin, and secretin as well as in response to ingestion. Release occurs into the gastric lumen and into the blood where, in the rat, gastric leptin release can account for 25% of the circulating peptide. Although leptin is transported from the blood to the brain, changes in plasma levels of this magnitude are not thought to be sufficient for meaningful alterations in leptin-regulated hypothalamic feeding systems. Instead, gastric leptin has been proposed to serve as a short-term satiety signal with a peripheral site of action (Bado *et al.*, 1998). Vagal afferent fibers innervating the stomach have been shown to contain leptin receptors (Buyse *et al.*, 2001), and leptin does activate vagal afferents, providing a potential signaling pathway (Wang, Martinez, Barrachina, & Tache, 1998).

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The GI tract receives both spinal and vagal innervation, providing the potential for direct neural within-meal feedback signaling between gut and brain. In this section, we outline the nature and extent of gut neural innervation, and identify the sources and modes of gut neural signaling that may play roles in meal termination.

Spinal afferents project from GI mucosa, smooth muscle, and submucosal layers and form a network around aspects of the GI vasculature (Furness, Papka, Della, Costa, & Eskay, 1982; Sternini, Reeve, & Brecha, 1987). The traditional view of the primary function of GI spinal afferents has been that they are activated only in response to potentially harmful or noxious stimuli (Holzer, 1995). More recent work has suggested that these afferents also are involved in aspects of duodenal nutrient signaling in that the inhibition of gastric emptying in response to monosaccharides depends in part upon a spinal capsaicin sensitive pathway (Raybould, 2002; Raybould & Holzer, 1992). Consistent with such a role is a recent finding demonstrating that celiac superior mesenteric ganglionectomy attenuates the inhibitory action of intraduodenal nutrients on feeding (Sclafani, Ackroff, & Schwartz, 2003). However, the overall role of spinal afferents in signaling the GI presence of nutrient products is not well understood and is an area requiring additional work.

Vagal afferent signaling appears to be the primary neural mode of transmission for nutrient feedback signaling. In the rat, the primary species used in such research, afferent fibers make up the majority of the subdiaphragmatic vagus (Prechtl & Powley, 1990), accounting for roughly 75% of the total number of nerve fibers. The subdiaphragmatic vagus has two major trunks traveling along the esophagus. The ventral vagal trunk forms the left gastric, the hepatic, and the accessory celiac branches. The dorsal trunk bifurcates into the right gastric and the celiac branches. Vagal motor axons are most prominent in the gastric branch, but still amount to only 36% of the total fibers count (Prechtl & Powley, 1990). Although the nomenclature does indicate the major organ of termination for the branches, the hepatic vagus also provides significant innervation to the distal gastric antrum, the pylorus, and the proximal duodenum. In fact, the hepatic branch is likely the source for the majority of the vagal innervation of the first 3 cm of duodenum in the rat (Phillips, Baronowsky, & Powley, 1997).

Within the muscle wall of the GI tract, two distinct vagal afferent terminations have been identified and labeled according to their different morphologies and locations. Intramuscular arrays (IMA) are found within the longitudinal and circular muscle, and consist of an array of free terminals running in parallel with the muscle fibers (Berthoud & Powley, 1992; Wang & Powley, 2000). Intraganglionic laminar endings (IGLE) are localized to the myenteric plexus and form a series of highly arborizing endings within myenteric ganglia (Rodrigo, Hernandez, Pedrosa, Peres Anton, & Vidal, 1975). IGLEs are widely distributed throughout the GI tract of the rat, present in the esophagus, stomach, and throughout the intestine (Berthoud, Patterson, Neumann, & Neuhuber, 1997). IMAs, on the other hand, are found predominantly within the gastric fundus and corpus and around the esophageal and pyloric sphincters (Wang & Powley, 2000). Polymorphic afferents, consisting of individual afferent fibers that have one or more collaterals terminating in IMAs and one or more terminating in IGLEs, also have been described (Berthoud & Powley, 1992). Mechanoreceptive roles for both terminations have been proposed, with IMAs serving as stretch or length receptors with ability to

respond to passive stretch, and IGLEs serving as tension receptors responsive to active muscle contraction (Phillips & Powley, 2000). The localization of IGLEs has led to proposals that they have the ability to respond to paracrine chemical stimulation arising from myenteric neurons (Berthoud *et al.*, 1997; Schwartz, 2000). Such a response characteristic would be consistent with electrophysiological data showing that single vagal afferents can respond to both mechanical and neuropeptide stimulation (Schwartz, McHugh, & Moran, 1991).

Vagal afferent sensors also have been identified within the duodenal mucosa (Berthoud, Kressel, Raybould, & Neuhuber, 1995). Studies using DiI injections into the nodose ganglia have revealed that labeled vagal afferent fibers and terminations are found in two primary locations: around crypts of Lieberkuhn and within the lamina propria of the villa. Within the lamina propria, fine fibers with multiple varicosities run along the longitudinal axis of the villa. Although fiber terminations are found in close approximation to the epithelial cells, they do not appear to penetrate into the duodenal lumen. This localization has led to the idea that intestinal nutrients do not have direct contact with vagal afferent terminations; consequently, if there is direct nutrient contact, it would occur at a postabsorptive site. Alternatively, signaling could involve a chemical message stimulated by the nutrient that in turn activates the vagal afferent terminations. Such an idea is consistent with the presence and transport of multiple peptide and neurotransmitter receptors within vagal afferent fibers (Moran & McHugh, 1992).

Vagal afferent activity is modified by a variety of modes of stimulation within the GI tract, the sufficient stimuli for producing activation differing by GI compartment. For the most part, vagal afferents innervating the stomach are responsive to the mechanical properties of the luminal contents. Two populations of distention-sensitive vagal afferents have been identified. The first, classic, slowly adapting mechanosensors, increase their activity in relation to increasing gastric volume or during gastric muscle contractions (Davison & Clarke, 1988; Schwartz, McHugh, & Moran, 1991). Activity decreases only slowly during a maintained distention, and there is an off response (i.e., a decrease in activity below baseline levels when the mechanical stimulus is removed). This distention-induced increase in activity is independent of the nutrient character and concentration of the gastric contents (Mathis, Moran, & Schwartz, 1998). A second population of afferents with apparent mucosal receptors has been identified. These fibers adapt rapidly to mechanical stimulation and demonstrate some chemosensitive properties (Blackshaw & Grundy, 1989; Clarke & Davison, 1978). A subset of these fibers responds to a variety of organic and inorganic acids, but this activity does not appear to be related to stimulus concentration or pH. Thus, as had been suggested by studies of the controls of gastric secretion (Grossman, 1967; Longhi, Greenlee, Bravo, Guerrero, & Dragstedt, 1957), some chemical characteristics of gastric contents do result in alterations in electrophysiological activity in a subpopulation of gastric vagal afferents. However, gastric vagal afferent activity does not appear to provide much information to the brain about the nutrient character or concentration of gastric contents, nor, as discussed above, does this information appear to affect ongoing food intake.

In contrast, vagal afferents innervating the duodenum respond to the mechanical properties as well as the chemical characteristics of duodenal nutrients. Duodenal distention induces activity in mechanoreceptive duodenal vagal afferents, with a pattern of activation consistent with actions through a classic, slowly adapting mechanoreceptor (Schwartz, Tougas, & Moran, 1995). Duodenal vagal

afferent chemosensation has been demonstrated for a range of nutrient and concentration-dependent characteristics. Mei (1978), recording in the cat from individual nodose ganglion neurons that did not respond to gastric stimulation, first demonstrated concentration-dependent neuronal stimulation in response to duodenal carbohydrate infusions. Activity in these fibers was not driven by distention or other forms of mechanical stimulation, nor was change in activity simply linked to intraluminal osmolality. This response profile led Mei to posit that these vagal fibers were glucoreceptive fibers (Mei, 1978). Other glucose responsive neurons have been identified that respond to a greater range of stimuli, and are especially sensitive to changes in osmolality (Mei & Garneir, 1986). These osmosensitive neurons are polymodal in that they also respond to stroking and alterations in pH and temperature.

Nodose ganglion neuronal responses to small intestinal lipid infusion also have been identified (Melone, 1986). One class of neurons responded to short-chain lengths, while another was activated only by long-chain fatty acids. In both cases, increases in activity were concentration dependent. Alterations in activity in duodenal and jejunal vagal afferent fibers also can be demonstrated in response to duodenal protein or fat infusions. Grundy and colleagues, recording from mesenteric paravascular nerve bundles, have demonstrated nutrient-specific responses to upper intestinal infusions of casein hydrolyzate (Eastwood, Maubach, Kirkup, & Grundy, 1998) and fatty acids of different chain lengths (Lal, Kirkup, Brunsden, Thompson, & Grundy, 2001). Randich *et al.* (2000) have identified responses to jejunal and ileal lipid infusion in single celiac branch or cervical vagal afferents.

Thus, in contrast to the limited chemosensitivity of gastric vagal afferents, intestinal afferents show a wide range of responsivity characterized by nutrient specificity and concentration dependence. These response characteristics provide a basis for nutrient sensing in the upper intestine that can contribute significantly to the controls of food intake.

Vagal afferent fibers also respond to a variety of transmitters and peptides (Eastwood & Grundy, 2000; Hillsley & Grundy, 1998; Kreis, Jiang, Kirkup, & Grundy, 2002), and this property may underlie much of the nutrient responsivity of upper intestinal vagal afferents. Work with CCK has identified several important characteristics of peptide-induced vagal afferent transmission that have provided the basis for the hypothesis that peptides can modify afferent activity in multiple ways. Responses to CCK are found in both mechano- and chemosensitive fibers. Slowly adapting gastric mechanoreceptive fibers that provide much of the feedback about gastric distention are responsive to local administration of CCK. CCK and gastric distention activate these fibers in a similar manner and responses to the two stimuli are additive. Importantly, activation by CCK sensitizes these fibers to distention even at times after the direct response to CCK has disappeared (Schwartz, McHugh, & Moran, 1993).

CCK also exerts multiple actions on the activity of slowly adapting mechanoreceptive fibers in the duodenum (Schwartz *et al.*, 1995). Thus, CCK activates these vagal afferents, CCK sensitizes these afferents to subsequent distention, and the peptide's effect combines additively with that induced by distention (Figure 4). CCK also mediates aspects of the nutrient responsivity of intestinal vagal afferents. CCK is released from intestinal endocrine cells in response to the intraluminal presence of nutrients, and has been proposed to activate nearby vagal afferent terminals through paracrine mechanisms. Such a role for CCK is indicated by data demonstrating that administration of a CCK antagonist blocks the vagal afferent

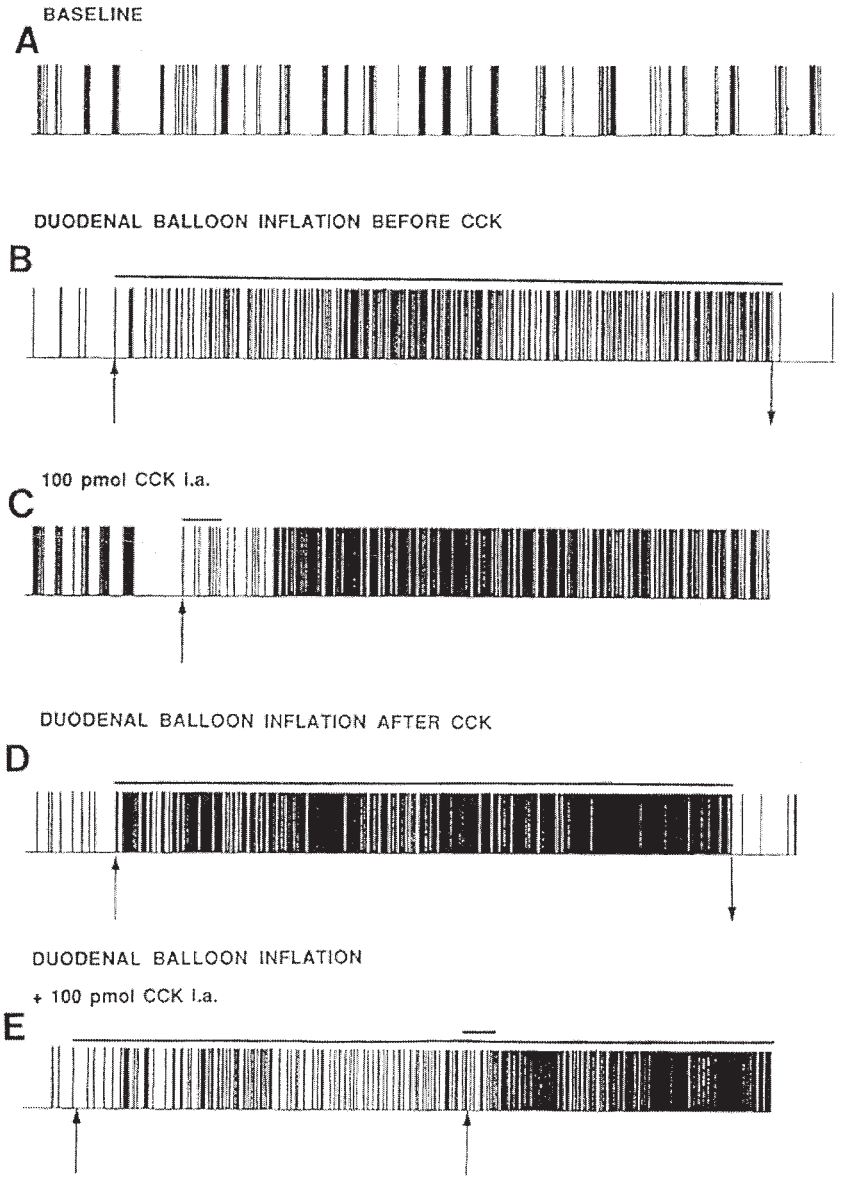

Figure 4. Electrophysiological activity of a single vagal afferent fiber with a duodenal receptive field. Activity increases from baseline (A) in response to 0.1 ml duodenal balloon inflation (B) and to a close arterial infusion of 100 pmol CCK (C). Prior activation by CCK increases the response to duodenal balloon inflation (D). The response to combined balloon inflation and CCK infusion is additive (E). Up arrows indicate time of stimulus presentation and the bar above the trace indicates the duration of stimulus delivery. (From Schwartz, Tougas, & Moran, 1995.)

responses both to intraintestinal casein hydrolysate (Eastwood *et al.*, 1998) and long-chain fatty acids (Lal *et al.*, 2001). A similar mechanism for serotonin in carbohydrate-induced vagal activation has been proposed (Raybould, 2002).

This combination of actions is consistent with a role for CCK in the overall integration of gastric and intestinal feedback in satiety. CCK, released in response to intestinal nutrients, directly stimulates both chemosensitive and mechanosensitive

vagal afferent fibers. CCK directly activates mechanosensitive afferents and amplifies the response to distending stimuli. This integration of actions by CCK has a clear parallel in behavioral experiments. The anorectic efficacy of CCK is significantly increased when combined with a nonnutrient gastric load (Moran & McHugh 1982; Schwartz, McHugh, & Moran, 1991). CCK provides direct inhibitory feedback through its activation of vagal afferent fibers, enhances gastric distention by inhibiting the emptying of the gastric load, and sensitizes vagal afferents that respond to the distensive properties of a gastric load.

Interruption of vagal afferent transmission eliminates important aspects of nutrient feedback signaling in the control of ingestion. For example, vagotomy eliminates the ability of gastric volume to inhibit food intake. As discussed above, gastric loads maintained in the stomach with a closed pyloric cuff produce volume-dependent reductions in food intake (Phillips & Powley, 1998). These reductions are eliminated by total subdiaphragmatic vagotomy and significantly reduced by disconnection of the gastric vagal branches (Phillips & Powley, 1998). Similarly, suppression of meal size by intestinal nutrient infusions is eliminated by selective deafferentation of the vagal celiac branch (Walls, Phillips, Wang, Holst, & Powley, 1995). Evidence that these same pathways are involved in the control of meal size under more normal ingestion conditions derives from a variety of findings. Chemical deafferentation with capsaicin results in overconsumption on the initial exposure to novel, high-fat solid or liquid diets (Chavez, Kelly, York, & Berthoud, 1997; Kelly, Chavez, & Berthoud, 1999). Specific surgical vagal deafferentation, which disconnects all subdiaphragmatic vagal afferents, resulted in chronic alterations in meal patterns in rats maintained on a liquid diet (Schwartz, Salorio, Skoglund, & Moran, 1999). Although total food intake was not altered, rats with vagal deafferentation consumed significantly larger meals than rats with sham surgeries. This increase in meal size was accompanied by a compensatory decrease in meal number. Similar patterns of food intake have been described for rats following area postrema lesions that destroy some vagal afferent projection sites (Stricker, Curtis, Peacock, & Smith, 1997). These data provide functional validations of the role of vagal feedback signaling in the controls of meal size.

The pattern of results concerning sources and stimuli for within-meal peptide and neural afferent signaling dovetail with the behavioral findings reviewed above. First, information about the quantity and composition of ingested nutrients arises both from gastric and intestinal sites. Just as the feeding inhibitory effect of gastric nutrients reflects the volume rather than the nutrient content, signaling arising from the stomach is volume- rather than nutrient-dependent. In contrast, duodenal signaling reflects both the amount and character of the intraluminal contents and has both direct neural and peptide mediation. Second, there are sites at which the feedback from gastric and postgastric signals is summated. The clearest instance of such integration occurs at the level of the vagal afferent fiber. Single gastric vagal afferents respond both to mechanical stimuli and nutrient elicited peptide release providing a mechanism for the summation of gastric and intestinal feedback signals. This is not to say that the vagal afferent fiber is the sole site for signal integration. Significant integration of oral, gastric, and duodenal signaling can be shown to occur at the level of the nucleus of the solitary tract as well (Emond, Schwartz, & Moran, 2001).

Although the general response patterns of feedback signaling are qualitatively consistent with the respective gastric and postgastric influences on food intake, it is not yet possible to provide a satisfactory account of how the system tracks

cumulative intake under normal conditions. On the gastric side, we require a model by which an accurate assessment of gastric volume can be derived from an array of afferent fibers with different thresholds and dynamic ranges of response to distention. For meals beyond the first of the day, the gastric contribution to meal-size control reflects the difference between the current volume and the appreciable volumes that may remain in the stomach from earlier meals (van der Velde *et al.*, 1999). Additional experiments are required to provide an indication of whether initial volumes are discounted peripherally (e.g., by diminished responding over the intermeal interval to given levels of distention), or whether a comparison between initial and later gastric volume-related signals must be performed by a central mechanism. On the postgastric side, information about the amount of nutrient that has emptied during the meal is not likely to be read simply from chemoreceptors sensitive to intraluminal nutrient quality and concentration (much as oral taste receptors do not directly report cumulative intake). In principle, a combination of chemoreceptive and intestinal distension-related feedback could permit an assessment of the current intraluminal content. But because a portion of the nutrients that had emptied already will have been absorbed (Strubbe & Steffens, 1977), feedback about the total amount emptied must be derived, at least in part, from the recent history of signals driven by intestinal stimulation. It is currently not known whether the compiled signal, bearing directly on the progress and termination of the meal, arises from the peripheral afferent input or from central mechanisms integrating the pattern of incoming information. Overall, it is clear that the simple judgments derived from the behavioral studies reviewed above regarding the accurate monitoring of cumulative intake within a meal present numerous analytic and interpretive challenges for future research.

Gut Peptide Signaling Across Meals

Up to this point we have focused on within-meal feedback signaling involved in the termination of an individual feeding bout. Recently, actions of GI peptides have been identified that may contribute to the overall patterning of food intake across meals. Such roles for two members of the NPY family of GI peptides have been suggested. These are peptide YY (3–36) (PYY(3–36)) and pancreatic polypeptide (PP). PYY(3–36) is an alternate form of PYY that is secreted from the lower intestine in response to the intraluminal presence of digestion products, reaching peak plasma levels 60–90 min following meal ingestion (Onaga, Zabielski, & Kato, 2002). Recent data from Batterham and colleagues (Batterham *et al.*, 2002) have indicated an anorexigenic action for PYY(3–36). Peripherally administered PYY(3–36), at doses that produced plasma levels similar to those found postprandially, was shown to exert prolonged inhibitory effects on food intake. A brain site for this feeding inhibitory action of PYY(3–36) has been suggested by the demonstration that PYY(3–36) injected directly into the hypothalamic arcuate nucleus at very low doses potently inhibits food intake. PYY(3–36) is an agonist at the presynaptic Y2 receptor subtype (Michel *et al.*, 1998), and mediation through a Y2 site was supported by findings indicating that the actions of PYY(3–36) were mimicked by administration of a specific Y2 agonist, and by data showing that PYY(3–36) did not affect food intake in Y2 null mice. This pattern of results suggests that postprandially released PYY(3–36) may directly access brain sites, and bind to presynaptic Y2 receptors to inhibit the release of endogenous NPY, which, in turn,

suppresses subsequent food intake over an extended time period. Importantly, infusions of PYY(3–36) that resulted in normal postprandial plasma levels in humans reduced ratings of appetite and significantly decreased intake during later test meals (Batterham *et al.*, 2002).

Peripheral injections of PP have been shown recently to have a similar inhibitory effect on food intake, although the mode of action for PP and the underlying mechanisms seem to be different from those proposed for PYY(3–36) (Asakawa *et al.*, 2003). Rather than directly accessing brain sites, PP appears to have a peripheral site of action. Data demonstrating that PP inhibits hepatic vagal afferent activity, and that vagotomy blocks the feeding inhibitory actions of PP, suggest that PP directly interacts with peripheral vagal afferent terminals, through which the influence of the peptide is transmitted to the brain. Campbell and colleagues recently identified Y4 receptor sites on lateral hypothalamic orexin neurons and showed that PP injected at this central site stimulates rather than inhibits food intake (Campbell *et al.*, 2003). Taken together, these results support a peripheral vagal mediation of the feeding inhibitory action of peripheral PP.

Although peripheral PP may not directly access hypothalamic sites, the feeding inhibitory action appears to depend upon alterations in the expression of a number of central and peripheral peptides involved in feeding control. Peripherally administered PP reduces deprivation-induced mRNA expression of the hypothalamic orexigenic peptides NPY and orexin, and of ghrelin within the stomach (Asakawa *et al.*, 2003). These data suggest that modulation of a vagal afferent pathway can have effects on gene expression in brain and gut systems involved in the control of food intake.

These findings with PYY(3–36) and PP, if confirmed by other investigators, identify an understudied role of GI peptides in food intake—the modulation of the post-feeding interval. Such an action would be consistent with their patterns of secretion. As noted above, plasma PYY(3–36) rises after rather than during a meal (Onaga *et al.*, 2002). Although plasma PP can be elevated in response to oral and gastric stimulation (Taylor, Feldman, Richardson, & Walsh, 1978), it remains elevated for significant periods following a meal (Taylor, 1985). More detailed behavioral analyses of how these peptides affect the patterning of meals are necessary to confirm their potential actions in prolonging the intermeal interval.

In contrast to the putative actions of PYY(3–36) and PP as inhibitors of food intake, ghrelin, an endogenous agonist for the growth hormone secretagogue receptor (Kojima *et al.*, 1999), potently stimulates food intake (Tschop, Smiley, & Heiman, 2000; Wren *et al.*, 2000). Ghrelin is a peptide produced mainly within the oxynic gland of the stomach. A physiological role for ghrelin in controlling food intake is suggested by data showing decreases in food intake and body weight both in lean and obese mice in response to repeated administration of a ghrelin antagonist (Asakawa *et al.*, 2003). Ghrelin increases food intake in a way that suggests that it is involved in stimulating the initiation of meals. A detailed behavioral analysis revealed that the hyperphagic response to central ghrelin administration was largely accounted for by decreased latency to the first meal and an increase in meal number (Faulconbridge, Cummings, Kaplan, & Grill, 2003). The profile of ghrelin secretion is consistent with such an action. Plasma ghrelin levels rise shortly before meals are initiated and rapidly fall when food is consumed (Shiiya *et al.*, 2002). Introduction of nutrients into the GI tract causes plasma ghrelin to decrease (Shiiya *et al.*, 2002) and postgastric stimulation is necessary for this decrease to occur (Williams, Cummings, Grill, & Kaplan, 2003). Although such experiments begin to

identify aspects of the control of ghrelin secretion, the critical signals for the premeal elevation in plasma ghrelin are yet to be determined.

Ghrelin's site of action has been proposed to be within the brain. Central ghrelin administration stimulates food intake at significantly lower doses than does peripheral ghrelin (Tschop *et al.*, 2000), and regulated mechanisms exist for the blood-brain transport of ghrelin (Banks, Tschop, Robinson, & Heiman, 2002). Ghrelin administration activates hypothalamic signaling systems involved in the controls of food intake (Lawrence, Snape, Baudoin, & Luckman, 2002); roles for NPY and the endogenous melanocortin antagonist, agouti-related peptide (AgRP), in the feeding stimulatory actions of ghrelin have been proposed (Seoane *et al.*, 2003). Recent data have questioned a direct central site of action for circulating or peripherally administered ghrelin. Subdiaphragmatic vagotomy or chemical vagal deafferentation with capsaicin blocks the ability of peripherally administered ghrelin to stimulate food intake (Date *et al.*, 2002). These data suggest a peripheral site of action and vagal mediation. Consistent with this view are demonstrations of the presence of growth hormone secretagogue receptors within the vagus and ghrelin-induced suppression of overall activity within the gastric vagal branch (Date *et al.*, 2002).

Interactions Between the Short- and Long-Term Controls of Ingestive Behavior

The satiating efficacy of gut peptides can be modulated by alterations in central peptide signaling, and this modulation may serve to explain how meal size varies with changes in metabolic state. Exogenous leptin reduces daily food intake (Campfield, Smith, Guisez, Devos, & Burn, 1995). A number of investigators have demonstrated that this reduction arises specifically from a decrease in average meal-size with no alteration in meal frequency (Eckel *et al.*, 1998; Flynn, Scott, Pritchard, & Plata-Salaman, 1998; Kahler *et al.*, 1998). Conversely, after a period of food deprivation, a time at which endogenous leptin levels are low, the initial meal size often is significantly larger than meals normally consumed (Bivens, Thomas, & Stanley, 1998). How are such changes in meal size mediated?

There is now a growing body of work suggesting that leptin, and leptin-induced changes in hypothalamic signaling, result in alterations in brain receptivity to within-meal satiety signaling. Thus, leptin can be shown to modify the efficacy with which gastric distention and CCK inhibit food intake (Emond, Landenheim, Schwartz, & Moran, 2001; Emond, Schwartz, Ladenheim, & Moran, 1999). These changes in behavioral sensitivity appear to be mediated by alterations in neuronal responses to gastric distention and CCK with the site of action within the dorsal hindbrain. Leptin treatment increases the number of NTS cells that express c-Fos in response to CCK (Emond *et al.*, 1999; Wang, Martinez, Barrachina, & Tache, 1998) or to gastric distention (Emond *et al.*, 2001). Leptin also increases the electrophysiological responsivity of individual NTS neurons to gastric distention (Schwartz & Moran, 2002).

NPY modulates brainstem responses to CCK in ways consistent with its ability to increase food intake. NPY decreases the number of NTS neurons expressing c-Fos in response to CCK (McMinn, Sindelar, Havel, & Schwartz, 2000), and NPY diminishes the electrophysiological responses of NTS neurons to gastric distention (Schwartz & Moran, 2002). Thus, manipulations that alter hypothalamic signaling produce changes in the dorsal hindbrain responses to satiety signals of GI origin.

Orexigenic states (low leptin, high NPY) reduce the efficacy of GI satiety signals while anorexigenic states (high leptin) increase their efficacy.

Complementing the action of leptin on the satiating potency of CCK, CCK appears to modulate longer-term effects of leptin administration on body weight and energy expenditure. Matson and Ritter (1999) and Matson, Reid, and Ritter (2002) have demonstrated increases in leptin-induced body weight loss when the CCK is given in combination with leptin. An amplification of leptin-induced activation of the hypothalamic paraventricular nucleus (PVN) by CCK is a possible mechanism. Just as leptin elevates CCK-induced c-Fos activation within the NTS, CCK elevates the level of leptin induced c-Fos activation within the PVN (Emond *et al.*, 1999). Other GI peptides also have been shown to affect energy expenditure. The clearest examples of such an action are PP and ghrelin, which modulate (increase and decrease, respectively) thermogenesis. The thermogenic effects have been shown to derive from their influences on sympathetic activity (Asakawa *et al.*, 2003; Yasuda, Masaki, Kakuma, & Yoshimatsu, 2003). Thus, gut peptides can have multiple catabolic or anabolic effects on energy balance. They not only alter food intake but, in some cases, can also modulate energy expenditure.

Summary

The GI tract provides various signals that are involved in multiple aspects of feeding control. Within individual meals, gut signaling provides the critical feedback for meal termination. Signals arise both from the gastric and intestinal compartments and, while signals from either independently affect food intake, feedback from both the stomach and the intestine is necessary to allow the animal to meter intake under normal feeding conditions and to fully account for the amount consumed in the meal. Both peptide and neural signaling contribute to the feedback control of food intake within meals. Roles for several peptides in meal termination can be demonstrated, and peptides can affect intake as blood-borne signals or through actions on vagal afferent fibers. Overall intake control within a meal reflects a neural integration of gastric and intestinal signaling that can occur at multiple levels including within single vagal afferent fibers. Increases in intake observed in experiments in which a peptide or neural action is blocked (by receptor antagonism, specific lesion, and in genetically modified mice) strongly support the physiological relevance of a number of GI signals in satiation.

As well as playing a role in terminating individual meals, GI signals arising from ingested nutrients also modulate the pattern of food intake over the course of the day. Both inhibitory and excitatory peptidergic influences have been proposed to mediate the influence of one meal on the timing and/or size of subsequent meals. Peptides released from GI sites also affect intake over yet longer time courses. Interactions have been demonstrated between within-meal GI feedback systems and central signaling pathways involved in energy balance. The degree to which the efficacy of within-meal signaling can be modulated allows for differences in meal size that depend upon metabolic status. Conversely, within-meal signaling can alter the efficacy of longer-term signals, thereby affecting overall metabolic status. Indeed, the growing range of proposed actions and interactions is making it difficult to identify any aspect of ingestive control to which GI signals do not contribute in appreciable measure.

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12

The Estrogenic Inhibition of Eating

Nori Geary

Studies of laboratory, agricultural, and wild mammals, as well as women, suggest that there are functionally important ties between the hypothalamic–pituitary–gonadal (HPG) axis, eating and body weight control, including, for example, changes in eating during the ovarian cycle, during pregnancy, and during lactation. Understanding the influence of the reproductive axis in the control of eating is important for several reasons. Many women are concerned with their eating and body weight throughout their lives, so increased understanding of the normal physiological influences on eating through the menstrual cycle as well as during menarche and menopause may lead to better well-being and health. Increased understanding of how HPG function affects eating and body adiposity may, for example, help inform decisions related to the use of contraceptives that affect HPG function or the use of hormone-replacement therapy after menopause. Understanding the sexual differentiation of eating also may lead to improvements in treatments for several serious health problems. Women are several-fold more likely than men to suffer from extreme obesity, defined as a body mass index (BMI, mass in kg divided by [height in m]^2) of more than 40 (Flegal, Carrol, Ogden, & Johnson, 2002; Freedman, Khan, Serdula, Galuska, & Dietz, 2002; Laurier, Guiguet, Chau, Wells, & Valleron, 1992). Furthermore, even less pronounced obesity is often associated with binge eating, the incidence of which is about 1.5 times higher in American women than men (American Psychiatric Association, 1994). And women are about nine times more vulnerable to anorexia nervosa and bulimia nervosa. Nevertheless, whether and how pathophysiological changes in sexually differentiated controls of eating might contribute to disturbed eating and obesity remains unknown.

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Neurobiology of Food and Fluid Intake, 2nd Ed., Volume 14 of *Handbook of Behavioral Neurobiology,* edited by Edward Stricker and Stephen Woods, Kluwer Academic / Plenum Publishers, New York, 2004.

This chapter focuses on the inhibitory effects of estradiol on eating during the ovarian cycle. This inhibition, sometimes called the estrogenic inhibition of eating, is the best understood interaction between HPG axis function and the control of eating. The behavioral expression of the estrogenic inhibition of eating, its underlying mechanisms, and its physiological implications, especially its relevance for energy balance and body weight, are reviewed in detail. Although most of what is known comes from studies in rats and mice, enough is known about eating in women to support the conclusion that estradiol also has an inhibitory effect on eating in women.

Cyclic Changes in Eating During the Ovarian Cycle

Introduction

Food intake decreases markedly during the peri-ovulatory phase of the ovarian cycle in most mammals, including mice, rats, pigs, goats, cattle, dogs, nonhuman primates, and women (for reviews, see Asarian & Geary, 2002; Blaustein & Wade, 1976; Dye & Blundell, 1997; Houpt, 1991; Kemnitz, Gibber, Lindsay, & Eisele, 1989; Wade, 1972). The duration and magnitude of this decrease parallel species-specific variations in ovarian cycling. Food intake also has been reported to increase during the post-ovulatory phase of the cycle in several nonhuman primate species and in women. This section describes these phenomena in mice, rats, and women.

Mice and Rats

Physiology of the Estrous Cycle. Mice and rats, like women, have regular, spontaneous ovarian cycles that are not dependent on seasonal cues or stimulation by conspecifics. The modal cycle period is 4 or 5 days (Blandau, Boling, & Young, 1941; Long & Evans, 1922; Freeman, 1994). The neuroendocrine controls of the rat ovarian cycle have been well characterized (Brown-Grant, Exley, & Naftolin, 1970; Butcher, Collins, & Fugo, 1974; Cecchini, Chattoraj, Fanous, Panda, Brennan, & Edilin, 1983; Freeman, 1994; Smith, Freeman, & Neill, 1975). Plasma levels of some of the principal HPG axis hormones through 4-day rat cycles are shown in Figure 1 (upper three panels). Secretion of the ovarian hormones estradiol and progesterone increases beginning late in the first day of the cycle (diestrus 1, see below). Progesterone secretion ceases temporarily about a day later before increasing rapidly on the third day (proestrus). In contrast, estradiol secretion increases steadily from the first day until it peaks in the middle of the diurnal period of the third day. Estradiol secretion then stops, and plasma estradiol concentration drops rapidly to a minimum. The patterns of secretion of estradiol and progesterone are controlled by the anterior pituitary hormones, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin, of which LH is the most important. The secretory patterns of these three pituitary hormones are very similar. Basal levels persist until midway through the diurnal period of the third day, when they rise very rapidly, peak at about dusk, and then fall (LH falls precipitously immediately after its peak, FSH falls slightly slower, and prolactin remains elevated for about 12 hr before falling). These patterns are controlled by the pulsatile release of hypothalamic gonadotropin releasing hormone (GnRH) and also by positive and negative neural feedback signals arising from actions of ovarian steroids in both the hypothalamus and pituitary. The pituitary hormones, especially LH, are the primary stimuli for ovarian secretion. Although plasma

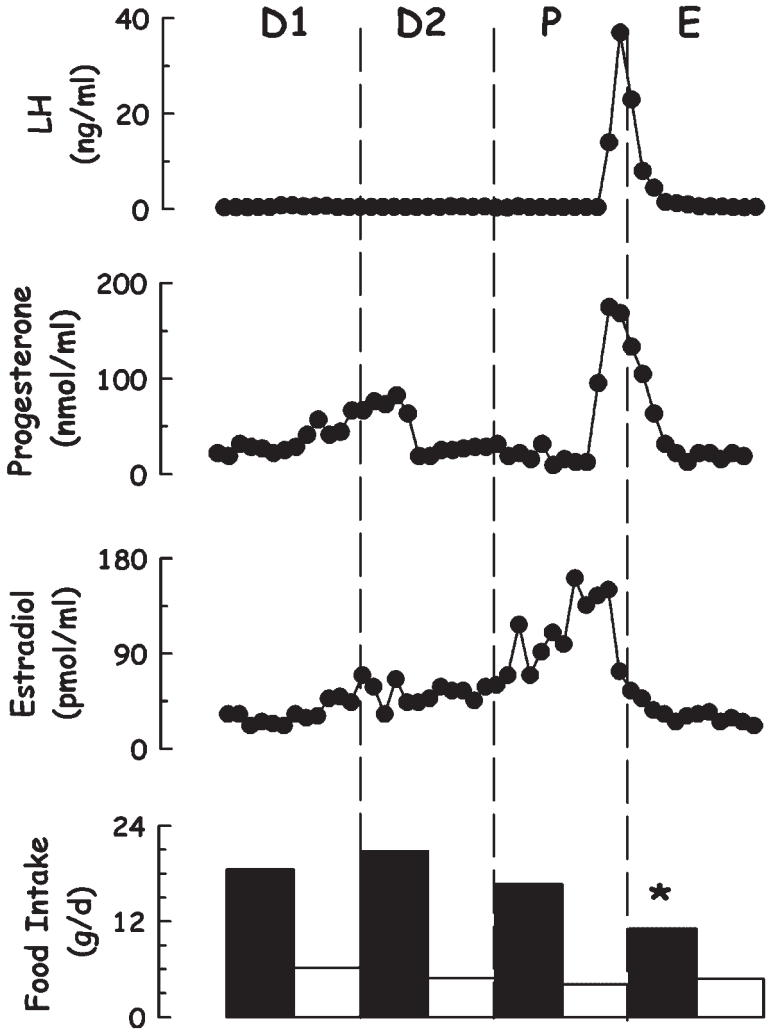

Figure 1. Changes in plasma hormone concentrations (upper three panels) and in food intake (lowest panel) during the estrous cycle of the rat. The days of the cycle are labeled beginning at dark onset as diestrus 1 (D1), diestrus 2 (D2), proestrus (P), and estrus (E). The widths of the bars in the bottom panel represent the daily 12-hr dark (filled) and light (unfilled) periods. The heights of the bars indicate nocturnal and diurnal food intakes.

Note: *Significantly different from nocturnal food intake in each other day. (Hormone data are adapted from Smith, Freeman, & Neill, 1975; food intake data are from Eckel *et al.*, 2000; used with permission.)

LH concentration remains at a constant, low level through the first 3 days of the cycle, it nonetheless stimulates a progressive increase in estradiol secretion. The pre-ovulatory LH surge then drives estradiol secretion rapidly back to its nadir. Because HPG function is tightly linked to the circadian system (Kriegsfeld, LeSauter, Hamada, Pitts, & Silver, 2002), the LH surge always occurs around dusk and the cycle period is a whole number of days. Either the pre-ovulatory or the postovulatory phase of the cycles may be lengthened in cycles with periods greater than 4 days.

In many mammals there are obvious increases in locomotor activity, general arousal, and sexual receptivity associated with the peri-ovulatory phase of the ovarian cycle. For this reason, Heape (1901) coined the term *estrus*, from the Greek *oistros*, denoting the frenzy that ensues from a bee sting, to describe this phase.

The ovarian cycle in animals that display estrus is known as the estrous cycle. In rats, the period of increased sexual receptivity lasts about 16 hr, beginning near dusk on proestrus, through the ensuing dark period, and ending early in the following diurnal period (Blandau *et al.*, 1941; Drewett, 1973).

The standard divisions of the estrous cycle are based on changes in vaginal cytology (see Freeman, 1994, for an authoritative review). The initial phase, diestrus, is characterized by leukocytes and increasing numbers of nucleated epithelial cells and lasts about 55–57 hr in rats. The disappearance of the leukocytes marks proestrus, which lasts 12–14 hr. Estrus is defined by the predominance of cornified squamous epithelial cells and lasts about 25–27 hr. Finally, during metestrus, which lasts only 6–8 hr, cornified epithelial cells disappear and leukocytes appear. By convention, the 4 days of the typical cycle are named diestrus 1, diestrus 2, proestrus, and estrus, based on the dominant diurnal vaginal cytology. This convention can be confusing for two reasons. First, the duration of behavioral estrus, that is, of increased sexual receptivity, is several hours shorter than the duration of estrous vaginal cytology (Blandau *et al.*, 1941; Drewett, 1973; ter Haar, 1972). Second, if days are labeled from midnight to midnight, behavioral estrus and cytological estrus are spread between two different days, the evening of nominal proestrus and the first part of nominal estrus. To avoid this latter problem here, estrous cycle days are defined as the 24-hr period beginning at the onset of the dark phase.

Eating During the Estrous Cycle. Slonaker (1925) first reported that rats eat less during estrus. Brobeck, Wheatland, and Strominger (1947) and Kennedy and Mitra (1963) considered the simultaneous increase in activity and decrease in food intake to be an interesting challenge to the normal equilibrium of energy exchange. Whether this transient perturbation of energy balance actually provokes any homeostatic counter-regulatory response has never been demonstrated. The small but reliable change in body weight during estrus appears to reflect the decreased weight of ingesta, especially water, rather than a change in body fat content (Tarttelin & Gorski, 1971).

Figure 1 (lower panel) shows typical diurnal and nocturnal food intakes through the 4 days of the estrus cycle in the rat. Note that (1) plasma estradiol concentration begins to increase during diestrus 2 and reaches a maximum during proestrus, whereas eating decreases only during estrus, 2 days later, and (2) estradiol concentration has already decreased before eating changes. Thus, eating decreases after estradiol concentration increases, not during the period of the increase. Nevertheless, these data are consistent with the idea that estradiol is the cause of the decreased eating. This conclusion is supported by observations that most physiological consequences of estradiol are caused by binding of complexes of estradiol and intracellular estradiol receptors to estradiol response elements on the DNA, whose activation leads to increased expression of many genes and consequently changes synthesis of many proteins in target tissues throughout the periphery and the brain. These physiological cascades typically require from 12 hr to a day or two. Thus, they appear likely to account for the latency between increases in estradiol secretion and most of its physiological and behavioral effects (Blaustein & Erskine, 2002; Pfaff, 1999; Pfaff, Schwartz-Giblin, McCarthy, & Kow, 1994). For example, in ovariectomized rats, estradiol administration induces increases in neuronal progestin receptors and in the ability of progesterone injection to increase sexual receptivity about 18 hr later (Pfaff, 1999; Pfaff *et al.*, 1994). Thus, assuming

a similar phase delay, the decrease in eating during estrus can plausibly be attributed to increased estradiol secretion during the preceding diestrus and proestrus. As described below, tests of estradiol administration in ovariectomized rats also support this hypothesis.

In most studies, rats offered separate sources of carbohydrate, fat, and protein have decreased total energy intake during estrus (Geiselman, Martin, VanderWeele, & Novin, 1981; Heisler, Kanarek, & Homoleski, 1999; Leibowitz, Akabayashi, Alexander, & Wang, 1998; but see Bartness & Waldbillig, 1984). Perhaps because different macronutrient sources were used, however, there was no unanimity among these studies as to whether intake of any specific macronutrient is preferentially affected.

Women

Physiology of the Menstrual Cycle.

The ovarian, or menstrual, cycle of women has a mean duration of 28 days, with periods between 15 and 45 days common (Chiazze, Brayer, Macisco, Parker, & Duffy, 1968; Griffin & Ojeda, 2000; Hotchkiss & Knobil, 1994). The cycle is divided into four phases: (1) the follicular phase, which is characterized by endometrial proliferation and development of the ovarian follicle; (2) the ovulatory phase, during which LH and FSH surge, the follicle ruptures, and ovulation begins; (3) the luteal phase, corresponding to the secretory phase of the endometrium and development of the corpus luteum; and (4) menstruation, or shedding of the endometrium, a process unique to the Old World monkeys, apes, and humans. The follicular stage has the most variable duration, with 8–14 days between the end of menstruation and the LH surge. Ovulation is completed 2–4 days after the LH surge. The 4 days surrounding the LH surge often are labeled the peri-ovulatory phase. The luteal phase has the most constant length, 14 days, and menstruation typically lasts 4–5 days. These phases are also associated with characteristic changes in vaginal cytology.

The changes in plasma levels of LH, progestins, and estradiol during the menstrual cycle in women are shown in Figure 2 (upper three panels). Although there are striking differences between the patterns of hormone levels across the rat and human cycles, the underlying neuroendocrine control system appears very similar in both species (Freeman, 1994; Hotchkiss & Knobil, 1994; Richards, Russell, Ochsner, & Espey, 2002).

Eating During the Menstrual Cycle.

Food intake also varies in women across the menstrual cycle. Figure 2 (lower two panels) shows data from two careful studies in which food intake was measured by weighing and ovarian cycling was monitored by measurements of LH or of body temperature. As do rats and many other animals, cycling women eat least during the peri-ovulatory period. Maximum food intake, however, does not occur earlier in the cycle, as in rats, but later, in the luteal phase. Average daily food intake during the follicular phase often has been reported to be less than during the luteal phase (see Figure 2 for an example). Because estradiol levels begin to increase during the last several days of the follicular stage, it might be expected that food intake would decrease gradually during the late follicular period before reaching a nadir around ovulation. Such a progressive decrease has not been reported in women. This finding may be an artifact of data averaging; the representative individual data displayed by Gong, Garrel, and Calloway (1989) do show a gradual decrease in food intake through the follicular

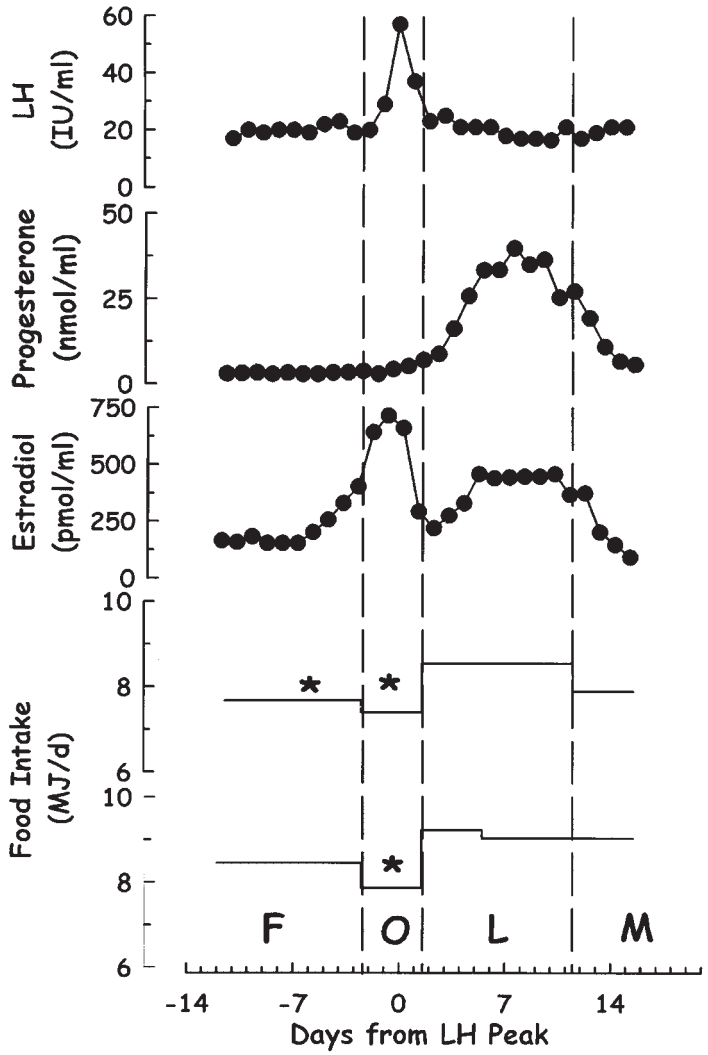

Figure 2. Changes in plasma hormone concentrations (upper three panels) and in food intake (lower two panels) during the menstrual cycle in women. The phases of the cycle are labeled as follicular (F), peri-ovulatory (O), luteal (L), and menstrual (M). Hormone concentrations are aligned with the LH (day 0); progestins are sums of 17-OH progesterone and progesterone. Food intake data (lower two panels) are shown as the average daily intake over the periods measured.

Note: *Significantly different from food intake during the luteal phase. (Hormone data are adapted from Thorneycroft, Mishell, Stone, Kharma, & Nakamura, 1971, and Ross *et al.*, 1970; food intake data are from, upper, Gong *et al.*, 1989, and, lower, Lyons, Truswell, Mira, Vizzard, & Abraham, 1989; used with permission.)

phase. Progressive decreases in eating through the follicular phase have been reported in Old World monkeys, which have similar gradual increases in estradiol through the follicular phase (Czaja, 1978; Rosenblatt, Dyrenfurth, Ferin, & VandeWiele, 1980). The follicular/peri-ovulatory decrease in food intake in women is substantial. In the two studies shown in Figure 2, women ate about 0.8 mJ/day (190 kcal/day) less during the follicular and peri-ovulatory phases than during the luteal and menstrual phases. Integrated over the 10–18-day duration of these phases of the menstrual cycle, this difference in intake is sufficient to affect energy

balance and adiposity. This apparent effect of cyclic changes in eating on energy balance contrasts with the energetically insignificant cyclic increase in energy expenditure during the luteal phase of the cycle in women (Bisdee, James, & Shaw, 1989; Tai, Castillo, & Pi-Sunyer, 1997).

Food intake is sometimes larger during the luteal phase than during the menstrual or early follicular phases in women (Buffenstein, Poppit, McDevitt, & Prentice, 1995; Dye & Blundell, 1997; Fong & Kretsch, 1993) and nonhuman primates (Czaja, 1978). This increase in eating is not closely associated with estradiol secretion because, after a brief decrease in estradiol secretion in the first third of the luteal phase, estradiol levels are elevated for the remainder of the luteal phase. A second cyclic decrease in eating might be expected to accompany this increase in estradiol, but no such effect has been reported either in women or in nonhuman primates (Bielert & Busse, 1983; Czaja, 1978; Kemnitz, Gibber, Lindsay, & Eisele, 1989; Krohn & Zuckerman, 1937; Rosenblatt *et al.*, 1980). The increase in eating during the luteal phase has been attributed to increased plasma progesterone, but, as described below, there is little support for this suggestion.

Although there are very few studies of eating during the menstrual cycle in women that are as well controlled as those shown in Figure 2, similar patterns have been seen in studies using less reliable or sensitive methods to measure eating (e.g., food diaries or retrospective self-reports) and ovarian cycling (e.g., counting 14 days backwards from menses to identify ovulation) (see Buffenstein *et al.*, 1995; Dye & Blundell, 1997, for comprehensive reviews). Women's macronutrient selection during the ovarian cycle has been investigated in several such studies, but, as with rats, no clear trend has emerged (for reviews, see Barr, Janelle, & Prior, 1995; Dye & Blundell, 1997; Rogers & Smit, 2000). Similarly, there is no clear evidence that shifts in macronutrient selection occur in women with eating disorders (Dye & Blundell, 1997; Yanovski, 2003).

Eating Following Interruption of Ovarian Cycling

Rats and mice

In mice, rats, nonhuman primates, and many other species, interruption of the ovarian cycle, usually by surgical ovariectomy, causes food intake to increase to a level that is higher than that displayed at any phase of the normal cycle (Blaustein & Wade, 1976; Drewett, 1973; Houpt, 1991; Kemnitz *et al.*, 1989; Wade, 1972). The increased eating induced by ovariectomy is associated with increased adiposity; mice and rats typically increase body weight by 10–25% over a month or so (Leshner & Collier, 1973; Wade & Gray, 1979; Wade, Gray, & Bartness, 1985; Wallen, Belanger, & Wittnich, 2001). Figure 3 shows typical data. In rats, food intake returns to near normal 1–2 months postovariectomy, but the increase in body weight is maintained permanently (Blaustein & Wade, 1976; Wallen *et al.*, 2001).

As Drewett (1973) originally pointed out, comparison of food intake in intact, cycling, and ovariectomized rats suggests that normal HPG function exerts two inhibitory influences on eating: a cyclic (or phasic) inhibition that causes the minimal peri-ovulatory level of eating, and a tonic inhibition that causes the decreased eating during the rest of the ovarian cycle in comparison to what occurs in ovariectomized animals (Figure 4). Subsequent sections of this review indicate that both of these inhibitions are caused by estradiol and that they have partially independent

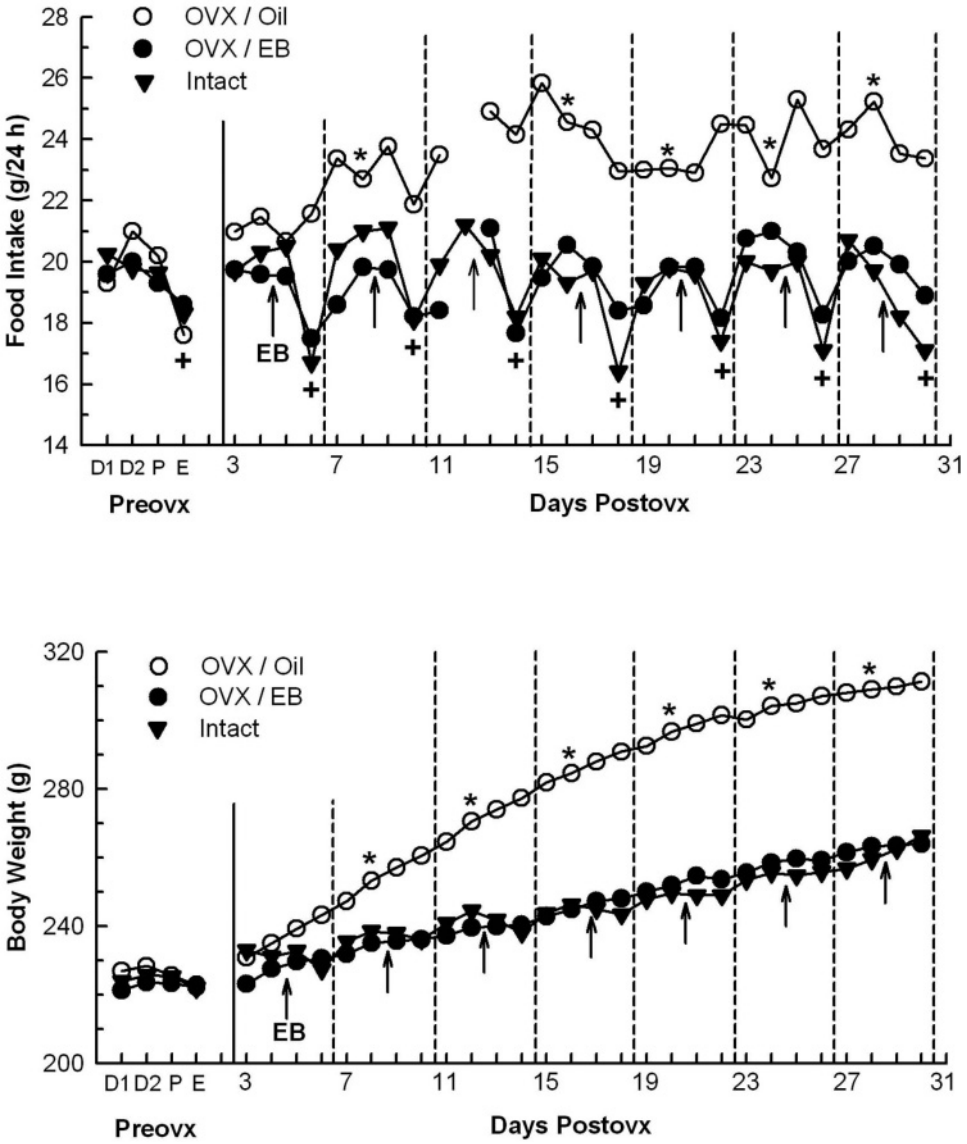

Figure 3. Normalization of daily food intake (upper panel) and body weight gain (lower panel) in ovariectomized rats by a near-physiological cyclic regimen of estradiol treatment. Data to left of solid vertical line are from the last ovarian cycle pre-ovariectomy (Preovx; D1, diestrus 1; D2, diestrus 2; P, proestrus; E, estrus). Data to the right of the solid vertical lines are intact rats, ovariectomized rats that received estradiol benzoate (EB, 2 μg injected subcutaneously in 100 μl sesame oil once each fourth day, indicated by arrows), and ovariectomized rats that received only the oil vehicle. Dashed vertical lines divide 4-day treatment cycles, aligned so that the last day of each cycle is the second day after estradiol treatment, corresponding to estrus in intact rats.

Note: *Significantly different from estradiol-treated rats and from intact rats (contrasts between D2 in intact rats and day 2 of the treatment cycle in ovariectomized rats); +E or day 4 value significantly different from D2 or day 2 of the same cycle. (From Asarian & Geary, 2002; used with permission.)

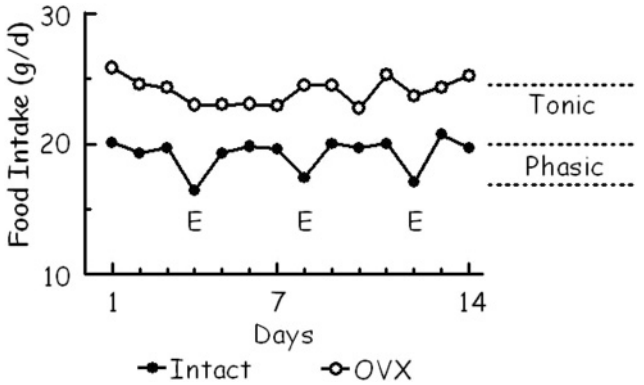

Figure 4. The tonic and phasic estrogenic inhibitions of eating in rats. The phasic inhibition is revealed by the cyclic decrease in food intake that occurs in cycling female rats during estrus (E), usually each fourth day. The tonic inhibition is revealed by the increase in food intake in ovariectomized rats in comparison to the higher, relatively constant level of food intake during non-estrus days of the ovarian cycle. These concepts were suggested originally by Drewett (1973). (Data from Eckel, Houpt, & Geary, 2000; used with permission.)

mechanisms. Therefore, I call them here the tonic and phasic estrogenic inhibitions of eating.

Women

Eating following ovariectomy in women (usually referred to as oophorectomy) has not been studied. Acute failure of normal ovarian function, however, appears to prevent the cyclic decrease in eating. In women who displayed intermittent anovulatory cycles, food diaries indicated that cyclic differences in eating occurred during cycles in which the women ovulated but not during cycles in which they did not ovulate (Barr *et al.*, 1995; Rock, Gorenflo, Drewnowski, & Demitrack, 1996). The normal cyclic variation in food intake was also absent in women with high dietary restraint, that is, women who reduce intake by exercising strong cognitive inhibition of eating (Dye & Blundell, 1997; Schweiger *et al.*, 1992). Whether this lack of peri-ovulatory decreases in eating in restrained women is associated with reductions in plasma estradiol concentrations has not been established, however, so these data provide no direct support for or against the hypothesis that estradiol is sufficient to account for the cyclic variation in eating during the menstrual cycle. Similarly, plasma estradiol measurements could potentially illuminate the inconsistent effects of hormonal contraceptive treatments on eating (Eck *et al.*, 1997; Pelkman, Chow, Heinback, & Rolls, 2001).

At first glance, the menopausal transition seems to provide a natural experiment testing the role of permanent interruption of ovarian function on eating and body weight in women. But the collection and interpretation of menopausal data are very complicated. For example, (1) the menopause is a long, gradual process rather than an abrupt transition (Wise, 1999), (2) estradiol secretion does not cease abruptly following the last menses but decreases gradually for at least a year (Longcope, 2001), and (3) menopause is accompanied by metabolic, behavioral, and psychological changes that may affect eating (reviewed in Poehlman, 2002). Thus, it is not surprising that there are no reports that menopause or postmenopausal hormone therapy affect eating.

Modest increases in total body adiposity and shifts of fat distribution toward the visceral pattern do accompany menopause (Hernandez-Ono *et al.*, 2002; Lovejoy, 2003; Pasquali *et al.*, 1994; Poehlman, 2002; Ruebinoff *et al.*, 1995). The increase in adiposity is only a few kilograms and has little effect on body weight because lean body mass decreases by a similar amount. The increased adiposity is mainly in the form of visceral fat, however, and measurably increases risk for diabetes and coronary diseases. The effects of hormone therapy on body composition and body fat distribution in postmenopausal women remain controversial (Hassager & Christiansen, 1989; Poehlman, 2002; Sumino *et al.*, 2003).

In summary, studies of changes in ovarian function (1) support the hypothesis that estradiol phasically inhibits eating during the peri-ovulatory phase of the ovarian cycle in women as it does in rats and many other species, but (2) do not support the hypothesis that estradiol tonically inhibits eating during the rest of the cycle in women as it does in many animals, and (3) do not support the hypothesis that estradiol has the same marked effect on body weight in women as it does in many species, although it may well have important effects on body fat distribution and lipid metabolism.

Spontaneous Meal Patterns in Cycling and Ovariectomized Animals

The ovarian inhibition of eating in rats and mice is expressed solely as decreases in meal size. There are three important observations. (1) The peri-ovulatory decrease in food intake in rats and mice is due only to decreased meal size (Asarian & Geary, 2002; Blaustein & Wade, 1976; Drewett, 1974; Eckel, Houpt, & Geary, 2000; Petersen, 1976; Rashotte, Ackert, & Overton, 2002). Indeed, meal frequency usually *increases* during estrus, although seldom sufficiently to prevent total food intake from being decreased. An interesting exception occurs in the Fisher 344 rat, in which meal frequency does increase sufficiently during estrus to compensate for the decreased meal size; this effect may occur because of the low energy reserves in these small and lean rats (Varma *et al.*, 1999). (2) The increase in food intake produced by ovariectomy is caused only by increased meal size (Asarian & Geary, 2002; Blaustein & Wade, 1976; Kenney & Mook, 1974). In this case, meal frequency decreases. (3) The change in meal size produced by ovariectomy is permanent. Blaustein and Wade (1976) showed that total food intake returned to the control level about a month after ovariectomy, but that this was due to a decrease in meal frequency with no correction of the increased meal size. Because meal size and meal initiation are under partially independent control (i.e., Geary & Schwartz, 2004; Geary & Smith, 2000; Smith, 1998; Woods & Stricker, 2003; Schwartz, Woods, Porte, Seeley, & Baskin, 2000), the selectivity of the ovarian inhibition of eating on meal size has directed the analysis of mechanism, as described below.

That meal frequency decreases in ovariectomized rats only after body weight has increased suggests that this change is stimulated by increased adiposity and is independent of estradiol's effect on meal size. This hypothesis leads to the prediction that if rats were made obese prior to ovariectomy, meal size would increase as usual postovariectomy but meal frequency would decrease immediately, so that there would be little effect on total daily food intake. Noel and Fleming (1977) showed that inducing overweight by force-feeding did reduce the hyperphagia and

weight gain produced by ovariectomy, but they did not measure spontaneous meal patterns.

Estradiol and the Ovarian Inhibition of Eating

The Estrogenic Inhibition of Eating

Both the phasic (cyclic) and tonic inhibitions of eating during the ovarian cycle appear to be solely due to a physiological inhibition of eating by estradiol. As described above, increased estradiol precedes the peri-ovulatory (or estrous) decrease in eating. There is a similar negative association between estradiol level and eating following ovariectomy (which, as noted above, is maintained indefinitely when meal size rather than total food intake is considered). Furthermore, estradiol administration is sufficient to normalize eating and body weight in ovariectomized animals (Blaustein & Wade, 1976; Geary & Asarian, 2000; Tarttelin & Gorski, 1973; Wade, 1972; Wade & Gray, 1979). The inhibitory effect of estradiol is dose related in the physiological range and in the pharmacological range (Wade, 1975; Wallen *et al.*, 2001). Finally, the most crucial result is that a cyclic regimen of estradiol treatment, which produced a near-normal 4-day cycle of plasma estradiol levels in ovariectomized rats, produced nearly normal tonic and cyclic decreases in spontaneous meal size and frequency, daily food intake, and body weight (Asarian & Geary, 2002). Some of these data are shown in Figures 3 and 5. It would be possible to use pharmacological suppression of ovarian secretion and hormone treatment to conduct similar studies in women, as has been elegantly done in the analysis of premenstrual syndrome (Rubinow, Schmidt, Roca, & Daly, 2002; Schmidt, Nieman, Danacesu, Adams, & Rubinow, 1998).

Other HPG Hormones

There are no data indicating that any of the other principal HPG axis hormones, GnRH, LH, FSH, progesterone, prolactin, and testosterone, mimic or mediate the effects of estradiol. Thus, estradiol appears to be the necessary as well as the sufficient contribution of HPG function to the control of eating during the ovarian cycle.

GnRH, LH, FSH, and Prolactin. Although increases in levels of GnRH in the hypothalamus and of LH, FSH, and prolactin in the plasma all correlate with the peri-ovulatory decrease in food intake during the ovarian cycle, they are very unlikely to cause it. First, following ovariectomy, plasma levels of LH, FSH, and prolactin all increase due to the lack of feedback control normally exerted by estradiol. At least some of this increase is thought to be due to increased GnRH (Freeman, 1994). Thus, if any of these hormones inhibited eating, then eating should decrease in ovariectomized rats, not increase. Second, elimination of LH, FSH, and prolactin by hypophysectomy does not increase food intake and body weight, unlike the effect of eliminating estradiol by ovariectomy (Wade & Zucker, 1970a). Furthermore, estradiol still inhibits eating in hypophysectomized rats, indicating that neither GnRH, LH, FSH, nor prolactin is necessary for the inhibitory effect of estradiol on eating (Wade & Zucker, 1970a).

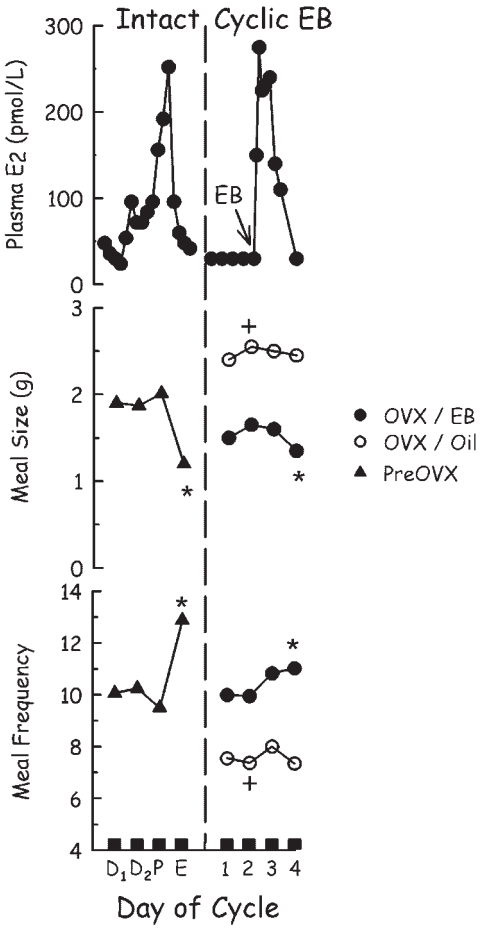

Figure 5. Cyclic estradiol treatment is sufficient to produce normal cyclic and tonic spontaneous feeding patterns in ovariectomized rats. Top panel shows plasma estradiol concentrations, left, during the estrus cycle and, right, after subcutaneous injection of 2 μg estradiol benzoate on day 2 (arrow). Middle panel shows mean spontaneous meal size during the dark period, left, in intact cycling rats (triangles) and, right, in estradiol-treated (filled circles) and untreated (open circles) ovariectomized rats. Lower panel shows corresponding mean spontaneous meal frequencies. Meal pattern data are averages of six cycles of estradiol treatment (cycles 2–7 in Figure 4).

Note: Abbreviations: see cycles 2–7 in Figure 3.
+Meal size and frequency in oil-treated ovariectomized rats significantly different from that in intact rats and estradiol-treated ovariectomized rats (contrasts between D2 in intact rats and day 2 of the treatment cycle in ovariectomized rats);
*Meal size and frequency on E or day 4 significantly different from D2 or day 2 in the same group. (Estradiol concentrations in intact rats from Smith *et al.*, 1975; other data from Asarian & Geary, 2002; used with permission.)

Prolonged peripheral or central treatment with prolactin stimulates eating (Heil, 1999; Noel & Woodside, 1993). But increased levels of prolactin cannot account for the entire effect of ovariectomy because (1) ovariectomized rats increase food intake within a few days, whereas about 10 days of treatment with prolactin is required before eating increases, and (2) prolactin treatment is equally effective in intact rats with normal estradiol levels and ovariectomized rats lacking estradiol (Heil, 1999), suggesting that the two phenomena are distinct. The latter finding also indicates that the disruption of ovarian cycling caused by prolactin

treatment in intact rats does not increase eating by releasing it from the normal inhibitory effect of estradiol. Prolactin may be necessary to maintain normal levels of eating by both male and female animals because transgenic mice lacking prolactin receptors do not maintain normal levels of body weight in adulthood (Freemark, Fleenor, Driscoll, Binart, & Kelly, 2001). In contrast, it may be noted that prolactin releasing peptide (PrRP) has been reported to decrease eating in male rats (Lawrence, Celsi, Brennand, & Luckman, 2000; Lawrence, Ellacott, & Luckman, 2002; Seal *et al.*, 2001). It is not clear, however, whether this is a sexually differentiated phenomenon or, indeed, whether PrRP plays a physiological role in HPG axis function (Lawrence *et al.*, 2000; Maruyama *et al.*, 1999).

Progesterone. Although pharmacological doses of progesterone can reverse the inhibitory effect of estradiol on eating, progesterone in physiological amounts or patterns does not appear to have any effect on eating in rats (Geary, Trace, McEwen, & Smith, 1994; Wade, 1972, 1975) or in nonhuman primates (Bielert & Busse, 1983; Cjaza, 1978; Kemnitz *et al.*, 1989). These data fail to support the hypothesis that progesterone causes the increases in food intake that occur during the luteal phase of the menstrual cycle in nonhuman primates and women.

Testosterone. Testosterone also affects eating. Orchiectomy at least transiently decreases food intake and permanently decreases body weight in rats and mice, and testosterone treatment partially reverses these effects (Chai *et al.*, 1999; Gentry & Wade, 1976; Petersen, 1978; Slusser & Wade, 1981; Wallen *et al.*, 2001). The comparatively small magnitude of the decrease in food intake suggests that metabolic changes contribute importantly to the weight change. All these phenomena are reminiscent of the effects of ovariectomy and estradiol treatment, except opposite in sign. Meal pattern analysis, however, indicates that the effects of testosterone and estradiol on eating differ more fundamentally: ovariectomy and estradiol selectively affect meal size, whereas orchiectomy and testosterone selectively affect meal frequency (Chai *et al.*, 1999).

Orchiectomy also decreases locomotor activity, and this effect is reversed by testosterone treatment. This action of testosterone appears to be due to the aromatization of testosterone to estradiol because (1) estradiol stimulates activity about 100 times more potently than testosterone in orchiectomized rats, (2) treatment with a non-aromatizable form of testosterone has no effect on activity, and (3) treatment with an estradiol antagonist blocks the effect of testosterone on activity (Gentry & Wade, 1976).

Development of the Estrogenic Inhibition of Eating

HPG axis hormones have organizational and activational effects that contribute to sexually differentiated structures and functions (Gorski, 2000). Organizational effects refer to permanent consequences of HPG hormones acting during early development that are expressed subsequently independent of hormone levels. In contrast, activational effects depend on ongoing hormonal signaling and vary depending on hormonal milieu. Of course, activational effects later in life may depend on morphological substrates resulting from earlier organizational effects.

Estradiol's inhibitory actions on eating appear to be activational effects. Exogenous estradiol inhibits feeding only in postpubertal rats (Wade, 1974;

Wade & Zucker, 1970). This observation suggests that the estrogen receptor (ER) mechanism mediating the estrogenic inhibition of eating is not competent prior to puberty, which occurs in rats at about 35–40 days of age. This hypothesis parallels that invoked to explain the development of ovarian cycling, which in rats and women appears to depend both on increases in the ovary's capacity to secrete estradiol and on the magnitude of estradiol's positive feedback effect on LH secretion (Ojeda & Urbanski, 1994). Another possibility is that the brain substrate for the estrogenic inhibition of eating is complete in prepubertal rats, but inhibitory influences are overwhelmed by unusually potent stimulatory controls of eating. The high level of growth hormone in juvenile rats may produce such a stimulatory control. Food intake, expressed as gram eaten per gram body weight, is at the highest level of the life span between weaning and puberty (Kennedy, 1969; Sieck, Nance, Ramaley, Newman-Taylor, & Gorski, 1977). When juvenile rats are hypophysectomized, their daily food intakes and growth rates drop precipitously and, despite the low level of food intake, estradiol effectively inhibits eating (Wade & Zucker, 1970). Treatment with growth hormone blocks estradiol's effect (Wade, 1974). Also consistent with the hypothesis that the substrate for the estrogenic inhibition of eating is present in juvenile rats is the finding that rats display 4-day cyclic decreases in meal size between 21 days of age and puberty (Sieck *et al.*, 1977). These decreases, however, were more than offset by increases in meal number, so that total food intake was cyclically *increased*. The normal adult eating pattern appeared rapidly after puberty. The juvenile eating pattern depends on normal HPG function because it was eliminated by ovariectomy.

Estradiol and Eating, Locomotor Activity and Sexual Receptivity

Estradiol's stimulatory effect on locomotor activity does not appear to cause its inhibitory effect on eating. (1) The increase in meal frequency during estrus mitigates against the possibility that animals eat less because they are diverted from eating by their increased general activity. If this were the case, then such interfering stimuli would be expected to reduce the frequency of meal initiation as much or more than they affect meal size. (2) Increasing the opportunity for locomotor activity by giving rats access to running wheels did not change the magnitude of the decrease in meal size during estrus (Eckel, Houpt, & Geary, 2000). (3) Estradiol may act in the medial preoptic areas (MPAs) to increase activity (Fahrbach, Meisel, & Pfaff, 1985), but it does not appear to act there to decrease eating (see below).

The cyclic decrease in eating during estrus cannot be caused by increased sexual behavior because estradiol is sufficient for the former whereas estradiol and progesterone are jointly necessary for the latter. Furthermore, the period of decreased eating during estrus usually ends a few hours before the end of the nocturnal period, that is, before the end of the period of increased sexual receptivity (Drewett, 1973). Whether increased sexual behavior interferes with the decrease in eating has apparently never been investigated in rats or mice, but studies of other animals (Houpt, 1991; Hurnik, King, & Robertson, 1975), including baboons (*Papio ursinus*) in the wild (Bielert & Busse, 1983), indicate that sexual behavior and other social interactions do not prevent the decrease in eating during estrus.

Mice and rats typically increase body weight by 10–25% within a month of ovariectomy, mainly in the form of increased adipose tissue (Leshner & Collier, 1973; Wade & Gray, 1979; Wade *et al.*, 1985). Although the exact contributions of estradiol's effects on eating, locomotor activity, and metabolism to weight gain in ovariectomized rats have not been simultaneously delineated, the increased feeding alone usually appears sufficient to account for the weight changes. Richard (1986) measured eating in ovariectomized rats that received subcutaneous implants of either estradiol or vehicle for 36 days prior to carcass analysis. Vehicle-treated rats ate more and gained more weight than estradiol-treated rats, which maintained normal weight, and the ovariectomized rats' surplus energy intake (i.e., metabolizable content of the food minus that of feces) was more than enough to account for the increase in energy stored (including standard estimates of the cost of deposition of lean and adipose tissue). This comports well with the estimates of Asarian and Geary (2002), Chen and Heiman (2001), and Ainslie *et al.* (2001). In the latter study, the ovariectomy-induced increase in body weight was temporally associated with eating but not with locomotor activity or resting energy expenditure (measured by indirect calorimetry). That is, weight gain and food intake each were increased only in the first weeks post-ovariectomy, whereas locomotor activity was decreased similarly during and after the period of weight gain, and resting energy expenditure was unchanged throughout.

The story is sometimes more complicated. In some situations, ovariectomized rats that are pair-fed to estradiol-treated rats still gain weight, sometimes as much as *ad libitum* fed ovariectomized rats (Mueller & Hsiao, 1980; Roy & Wade, 1977). This result suggests that preventing ovariectomized rats from overeating can unmask some other effect that is sufficient to increase body weight. This other effect presumably is in metabolism rather than in physical activity because even *ad libitum* fed ovariectomized rats are very inactive. But it remains unclear what this metabolic effect is, how it is recruited by pair-feeding, and why it does not always occur (e.g., Toth, Poehlman, Matthews, Tchernoff, & MacCoss, 2001). The macronutrient composition of the diet may elicit a similar metabolic response: when ovariectomized rats were fed high-fat food, energy intake did not increase but body weight did (Bartness & Waldbillig, 1984).

Receptor Mechanisms in the Estrogenic Inhibition of Eating

Estrogen Receptor Type

Introduction. The "classical" nuclear ER, whose gene was cloned in 1985 (Green *et al.*, 1985), was renamed ERα when a second nuclear ER, ERβ, was discovered 10 years later (Kuiper, Enmark, Pelto-Huikko, Nilsson, & Gustafsson, 1996). Transgenic mice with mutations for either ERα or ERβ, known as αERKO and βERKO mice, respectively, have been produced (Krege *et al.*, 1998; Lubahn *et al.*, 1993) and used extensively in the analysis of estradiol's biological actions (Couse & Korach, 1999; Pollard, 1999). Results to date suggest that ERα is necessary for all of estradiol's genomic actions and that ERβ exerts modulatory roles (Couse & Korach, 1999; McDonnell & Norris, 2002). Rapid, nongenomic actions of estradiol

also have been described, and some of them appear to involve ERβ (Abraham, Han, Todman, Korach, & Herbison, 2003; McEwen & Alves, 1999).

αERKO and βERKO mice have been used in two types of investigations of estradiol's role in eating and adiposity, studies of the action of exogenous estradiol and longitudinal studies of changes in eating and adiposity through development. The former have been more informative than the latter. Furthermore, the role of ERα polymorphisms in human weight control has begun to attract attention.

Effects of Estradiol in ER-Deficient Mice. We (Geary, Asarian, Korach, Pfaff, & Ogawa, 2001) tested the effects of chronic estradiol treatment, by implantation of subcutaneous time-release pellets, in ovariectomized αERKO and βERKO mice. Figure 6 shows the results. Wild-type mice that did not receive

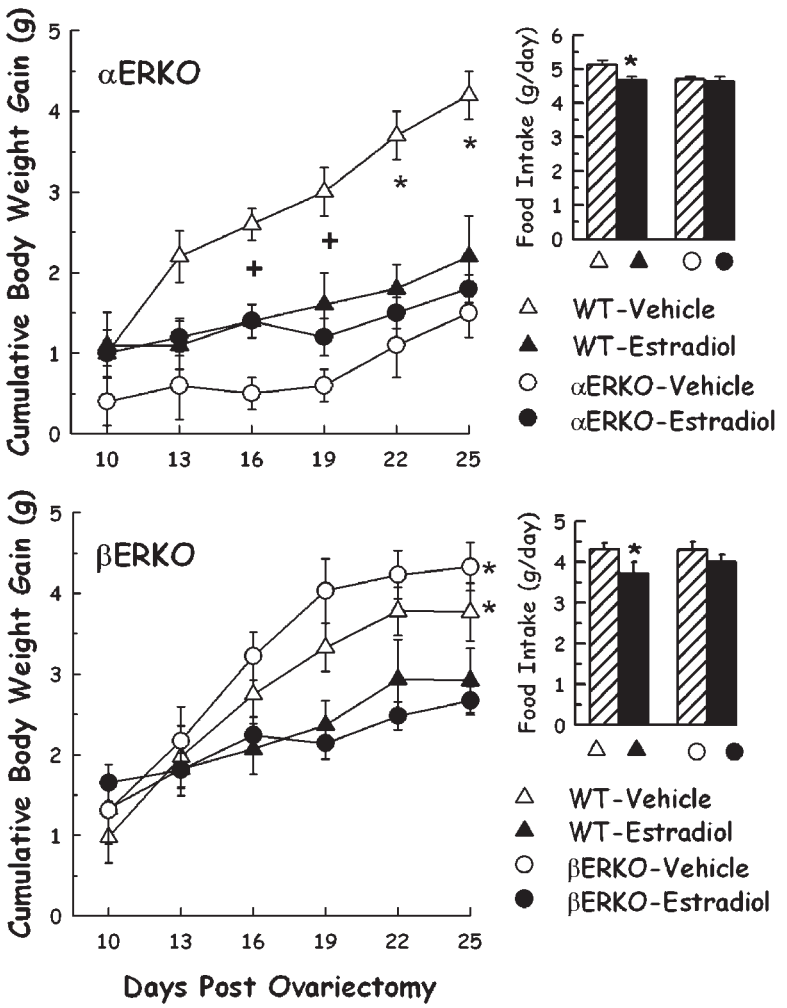

Figure 6. Chronic estradiol treatment reduces food intake and body weight gain in ovariectomized wild-type mice and βERKO mice (upper panels), but not in ovariectomized αERKO mice (lower panels). Time-release estradiol pellets or vehicle pellets were implanted subcutaneously during ovariectomy. Data are average daily food intakes and cumulative body weight gains through 2 weeks, beginning 10 day post-operatively, when both wild-type and ERKO mice had regained their preoperative weights.

Note: *Significantly less than vehicle-treated mice. (From Geary *et al.*, 2001; used with permission.)

estradiol increased food intake and body weight gain more than estradiol-treated wild-type mice, whereas food intake and body weight gain were not different in estradiol-treated and untreated αERKO mice. In contrast, food intake and body weight gain were similar in estradiol-treated wild-type and βERKO mice. These data indicate that (1) ERα is necessary and sufficient for the normal inhibitory effects of estradiol on eating and body weight in adult female mice, and (2) ERβ is neither necessary nor sufficient for either effect. The latter conclusion does not, of course, rule out a participatory role for ERβ in the normal physiology of these functions.

These studies of adult αERKO and βERKO mice do not distinguish the activational actions of estradiol from the organizational effects of estradiol. Whether disturbances in estradiol's normal organizational actions affect the eating behavior of αERKO or βERKO mice could be investigated by producing temporary, reversible inactivation of ERα or ERβ at different developmental stages.

Adiposity in ER-Deficient Mice. The adiposity phenotype of the αERKO mouse is complicated, perhaps due to disrupted organizational effects of estrogen-sensitive systems early in development. Both male and female αERKO mice display increased body weight and adiposity by 3 months of age (Heine, Taylor, Iwamoto, Lubahn, & Cooke, 2000; Ohlsson *et al.*, 2000). Adiposity was also increased in male and female aromatase-deficient mice, in which biosynthesis of estradiol is prevented (Jones *et al.*, 2000). Thus, estradiol appears to have important effects on the development of adiposity in both male and female mice. The cause of the phenomenon is unclear. It may be related to a metabolic effect of estradiol because the overweight was associated with reduced energy expenditure (Heine *et al.*, 2000; Ohlsson *et al.*, 2000). Overeating does not appear causal to adiposity. No increase in eating in αERKO females has been reported, and aromatase-deficient mice of both sexes actually decreased eating. Furthermore, although estradiol does circulate in males, it does not seem to produce the same effects on eating as it does in females. Estradiol levels are much lower in males, and estradiol treatment has been reported to produce less decrease in body weight in males than in females (and in androgenized females; Bell & Zucker, 1971), or to actually increase weight in males (i.e., Wallen, Belanger, & Wittnich, 2001). These effects of estradiol may be organizational rather than activational actions.

Adiposity in Women with ER Polymorphisms. Abnormal adiposity has been associated with the XbaI polymorphism of the human ERα gene, in which guanidine is substituted for adenine in exon one of the gene (Okura *et al.*, 2003; Speer *et al.*, 2001; Yamada, Ando, Niino, & Shimokata, 2002). In a cross-sectional epidemiological sample of over two thousand middle-aged and aged Japanese, premenopausal women with the GG genotype had increased fat mass and increased waist-hip ratios (an index of visceral adiposity) compared to premenopausal women with the AA genotype, and AG genotype women were intermediate (Okura *et al.*, 2003; Yamada *et al.*, 2002). The polymorphism did not affect adiposity in postmenopausal women or in men. Thus, polymorphisms of the human ERα gene may impair estrogen signaling and lead to increased visceral adiposity and its attendant health risks. It would be fascinating to learn whether this abnormality is also associated with increased eating.

NORI GEARY

INTRODUCTION. Estradiol is usually thought to act in the brain to inhibit eating. Its site or sites of action, however, have not been identified. Microinjections of small amounts of estradiol into the brain have been used to evaluate the hypotheses that the estrogenic inhibition of eating is initiated by ERα in the ventromedial hypothalamus (VMH), the MPA, and/or the paraventricular nucleus of the hypothalamus (PVN), but the evidence for each of these hypotheses is controversial.

An important methodological issue in this literature is the amount of estradiol implanted. Several earlier investigators implanted crystalline estradiol in 28-gauge or larger cannulas. This procedure was subsequently found to allow leakage of enough estradiol out of the brain into the periphery to produce effects such as increased uterine weight or cornification of vaginal epithelial cells (Butera & Beikirch, 1989; Palmer & Gray, 1986). Because inhibition of eating requires even smaller peripheral doses of estradiol than are required to produce cornification of vaginal epithelial cells (Drewett, 1973), implantation of pure or concentrated estradiol cannot be used to determine accurately the local sites of its action within the brain. In contrast, implants of estradiol diluted in cholesterol do produce localized results. For example, 28-gauge implants of 1:300 [^{3}H]-labeled estradiol in cholesterol into the VMH were shown to increase sexual receptivity (after peripheral progesterone injection) and to produce measurable label only within about 500 μm of the cannula tip (Davis, Krieger, Barfield, McEwen, & Pfaff, 1982; Davis, McEwen, & Pfaff, 1979). No implants of such dilute estradiol have been shown to affect food intake, but hypothalamic implants of 1:10 estradiol in cholesterol consistently fail to produce signs of peripheral leakage and appear to produce selective effects within the hypothalamus. Therefore, only research using implantation of small amounts of dilute estradiol is reviewed here.

PVN. Butera (1996) and Palmer and Gray (1986) concluded that ERs in the PVN initiate the estrogenic inhibition of eating. The strongest evidence was that 3-day food intake in ovariectomized rats was decreased by implants of 10% mixtures of estradiol benzoate in cholesterol into the PVN, but not into the VMH, MPA, or other areas (Butera & Beikirch, 1989; Butera, Xiong, Davis, & Platania, 1996). The potency of 10% estradiol in the PVN to inhibit eating in this study was, however, less than the potencies of undiluted or 25% estradiol implanted into the PVN. The effect of peripherally administered estradiol was not measured. In contrast, Hrupka, Smith, and Geary (2002) observed only a 10% decrease in food intake on day 2 and day 3 after PVN implants of 10% estradiol in comparison to cholesterol implants. The decrease was not significant in comparison to the pretest baseline on any single day or over the 3-day post-implantation period used by Butera and Beikirch (1989). Furthermore, peripheral (subcutaneous) administration of estradiol under the same conditions produced a larger inhibition of eating. In a subsequent study, Butera, Willard, and Raymond (1992) reported that subcutaneous estradiol treatment did not affect 3-day food intake in ovariectomized rats with bilateral lesions of the PVN. Dagnault and Richard (1994), however, demonstrated that subcutaneous estradiol reduced feeding and body weight as much in rats with PVN lesions as in control rats throughout a 28-day test.

Taken together, these data suggest that ER in the PVN may contribute to the estrogenic inhibition of eating, but are not sufficient to account for the full physiological effect of peripheral estradiol.

MPA. There is a single report that estradiol implanted into the MPA inhibits eating (Dagnault & Richard, 1997). In contrast, neither Butera and Beikirch (1989) nor Hrupka *et al.* (2002) saw any inhibition of food intake 3 hr to 3 days after MPA implantation of dilute estradiol in cholesterol. There are two unusual aspects to Dagnault and Richard's (1997) study. First, a water-soluble preparation of cyclodextrine-encapsulated estradiol was used, and neither the spread nor the release rate of estradiol from this novel preparation was measured. Second, the inhibition of eating occurred within 2 hr and was gone by 13 hr postinjection. Neither the short latency nor the brief duration of this effect match the usual action of estradiol on eating or the usual latency for nuclear, genomic ER-mediated effects, as reviewed above. The MPA, however, is one of the brain areas where direct administration with estradiol leads to an immediate decrease in electrophysiological activity (Kelly, Moss, & Dudley, 1977), suggesting the presence of nongenomic, presumably membrane-associated, ER. Thus, the normal estrogenic inhibition of eating does not appear to be initiated by ER in the MPA.

VMH. In rats and mice, there are ER-expressing neurons in the ventromedial nucleus, which have been implicated in the control of sexual behavior (Calizo & Flanagan-Cato, 2000; Pfaff, 1999; Pfaff *et al.*, 1994), and in the arcuate nucleus, to which no function has been ascribed. Although Wade and Zucker (1970b) reported that estradiol implants into the VMH selectively inhibited eating, others (i.e., Butera & Beikirch, 1989; Palmer & Gray, 1986) did not see any such effect. In addition, in several studies, peripheral estradiol still inhibited feeding in ovariectomized rats (Kemnitz, Goy, & Keesey, 1977; King & Cox, 1973; Thompson & Cox, 1979) or mice (Blaustein, Gentry, Roy, & Wade, 1976) with VMH lesions. Thus, the preponderance of data fail to support the idea that estradiol inhibits eating through activation of ER in the VMH.

Nucleus Tractus Solitarius (NTS). Recent studies using functional imaging techniques suggest that the estrogenic inhibition of eating may be mediated by stimulation of brainstem ER. Several food-associated stimuli lead to increased expression of c-Fos protein, a reliable marker for increased neural activity, in the NTS and other brain areas of rats. As described below, we have shown that the amount of c-Fos in some of these brain areas is larger in estradiol-treated ovariectomized rats than in untreated ovariectomized rats (Eckel & Geary, 2001; Eckel *et al.*, 2002) and mice (Geary *et al.*, 2001). One manipulation that produces this effect is intestinal fat. Intraduodenal infusion of a fat emulsion (1) inhibited sham feeding more in estradiol-treated than in untreated ovariectomized rats, (2) increased c-Fos expression more in the NTS in estradiol-treated than in untreated ovariectomized rats, and (3) increased c-Fos in many ERα-expressing NTS neurons in estradiol-treated ovariectomized rats (Asarian, Wolfe, & Geary, 2003; Geary, 2001). These data strongly suggest that at least some of the estrogenic inhibition of eating may arise through stimulation of ERα in the NTS. This possibility has not yet been tested directly.

Synthesis. The site of the ER mediating the estrogenic inhibition of eating has not yet been identified. There are several potential explanations for this failure. Most obviously, estradiol may inhibit eating by acting in some central or peripheral site that has not yet been investigated. Alternatively, ER in two or more sites may contribute jointly to the estrogenic inhibition of feeding. This is the case, for example,

for estradiol's effects on LH secretion (Freeman, 1994) and reproductive behavior (Pfaff *et al.*, 1994). Thus, estradiol may act in several sites to inhibit feeding, possibly with different sites mediating different aspects of its effects. If this is so, then estrogenic stimulation of a particular site may not duplicate the full effect of peripheral estradiol or, depending on testing conditions, may not produce any effect at all.

Peripheral Mechanisms of the Estrogenic Inhibition of Eating

Introduction

Two types of theory have linked peripheral physiological events to estradiol's inhibitory effects on eating. The first type is that estradiol affects peripheral physiological events in a way that leads to reduced eating. The most prominent example is Wade and Gray's (1979) hypothesis that estradiol increases the availability of fuels for energy metabolism and thereby reduces eating. This idea derives from the renaissance of interest in peripheral metabolic controls of eating during the 1970s (Friedman & Stricker, 1976) and produced considerable research. None of the studies designed to link estradiol's putative metabolic effects to reduced eating, however, provided any strong support for it. For example, the effects of estradiol administration on plasma lipoprotein lipase and triglyceride levels do not correlate temporally with its effect on eating (Gray & Greenwood, 1982; Nunez, Gray, & Wade, 1980; Palmer & Gray, 1986; Ramirez, 1981). Such negative results caused interest in this theory to wane (e.g., Wade *et al.*, 1985). Similarly, resting metabolic rate, the thermic effect of food, and core body temperature either do not change reliably through the menstrual cycle in women or display changes that are not associated with cyclic changes in estradiol secretion and food intake (Piers *et al.*, 1995; Tai *et al.*, 1997; Webb, 1986). It should be noted, however, that it is still possible that estradiol acts on other peripheral physiological events that may influence eating, such as gastrointestinal transit or fat absorption (e.g., Caballero-Plasencia, Valenzuela-Barranco, Martin-Ruiz, Herrerias-Gutierrez, & Esteban-Carretero, 1999; Chen, Doong, Chang, Lee, & Wang, 1995; Romanski, Nelson, & Jensen, 2000).

The second type of theory suggests that estradiol acts in the brain to change the processing of peripheral signals that affect eating. The rationale of these theories is that (1) the estrogenic inhibition of eating is expressed as decreases in meal size, and (2) many peripheral signals that influence eating do so by changing meal size. These peripheral signals include positive and negative feedback signals generated during meals by rapid, eating-generated events, such as flavor stimuli and gastrointestinal food stimuli as well as signals that are not directly related to meals, such as hormonal signals related to body adiposity (Geary, 2001b, 2004; Smith, 1998; Woods & Stricker, 2003). As reviewed in the subsequent sections, there is increasing evidence that estradiol increases the effect of specific meal-generated negative feedbacks from intestinal food stimuli that control meal size.

Flavor Selection

Flavors provide potent positive feedback signals during meals that stimulate further eating and increase meal size. There is little evidence, however, that estradiol inhibits eating by decreasing the positive-feedback effect of flavors.

Rats

Sweet. Sweet taste is frequently used as a model of food reward. Although some investigators have reported sexually differentiated effects of sugars such as sucrose and maltose on eating in rats (Clark & Ossenkopp, 1998; Kenney & Redick, 1980; Sclafani, Hertwig, Vigorito, & Feigin, 1987; Sclafani & Xenakis, 1984), others have not (Hirsch, Ball, & Godkin, 1982; Nance, Gorski, & Panksepp, 1976). Because gastrointestinal or postabsorptive effects may have accounted for some of these apparent discrepancies, we examined the effects of estradiol on sham feeding of sucrose in ovariectomized rats with gastric cannulas (Geary, Trace, & Smith, 1995; Mangiaracina & Geary, 2001). Gastrointestinal and postabsorptive effects are absent or minimal during sham feeding because ingested food drains from the cannulas. Estradiol treatment did not decrease sham intake of any sucrose solution tested, although estradiol did produce a small but reliable decrease in real intake of some solutions. These data indicate that estradiol does not inhibit eating by decreasing the rewarding effect of sweet taste.

An activational action of estradiol and progesterone may increase intake of mixtures of 3% glucose and 0.25–0.75% saccharine, which rats ingest avidly. Beginning around puberty, female rats consume more glucose-saccharine solution offered *ad libitum* with chow than male rats do; this difference is reduced by ovariectomy, and the effect of ovariectomy is reversed by treatment with both estradiol and progesterone but not by treatment with either hormone alone (Valenstein, Kakolewski, & Cox, 1967; Wade & Zucker, 1969a; Zucker, 1969). The finding that both estradiol and progesterone are required to stimulate glucose-saccharine intake suggests that this phenomenon may be a useful model of changes in eating during the luteal phase in nonhuman primates and woman.

Fat. Fat is another flavor preferred by rats. As in the case of sweet foods, there are data suggesting a selective estrogenic inhibition of fat intake (Geiselman *et al.*, 1981; Liebowitz *et al.*, 1998) and data suggesting no change or an estrogenic stimulation of fat intake (Bartness & Waldbillig, 1984; Heisler *et al.*, 1999). We used the sham feeding procedure to isolate the control of fat ingestion by flavor in ovariectomized rats (Mangiaracina & Geary, 2002). Estradiol treatment did not decrease sham intake of several concentrations of corn oil emulsifications, but did decrease real intake of some of the same concentrations. These data indicate that estradiol does not affect the positive feedback effect on eating of the flavor of this fat.

Conditioned Taste Aversions. Under some conditions, large doses of estradiol can support the formation of conditioned aversions (Chambers, 1985; DeBeun *et al.*, 1991; Merwin & Doty, 1994; Yuan & Chambers, 1999). Male rats are much more sensitive to this pharmacological effect of estradiol than females, apparently because of an organizational difference (reviewed in Geary, 2001). There is no evidence that physiological doses of estradiol decrease eating by producing gastrointestinal malaise or other aversive effects in female rats (Flanagan-Cato, Grigson, & King, 2001; Ganesan, 1994; Geary, 2001).

Women. Investigations of the contribution of changes in flavor perception or preference to changes in eating during the menstrual cycle have produced conflicting results. The many methodological difficulties of such studies undoubtedly contribute to this lack of consistency (Bowen & Grunberg, 1990; Bowen *et al.*,

2003; Buffenstein *et al.*, 1995; Drewnowski, 1997; Dye & Blundell, 1997; Frye, Crystal, Ward, & Kanarek, 1994). Some of the more important difficulties are that: (1) many studies involve threshold determinations rather than ratings of suprathreshold intensities of pleasantness or preference, which are probably more relevant to eating; (2) the relationship between sensory preferences or pleasantness and food intake is not always clear; and (3) there may not be good correspondence between the ratings of pure flavors and ratings of complex foods, even foods in which a particular flavor predominates, as in sweet foods. In addition, complex, potent, and poorly understood cognitive and social influences on human food preference and choice complicate both the interpretation of individual studies and the comparison of different studies (Bowen *et al.*, 2003; Drewnowski & Levine, 2003). Nevertheless, some influences of menstrual cycle variations on the control of eating have emerged. In one well-designed study, Bowen and Grunberg (1990) reported that both preference for and intake of sweet foods (e.g., coffee cake, chocolate) were significantly higher during the luteal phase than the follicular phase, whereas there were no differences in either measure for salty (e.g., ham, salted peanuts) or bland (e.g., cheese, unsalted peanuts) foods. When cycling monkeys (*Macaca mulatta*) were offered 0.5 M sucrose for 2 hrs/day, however, there was a trend for sucrose intake to decrease during the luteal phase (Kemnitz, Gibber, Eisele, & Lindsay, 1986). This latter result, of course, may have resulted from postingestive rather than orosensory controls of eating.

Several psychophysical studies indicate that (1) many women are more sensitive to a variety of bitter tastes than are men, and (2) the perceived intensity of tastes, especially bitter tastes, varies through the menstrual cycle, increases in pregnancy, and decreases after menopause (Drewnowski, 1997; Prutkin *et al.*, 2000). The relationships of these differences to eating have not yet been established. Interestingly, the intensity of bitter tastes appears to increase when progesterone levels are high, as during the luteal phase and early pregnancy. As many natural poisons are bitter, Prutkin *et al.* (2000) suggested that this increase could be an adaptation insuring that pregnant women are good poison detectors. Because there is evidence that people with increased sensitivity to certain bitter tastes also perceive viscous substances more intensely (Duffy & Bartoshuk, 2000; Prutkin *et al.*, 2000), it is possible that progesterone increases positive feedbacks arising from fatty foods. This is another potential mechanism for the increase in eating during the luteal phase.

Intestinal Satiation

Estradiol and Intestinal Satiation. Intraduodenal infusion of proteins, carbohydrates, and fats in amounts approximating those appearing in the intestines during meals inhibits sham feeding and produces the behavioral signs of postprandial satiation (Greenberg, 1998; Liebling, Eisner, Gibbs, & Smith, 1975; Kalogeris, Reidelberger, & Mendel, 1983; Reidelberger, Kalogeris, Leung, & Mendel, 1983). The effects of estradiol have been tested on intestinal infusions of two nutrients, a fat emulsion and l-phenylalanine (Asarian *et al.*, 2003, Geary, 2001). Nutrient infusion parameters that had inhibited sham feeding similarly in male rats (Greenberg, Smith, & Gibbs, 1990; Yox, Brenner, & Ritter, 1992) also produced similar effects in untreated ovariectomized rats. In estradiol-treated rats, however, fat inhibited sham feeding significantly more than did l-phenylalanine. These data indicate that (1) estradiol can increase the satiating potency of intestinal food stimuli, and (2) estradiol acts this way on some nutrients but not others. Furthermore, because the

satiating action of the fat emulsion is mediated by intestinal cholecystokinin (CCK), but the satiating action of l-phenylalanine is not, these data also suggest that estradiol treatment increases the satiating action of endogenous CCK (see below).

As described above, the intestinal infusions of fat whose satiating action was estradiol-sensitive also increased the expression of c-Fos protein, a marker of increased neuronal activity, in ERα-expressing cells in the subregion of the NTS that contain the terminal fields of the vagal afferent neurons that innervate the proximal small intestine (Asarian *et al.*, 2003). Thus, estradiol may act on ERα in a population of NTS neurons so as to amplify the negative feedback signals from intestinal fat that control meal size.

CCK, Estradiol, and Intestinal Satiation. CCK is released from the small intestine by intraluminal food stimuli and acts on abdominal CCK-1 (formerly known as CCK-A) receptors, and thereby contributes to the control of meal size in rats and humans (Beglinger, Degen, Matzinger, D'Amato, & Drewe, 2001; Cox, 1998; Smith, 1998). The initial evidence suggesting that CCK might mediate part of the effect of estradiol on eating arose from demonstrations that estradiol treatment increased the satiating action of exogenous CCK in ovariectomized rats (Butera, Bradway, & Cataldo, 1993; Geary, Trace, McEwen, & Smith, 1994; Linden, Uvnäs-Moberg, Forsberg, Bednar, & Södersten, 1990). These results motivated tests of the role of endogenous CCK in the estrogenic inhibition of eating that used pre-meal administration of the selective and potent CCK-1 receptor antagonist devazepide (Asarian & Geary, 1999a; Eckel & Geary, 1999; Huang *et al.*, 1993). The most important results of these tests were that (1) in intact rats, the satiating potency of endogenous CCK was increased during estrus but not during diestrus, and (2) in ovariectomized rats, the satiating potency of endogenous CCK was increased by cyclic estradiol treatment on the day of the treatment regimen that modeled estrus but not on the day that modeled diestrus. Some of these data are shown in Figure 7. Note that the data implicate endogenous CCK in only the phasic inhibitory effect of estradiol on eating and not in the tonic inhibitory effect. Thus, estradiol's tonic and cyclic inhibitory effects on eating apparently have at least partially different physiological mechanisms.

Estradiol probably increases the effects of endogenous CCK on satiation at a central site, perhaps the same site as for the effects of intestinal infusions of fat, described above. The mechanism is unlikely to involve CCK secretion because estradiol treatment also increases exogenous CCK's satiating potency in ovariectomized rats (Butera *et al.*, 1993; Geary *et al.*, 1994; Lindén, Uvnäs-Moberg, Forsberg, Bednar, & Södersten, 1990). Estradiol treatment failed to affect the number or affinity of CCK-1 receptors in the NTS (Geary, Smith, & Corp, 1996). This finding suggests that estradiol does not upregulate vagal CCK-1 receptors because vagal afferents, which project to the NTS, are pseudounipolar neurons, so NTS CCK-1 receptor profiles should reflect changes in vagal afferent CCK-1 receptors. Finally, that estradiol increases the number of NTS neurons expressing c-Fos protein in response to intraperitoneally injected CCK (Geary *et al.*, 2001; Eckel, Houpt, & Geary, 2002) also suggests that estradiol acts centrally to increase the satiating action of endogenous CCK.

Pancreatic Glucagon, Estradiol, and Intestinal Satiation. Endogenous glucagon released during meals apparently plays a physiological role in the control of meal size in rats, and prandial glucagon secretion is controlled in part by preabsorptive food stimuli (Geary, 1998, 1999). The satiating potencies both of

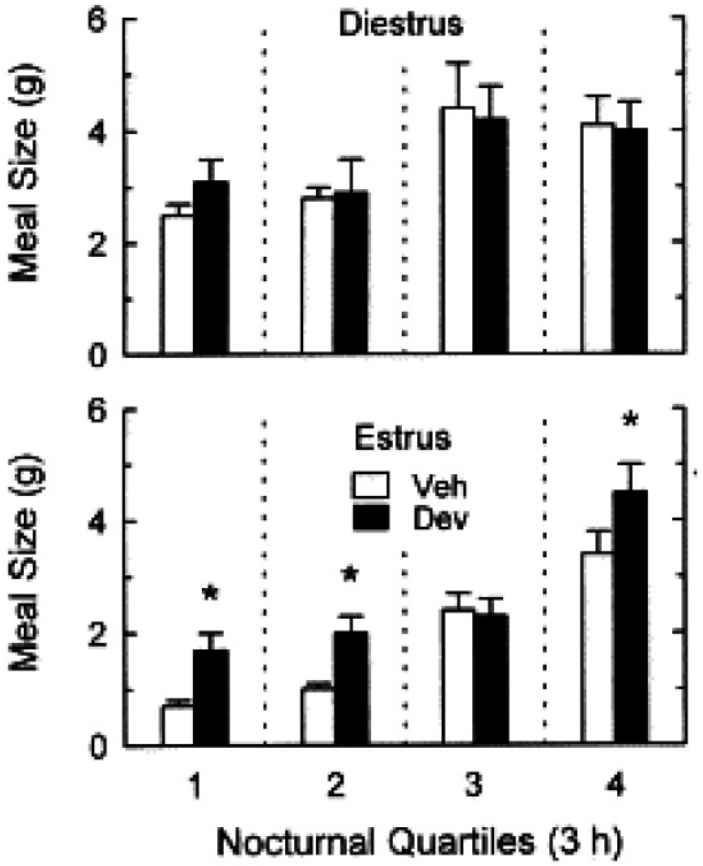

Figure 7. Intraperitoneal injection of the CCK-1 receptor antagonist devazepide (Dev; 1 mg/kg) just before the onset of the 12-hr dark period increased nocturnal spontaneous meal size during estrus (lower panel), but not during diestrus (upper panel), in intact cycling rats. Data are the mean sizes of spontaneous meals initiated during each 3-hr quartile of the dark phase. Veh, vehicle. Devazepide did not affect meal frequency during either estrus or diestrus (not shown). As a result, devazepide increased total nocturnal food intake 5.3 ±1.5 g during estrus versus only 0.8 ±0.6 g during diestrus, a significant difference. Note that during estrus, devazepide increased meal size both early in the dark, when control meals were small, and later in the dark, when control meals were as large as those during the early dark of diestrus. Thus, devazepide's effect was not an artifact of the smaller average meal size during estrus. *Note*: *Significantly different from vehicle during estrus. (From Eckel & Geary, 1999; used with permission.)

exogenous glucagon and of endogenous glucagon (assessed by administration of glucagon antibodies during meals) were increased by estradiol treatment in ovariectomized rats (Geary & Asarian, 2001). It is not yet clear whether a similar phenomenon occurs in intact cycling rats or whether glucagon participates in the cyclic or the tonic estrogenic inhibition of eating.

Adiposity Signals

Increased adiposity inhibits eating, apparently through tonic hormonal negative feedback signals. There is extensive evidence that insulin and leptin have this feedback function (Air, Benoit, Clegg, Seeley, & Woods, 2002; Niswender & Schwartz, 2003; Woods & Seeley, 2001; Woods & Stricker, 2003). The basal level of

each of these hormones is positively correlated with body adiposity, and chronic central administration of each inhibits eating and decreases body weight. Amylin, which is co-released with insulin from the pancreatic β-cells, may function similarly (Rushing, Hagan, Seeley, Lutz, & Woods, 2000; Rushing *et al.*, 2001). Furthermore, ghrelin, a hormone secreted from the stomach, may also signal adiposity. Basal plasma levels of ghrelin are inversely correlated with body weight, and chronic ghrelin administration can increase eating and body weight (Cummings *et al.*, 2002; Tschöp, Smiley, & Heiman, 2000; Tschöp *et al.*, 2001). At present, however, there is no strong evidence that any of these hormones are part of the estrogenic control of eating and body weight.

Leptin appeared to be a candidate link between the HPG axis and eating because (1) its inhibitory effect on eating is expressed as a decrease in meal size (Eckel *et al.*, 1998; Flynn, Scott, Pritchard, & Plata-Salamán, 1998; Kahler *et al.*, 1998), and (2) it apparently plays physiological roles in the control of GnRH secretion and in pubertal development (Chan & Mantzoros, 2001; Chehab, Qiu, Mounzih, Ewart-Toland, & Ogus, 2002). Nevertheless, the majority of the data do not support a role for leptin in the estrogenic inhibition of eating: (1) Neither ovariectomy nor chronic estradiol treatment altered leptin level as a function of fat mass in mice (Pelleymounter, Baker, & McCaleb, 1999). (2) Ovariectomy did not reduce leptin levels in rats (Ainslie *et al.*, 2001). (3) Chronic leptin administration did not affect estradiol levels in intact mice (Pelleymounter *et al.*, 1999). (4) Neither ovariectomy nor estradiol treatment affected the potency of exogenous leptin to reduce food intake and body weight in mice or rats (Chen & Heiman, 2001; Pelleymounter *et al.*, 1999). The only support for the hypothesis that leptin plays a role in mediating the estrogenic inhibition of eating comes from a report that intracerebroventricular injections of 0.5 or 3 μg leptin inhibited eating in intact rats but failed to do so in ovariectomized rats (Ainslie *et al.*, 2001). The relevance of this acute effect is questionable, however, given the unchanged potency of chronic leptin administered via the more physiological subcutaneous route. In addition, it appeared possible that the differential effect of leptin was an artifact of the anomalously low control intake of ovariectomized rats in comparison to the intact rats.

Clegg, Reidy, Blake Smith, Benoit, and Woods (2003) compared the effects of acute intracerebroventricular administration of leptin and insulin on food intake in age- or weight-matched male and female rats. Under their conditions, leptin reduced food intake in females but not in males, whereas insulin reduced food intake in males but not in females. In view of the foregoing discussion, it would be important to extend these data to chronic administration and to tests of antagonism of the endogenous hormones.

Central Signaling Molecules in the Estrogenic Inhibition of Eating

Introduction

There has been only limited progress in identifying the interneuronal signaling molecules that mediate estradiol's inhibitory effects on eating. The cases of neuropeptide Y (NPY), serotonin (5-hydroxytryptamine, 5-HT), and melanin-concentrating hormone (MCH) are instructive and are reviewed briefly here. Other signaling molecules that have been considered in relation to the estrogenic

inhibition of eating include corticotropin-releasing factor (Dagnault, Ouerghi, & Richard, 1993; Rivest, Deshaies, & Richard, 1989), endogenous opiates (Bodnar, Hadjimarkou, Krzanowska, Silva, & Stein, 2003; Wager-Srdar, Gosnell, Morley, & Levine, 1985), galanin (Liebowitz *et al.*, 1998), and α-melanocyte stimulating hormone (Polidori & Geary, 2002). Unfortunately, none of the phenomena has yet been investigated extensively, and many of the positive results have emerged from studies with idiosyncratic or frankly aphysiological experimental designs. Furthermore, the analysis of these effects on eating is complicated by the fact that all these signaling molecules also are implicated in the control of HPG axis hormones (Freeman, 1994; Griffin & Ojeda, 2000).

NPY

A wealth of evidence indicates that hypothalamic NPY increases eating (Kalra *et al.*, 1999). Three observations implicate hypothalamic NPY release in the estrogenic inhibition of eating: (1) Ovariectomy increased and estradiol treatment decreased levels of NPY mRNA in the arcuate nucleus of the hypothalamus (Ainslie *et al.*, 2001; Baskin, Norwood, Schwartz, & Koerker, 1995). (2) Food deprivation increased arcuate NPY mRNA level less in estradiol-treated rats than in untreated ovariectomized rats (Baskin *et al.*, 1995). (3) Chronic estradiol treatment decreased NPY levels and decreased NPY release in the PVN (Bonavera, Dube, Kalra, & Kalra, 1994). It cannot be assumed that these phenomena are related to NPY's influence on eating, however, because hypothalamic NPY also functions as part of the control of LH secretion (Leupen, Besecke, & Levine, 1997; Wehrenberg, Corder, & Gaillard, 1989). Because none of these studies included measures of eating, none provides a direct test of the hypothesis that NPY mediates the estrogenic inhibition of eating. Perhaps most interesting would be tests of the effects of NPY antagonism on eating at different stages of the estrous cycle or as a function of estradiol treatment. Finally, it should also be noted that neither Baskin *et al.* (1995) nor Bonavera *et al.* (1994) used a physiological pattern of estradiol treatment, which may have been crucial because acute, rather than chronic, estradiol treatment increased rather than decreased arcuate NPY mRNA (Bonavera *et al.*, 1994; Sahu *et al.*, 1992). Thus, it cannot be concluded with certainty that these data reflect physiological effects of estradiol on NPY function.

5-HT

Central 5-HT inhibits eating by reducing meal size (Blundell, 1992; Simansky, 1998). 5-HT may contribute to the estrogenic inhibition of eating. First, acute administration of fenfluramine, a 5-HT agonist that releases 5-HT and blocks its reuptake, decreased eating more during estrus than during diestrus and decreased eating more in estradiol-treated than untreated ovariectomized rats (Rivera, Dixon, & Eckel, 2003). Second, the stimulatory effect on eating of the selective 5-HT_{1A} receptor agonist 8-hydroxy-2-9(di-*n*-propylamino)tetralin (8-OH-DPAT) was less during proestrus and estrus than during diestrus in cycling rats (Uphouse, Salamanca, & Caldarola-Patuszka, 1991), and was decreased by estradiol treatment in ovariectomized rats (Salamanca & Uphouse, 1992). Because 5-HT_{1A} receptors function as autoreceptors, 8-OH-DPAT presumably stimulates eating by decreasing the activity of 5-HT neurons. Taken together, these studies suggest that the phasic estrogenic inhibition of eating during estrus is mediated by decreases in 5-HT_{1A}

autoreceptor function and increases in 5-HT synaptic activity. In contrast, the anorectic action of chronic administration of fenfluramine by osmotic minipump was not affected by chronic estradiol treatment in ovariectomized rats (Souquet & Rowland, 1990), suggesting that 5-HT does not contribute to the tonic estrogenic inhibition of eating.

Hypothalamic 5-HT may mediate the phasic estrogenic inhibition of eating by affecting the central processing of the CCK satiation signal. As described previously, eating appears to decrease cyclically during estrus in part because of an increase in the satiating potency of CCK. The involvement of 5-HT in this increase is suggested by reports that the satiating potency of exogenous CCK was decreased in male rats by either 5-HT_{1A} autoreceptor agonists (Poeschla, Gibbs, Simansky, & Smith, 1992) or by 5-HT1C synaptic receptor antagonists (Poeschla, Gibbs, Simansky, Greenberg, & Smith, 1993). On the other hand, neither 5-HT nor its metabolite 5-hydroxyindoleacetic acid varied in the hypothalami of cycling rats or of estradiol-treated ovariectomized rats (Gundlah, Simon, & Auerbach, 1998; Maswood, Stewart, & Uphouse, 1995). Further research is required to resolve these apparently discrepant results.

The amygdala is another potential site where 5-HT may mediate the estrogenic inhibition of eating. Parker, Bishop, and Coscina (2002) reported that bilateral infusions of the broad spectrum 5-HT receptor antagonist metergoline into the posterior basolateral amygdala increased eating less during diestrus than during estrus (or shortly afterwards—the rats were tested in the middle of the light phase after the night of estrus). The observations that estradiol increased eating- and CCK-induced expression of c-Fos in the central nucleus of the amygdala (Eckel & Geary, 2001, 2002) are also consistent with the hypothesis that the amygdala plays a role in the effects of estradiol on eating. On the other hand, King and his colleagues (King, Rollins, Grundmann, & Olivier, 2003; King *et al.*, 1999) have reported that female rats display more hyperphagia and weight gain following lesions of the posteriodorsal amygdala or adjacent stria terminalis than do male rats, but the effects of amygdaloid lesions and of ovariectomy appeared additive and, therefore, independent.

Finally, when rats were fed a more palatable food (Parker *et al.*, 2002) or were fed different macronutrients separately (Heisler *et al.*, 1999), cyclic variations in the serotonergic control of eating were not apparent. These phenomena are potentially of relevance to the hypotheses that 5-HT may control food selection during the menstrual cycle (see Dye & Blundell, 1997; Heisler *et al.*, 1999, for reviews).

MCH

Hypothalamic MCH may be involved in the control of both eating and LH secretion (Gonzalez, Baker, & Wilson, 1997; Mystkowski & Schwartz, 2000). Estradiol decreased hypothalamic MCH mRNA in ovariectomized rats (Murray, Baker, Levy, & Wilson, 2000), which is consistent with a role in the estrogenic inhibition of eating. Similarly, infection with the estradiol-secreting Leydig cell tumor, or treatment with equivalent doses of estradiol, decreased hypothalamic MCH mRNA more in male rats than in untreated, pair-fed male rats (Mystkowski *et al.*, 2000). However, this effect of estradiol on MCH expression in male rats seems unlikely to be related to the inhibition of eating by physiological amounts of estradiol because (1) the Leydig cell tumor does not decrease eating in female rats (Mordes, Longcope, Flatt, MacLean, & Rossini, 1984), (2) Leydig cell tumor anorexia is related to a strong taste aversion (Bernstein, Courtney, & Braget, 1986),

which physiological levels of estradiol do not produce (Flanagan-Cato *et al.*, 2001), and (3) Leydig cell tumor anorexia is expressed as a decrease in meal frequency (Emery, 1999), whereas estradiol's physiological effects on eating are expressed as decreases in meal size.

Future Directions

This section considers the progress and potential future directions in behavioral, mechanistic, and clinical studies of the estrogenic inhibition of eating.

Behavior

The most significant behavioral feature of the phasic and tonic physiological actions of estradiol on eating in rats and mice is that both effects are expressed as selective changes of spontaneous meal size. Spontaneous meal frequency—that is, meal initiation—is not directly affected. Two important points follow from these facts: (1) Amount of food intake alone is not a sufficient behavioral measure to characterize the estrogenic inhibition of eating because the same total food intake can result from strikingly different patterns of spontaneous meal size and meal frequency. Several examples have been reviewed, including the comparison of eating by intact, cycling rats and by ovariectomized rats after the initial period of hyperphagia abates, and the comparison of the effects of estradiol and of testosterone on eating. (2) Appropriate measures of eating must be included in physiological analyses in order to establish the relevance of physiological phenomena to the phasic or tonic estrogenic inhibition of eating. An example is provided by the satiating action of CCK. Behavioral analysis indicates that, at least in rats, changes in the satiating potency of CCK are involved only in the phasic, and not the tonic, estrogenic inhibition of eating.

A striking indication of the selectivity of the normal physiological effects of estradiol on meal size is provided by studies of a pathophysiological effect of estradiol on eating during illness. Estradiol has multiple effects on immune system function, including increasing the intensity of the acute phase response of the innate immune system (Geary, 2001). Anorexia is a prominent component of the acute phase response. The anorexia produced by injection of the gram-negative bacterial toxin lipopolysaccharide is increased during estrus and is increased by estradiol treatment in ovariectomized rats, in both cases due to decreases in spontaneous meal frequency (Geary, Asarian, Sheahan, & Langhans, 2004). These data identify another action of estradiol in addition to the physiological effects reviewed here. Germane to the present discussion is that this pathophysiological effect of estradiol on illness anorexia is expressed as a selective interaction with the control of meal frequency and not with the control of meal size. The conclusion is inescapable that estradiol—and probably other physiological controls of eating—can operate through selective and specific interactions with the differing neural substrates governing meal size and meal frequency.

One crucial behavioral question is whether the cyclic change in eating by women is also expressed as decreased meal size. If the estrogenic inhibition of eating in women could be demonstrated in the laboratory, then test meals could be analyzed with many powerful behavioral and physiological methods (Geary & Schwartz, 2004; Kissileff, 2000).

Mechanism

Some components of the mechanism of the estrogenic inhibition of eating are well established: (1) estradiol apparently acts on ERα to produce both the tonic and cyclic decreases in meal size; (2) the cyclic inhibition appears to involve ERα in the NTS, although it is not yet clear whether they play a necessary role; (3) estradiol apparently acts on post-oral controls of meal size; (4) estradiol produces an increase in the potency of some, but not all, intestinal satiation mechanisms; (5) an estradiol-dependent increase in the satiating potency of endogenous CCK is involved in the cyclic but not the tonic effect; and (6) an estradiol-dependent increase in the satiating action of endogenous glucagon may be part of the mechanisms of the cyclic or the tonic effect.

These data suggest numerous further questions. Where does estradiol act to inhibit eating? Does it affect other peripheral signals that control eating, such as those related to amylin, ghrelin, or hepatic metabolism? Which actions of estradiol mediate its tonic action on eating, and which mediate its phasic actions on eating? What genes activated by the estradiol–ERα complex affect eating? What neuronal network functions are affected by these genes? Which neuronal signaling molecules contribute importantly in the function of these networks? Which of these mechanisms exist in women, and what determines their functional potency?

Clinical

The clinical potential of the estrogenic inhibition of eating is 3-fold: (1) disturbances in normal estrogenic mechanisms may be sufficient to cause disordered eating in women or contribute to the vulnerability of some women to develop eating disorders. (2) Disordered eating may affect the operation of estrogenic controls of eating in a way that exacerbates the course of the disorder. (3) Even if it turns out that the physiological control of eating, including its sexually differentiated aspects, is normal in patients with disordered eating, understanding the normal role of estradiol in eating may suggest physiological mechanisms that could serve as therapeutic levers for their treatment (e.g., Tofovic, Dubey, & Jackson, 2001). Each of these potentials should be pursued by translational research to bridge basic research and clinical research and practice. CCK is again a useful example. Women with bulimia nervosa have been shown to have blunted prandial CCK secretion and reduced perception of satiation during meals (Devlin *et al.*, 1998; Geriacotti & Liddle, 1988). These effects may have been secondary in the disorder because therapies that reduced binge frequency also normalized CCK secretion and perception of satiation. The potential of therapies targeted at CCK satiation have not been tested. The potent effect of estradiol on the satiating potency of CCK in rats indicates that consideration of an estrogenic component should be included in this important work.

Conclusion

Progress in analyzing the estrogenic inhibition of eating parallels that of many other problems in behavioral neuroscience: although many fascinating observations have been made, the surface of the problem has barely been scratched, and although the ultimate relevance of the enterprise is not at all certain, the potential seems great. Two general lessons have emerged in this work—as they have in many other areas of biological research. First, biological function can be understood only

through converging views from multiple perspectives. No one perspective, be it genetic, physiological, or behavioral, reveals all the answers. Second, ultimate identification of biological function requires experimental methods that do not do violence to the normal operation of the system studied. Thus, every newly developed experimental technology brings with it the challenge, not simply of how to apply it to a particular problem, but, in Diamond's (1995) apt phrase, "how to be physiological" in that application.

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Anorexia

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13

Anorexia during Disease

Wolfgang Langhans

Introduction

Virtually all diseases in man and animals cause a variety of behavioral sickness symptoms such as lethargy, loss of appetite (i.e., anorexia) and thirst. In chronic diseases, anorexia often combines with an increase in resting energy expenditure (i.e., hypermetabolism) to cause cachexia (Ling, Schwartz, & Bistrian, 1997). Cachexia compromises host defense and delays recovery, and its consequences are often detrimental for the host. In the acute phase of a disease, however, a reduction of spontaneous food intake has been shown to be part of the host's defense mechanisms (Hart, 1988). The beneficial effects of anorexia in this situation supposedly are related to a conservation of energy (from not moving around in search for food), a limitation of the supply of food-derived micronutrients (e.g., iron and zinc) which are essential for the growth of pathogenic microorganisms (Hart, 1988), and an enhanced elimination of afflicted cells which are primed for apoptosis and particularly susceptible to energy deprivation (LeGrand, 2000). Consistent with this hypothesis, force-feeding decreases survival time and increases mortality in experimentally infected laboratory animals (Murray & Murray, 1979). This finding reflects the initial beneficial effect of the anorexia during infection. In sum, anorexia is a well-organized, adaptive response to disease that occurs largely independent of other sickness behaviors or disease-related phenomena such as fever, metabolic alterations, and other changes (see Langhans [2000] for review). In this chapter, I will first discuss the role of cytokines as primary endogenous mediators of the anorexia during many diseases. Then I will briefly introduce some

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Neurobiology of Food and Fluid Intake, 2nd Ed., Volume 14 of *Handbook of Behavioral Neurobiology,* edited by Edward Stricker and Stephen Woods, Kluwer Academic / Plenum Publishers, New York, 2004.

disease models that are widely used to investigate the mechanisms of immune reactions and anorexia. Thereafter, I will deal with some general characteristics of the anorexia during disease, and, finally, I will present a concept of the underlying pathophysiologic mechanisms.

Cytokines as Endogenous Mediators of Anorexia

Cytokines are extracellular messenger molecules that are conventionally grouped in families (see Oppenheim [2001] for review). Virtually all cells can produce some cytokines in response to a variety of stimuli, with production cycles that typically last from hours to days. Likewise, virtually all cells have receptors for cytokines. Some macrophage-derived proinflammatory cytokines act on virtually all cells (Figure 1), whereas others act primarily on immune cells. A key feature of cytokines is their high potency, which is related to their high receptor affinity (Oppenheim, 2001).

Given the cytokines' multiple actions and target sites, it is not surprising that their activities are tightly controlled by physiologic feedback loops. Despite structural differences, several cytokines share the same receptors. This feature probably accounts for some of their redundant effects. Many cytokine receptors are naturally expressed as both membrane and soluble forms. Soluble cytokine receptors are created by proteolytic cleavage of membrane receptors ("receptor shedding") or alternative splicing of the RNA encoding the cytokine receptor, creating a truncated, soluble receptor (Fernandez, 2000). As the affinities of the soluble cytokine receptors usually match those of the membrane receptors, they are potent binding

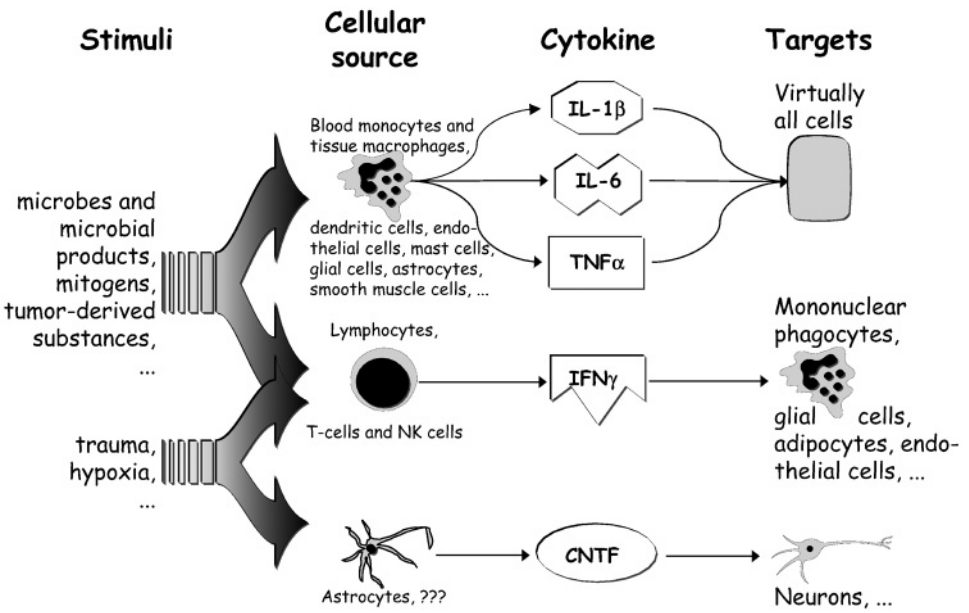

Figure 1. Stimuli for the release, cellular sources, and targets of some cytokines that are involved in the anorexia during many diseases. CNTF = ciliary neurotrophic factor, IFNγ = interferon-γ, IL-1β = interleukin-1β, IL-6 = interleukin-6, NK cells = natural killer cells, T-cells = T-lymphocytes, TNFα = tumor necrosis factor-α. See text for further details.

competitors. Most soluble cytokine receptors therefore act as cytokine antagonists and have generated interest as potential immunotherapeutic agents.

Cytokines are known to orchestrate nonspecific and specific immune reactions, and they are broadly categorized as being proinflammatory or anti-inflammatory; that is, they are involved in both pathogenesis of and protection against the disease. The primary disease process triggers the production and release of proinflammatory cytokines from various cells (Figure 1). For instance, microbial products or tumor-derived substances stimulate cytokine production through membrane receptors and post-receptor signaling cascades, resulting in activation of the transcription factor NFκB. Disease processes also activate negative regulators that limit further cytokine production and action, and result in a well-regulated overall response. In addition to their pleiotropic effects in the immune system, cytokines affect other physiologic functions and cause CNS-mediated effects such as fever, sleep, and an activation of the hypothalamic–pituitary–adrenal axis. Also, proinflammatory cytokines are the major endogenous mediators of the anorexia during disease, including interleukin-1 (IL-1), IL-2, IL-6, IL-8, tumor necrosis factor-α (TNFα), and interferon-γ (IFNγ). Each of these agents has been shown to inhibit eating after peripheral or central administration (e.g., Langhans & Hrupka, 1999; Plata-Salamàn, 1995). A synergistic suppressive effect on feeding has been described for IL-1β and TNFα (vanderMeer, Sweep, Pesman, Borm, & Hermus, 1995; Yang, Koseki, Meguid, Gleason, & Debonis, 1994) and for these two cytokines and IL-8 (Sonti, Ilyin, & Plata-Salamàn, 1996). Presumably the synergies are related to the cytokines' overlapping effects and to the fact that they act through converging intracellular signaling pathways.

Acute antagonism of particular cytokines and/or their receptors often attenuates anorexia in various models of disease, implicating the pertinent cytokines in the reduction of food intake (Arsenijevic *et al.*, 2000; Kent, Bret-Dibat, Kelley, & Dantzer, 1996; Porter, Hrupka, Altreuther, Arnold, & Langhans, 2000). Furthermore, several disease models or experimental manipulations of endogenous cytokines reduce food intake less in mice that are genetically deficient in a particular cytokine or cytokine receptor than in control animals (see below). Failures to establish a role of a particular cytokine in disease-related anorexia with genetic knockout (KO) mice (Arsenijevic *et al.*, 2000; Kozak *et al.*, 1995; Kozak *et al.*, 1997; Leon, Conn, Glaccum, & Kluger, 1996; Leon, Kozak, Peschon, Glaccum, & Kluger, 1997) are most likely due to the redundancy and overlapping actions of cytokines, which allow for developmental compensation. All in all, it is safe to say that proinflammatory cytokines play a prominent role in the anorexia of various diseases, but it is the complex interactions of several cytokines in a network rather than the effect(s) of single cytokines that cause the anorexia during disease.

Anorexia in Laboratory Animal Models of Disease

General Aspects

Several animal models of inflammation, infection, or cancer have been employed to investigate the pathophysiologic mechanisms of the anorexia during disease and the role of cytokines in this context. These models usually are characterized by acute and/or prolonged anorexia, hypermetabolism, cachexia (in the chronic models), and at least a transient increase in circulating concentrations of proinflammatory cytokines.

Cancer Anorexia and Cachexia

Cytokines have long been implicated in cancer anorexia and cachexia. Circulating levels of single cytokines or cytokine expression in various organs often are higher in cancer-bearing individuals than in healthy controls (see Langhans & Hrupka [2003] for review). Lobund–Wistar rats bearing prostate adenocarcinoma tumor cells are one animal model of cancer that displays a high correlation between early anorexia and an upregulation of IL-1β mRNA and other components of the IL-1 system in several brain regions including cerebellum, cortex, and hypothalamus (Plata-Salamàn, Ilyin, & Gayle, 1998). More important for the presumed causal relationship between proinflammatory cytokines and cancer anorexia, however, is that experimental tumors cause less pronounced anorexia and cachexia in some cytokine KO mice than in corresponding wild-type (WT) control animals (Cahlin *et al.*, 2000; Molotkov, Satoh, & Tohyama, 1998). Moreover, immunoneutralization or pharmacologic antagonism of proinflammatory cytokines attenuated cancer-induced anorexia and/or cachexia in many studies (Langstein, Doherty, Fraker, Buresh, & Norton, 1991; Laviano *et al.*, 1995; Smith & Kluger, 1993; Stovroff, Fraker, Swedenborg, & Norton, 1988; Strassmannn, Fong, Kenney, & Jacob, 1992; Torelli *et al.*, 1999). Thus, several proinflammatory cytokines appear to play a role in the anorexia that accompanies progressive tumor growth in man and animals. The type of tumor as well as its location and size presumably determine the organism's cytokine profile and, hence, specifics of the behavioral response.

Sterile Inflammation

Subcutaneous (sc) injection of turpentine in laboratory animals mimics sterile inflammation causing fever, lethargy, body weight loss, muscle breakdown, and anorexia (Wusteman, Wight, & Elia, 1990). Subcutaneous injection of turpentine increased plasma IL-6 (Cooper *et al.*, 1994) and failed to induce anorexia and other sickness symptoms in IL-6 KO mice (Kopf *et al.*, 1994; Kozak *et al.*, 1997). Similarly, sc turpentine also failed to reduce food intake in IL-1 receptor-1 KO mice (Leon *et al.*, 1996). These results indicate that IL-6 and IL-1 are involved in the anorexia in this model of sterile local inflammation. This observation is consistent with older findings showing that antagonism of either IL-6 or IL-1 receptor-1 by monoclonal antibodies antagonized body weight and food intake responses to sc turpentine in mice (Oldenburg *et al.*, 1993). Finally, TNFα appears to be involved in the hypermetabolic response to sc turpentine but not in the accompanying anorexia (Cooper *et al.*, 1994).

Inflammatory Bowel Disease

Intrarectal administration of irritants (e.g., trinitrobenzenesulfonic acid [TNB]) is used to model inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease. These diseases are usually associated with marked weight loss due to reduced food intake, malabsorption, and increased energy expenditure. Similarly, TNB-treated animals typically eat less food and lose body weight (Mchugh, Castonguay, Collins, & Weingarten, 1993). TNB produces high levels of proinflammatory cytokines locally, and TNB-induced anorexia is attenuated by chronic peripheral or central administration of human recombinant IL-1 receptor antagonist (Mchugh *et al.*, 1993). These data implicate an activation of the IL-1 system

in the feeding suppressive effect of intrarectal TNB treatment. The role of other proinflammatory cytokines in the response to this model of inflammatory bowel disease is still unknown.

Toxoplasma gondii Infection in Mice

Inoculation of mice with *T. gondii* cysts is an interesting model because it allows for differentiation of the anorectic and hypermetabolic phases of cachexia (Arsenijevic, Girardier, Seydoux, Chang, & Dulloo, 1997). Intraperitoneal (ip) inoculation of Swiss Webster mice with *T. gondii* cysts causes substantial weight loss within the first 2–3 weeks (Arsenijevic *et al.*, 1997), and all mice display hypermetabolism (Days 1–7) followed by anorexia (Days 7–14 or 7–21). Subsequently, some mice remain anorectic but no longer are hypermetabolic and show partial weight recovery ("gainers"), whereas others remain both anorectic and hypermetabolic and do not regain weight ("non-gainers"). Different patterns of cytokine expression characterize the different periods of infection as well as the gainers and non-gainers, making experimental *T. gondii* infection in mice a unique model to study phase-dependent and individual differences in the mechanisms of infection-induced anorexia and hypermetabolism. The initial hypermetabolic response to *T. gondii* infection was unaffected in TNFα-KO mice but completely absent in IFNγ-KO mice (Arsenijevic *et al.*, 1997), indicating that the latter but not the former cytokine is indispensable for the acute hypermetabolism in this infection model. On the other hand, cytokine expression patterns had largely normalized in gainers (which were still anorectic but not hypermetabolic) except for a remaining increase in TNFα and IL-10 expression, suggesting that these cytokines might somehow be involved in anorexia in this model (Arsenijevic *et al.*, 1997).

Peripheral LPS Administration as a Model of Systemic Bacterial Infection

General Aspects. Administration of live bacteria or bacterial lipopolysaccharides (LPS) is the most extensively used model of microbial infections, and some generalization of the results to other diseases (and disease models) is possible. LPS are purified Gram-negative bacterial cell wall constituents that are released after bacteriolysis or during periods of rapid bacterial proliferation (Rietschel *et al.*, 1998). Like systemic microbial infections, LPS administration triggers a profound proinflammatory cytokine response (Abram, Vuckovic, Wraber, & Doric, 2000; Imanishi, 2000), thus mimicking many of the host's reactions to severe Gram-negative bacterial infections and generalized inflammation, including the anorexia. Intraperitoneally administered LPS (doses of $\leq$150 μg/kg body weight) inhibit feeding by reducing the number of daily meals, and this effect is independent of a concomitant inhibition of water intake or gastric emptying (Langhans, 1996). On the other hand, intracerebroventricular (icv) administration of LPS (Plata-Salamàn & Borkoski, 1993), at doses that inhibit feeding similarly to ip LPS, reduce both meal number and meal size. This difference suggests that icv and ip LPS reduce food intake through different mechanisms. In line with this interpretation, ip but not icv LPS reduced food intake in IL-1β-converting enzyme-deficient mice (Burgess *et al.*, 1998; Yao *et al.*, 1999), suggesting that IL-1β is crucial for the anorexia in response to central but not peripheral LPS administration. Interestingly, the pyretic effects

of centrally and peripherally administered LPS are also different, whereas intravenous (iv) injection of LPS produced a biphasic fever, icv LPS produced a monophasic fever similar to the second phase of the biphasic fever after iv LPS (Morimoto, Murakami, Nakamori, & Watanabe, 1987). Peripherally administered cytokines also affect both meal number and meal size (Debonis, Meguid, Laviano, Yang, & Gleason, 1995; Langhans, Savoldelli, & Weingarten, 1993). The different effects of ip injected LPS and cytokines on meal patterns under the same conditions suggest that the interactions between LPS and cytokines are too complex to fit the concept of a simple linear causality from LPS through cytokines to anorexia.

LPS have no structural homolog among multicellular organisms and probably are the most powerful stimuli of innate immune responses. The activation of macrophages by LPS requires the cell-surface glycoprotein CD14 and is promoted by the LPS-binding protein (Schutt, 1999). In cells devoid of CD14, such as endothelial cells, the soluble form of CD14 present in serum can replace membrane-bound CD14 (Akira, 2000). CD14 transfers LPS in cooperation with the myeloid differentiation protein-2 to the toll-like receptor 4 (TLR-4), which is the "true" LPS receptor (Akira, 2000; Beutler, 2000). TLRs are a family of transmembrane proteins that are grouped into the same gene family (Akira, 2000; Muzio & Mantovani, 2000). The post-receptor signaling pathways of TLRs involve the myeloid adapter protein MyD88 and the Ser/Thr kinase IRAK that interacts with TRAF6 and leads to the activation of the transcription factors NFκB and activating protein-1 (Muzio & Mantovani, 2000). This signaling cascade overlaps substantially with the intracellular pathways of cytokine action. The transcription factors stimulate the transcription of the genes for proinflammatory cytokines, prostanoids, and other downstream mediators of LPS effects. Through these mechanisms LPS induce marked increases in circulating proinflammatory cytokine levels and reduce food intake. Some evidence indicates that LPS binding protein and soluble CD14 not only mediate LPS effects but can also mitigate excessive responses to LPS (Lamping *et al.*, 1998; Schutt, 1999) and might therefore hold some therapeutic potential to counteract LPS-induced effects.

With respect to food intake, we recently found that neither CD14 KO mice nor TLR-4 deficient mice significantly reduced their food intake in response to ip injected LPS (von Meyenburg, Hrupka, Schwartz, Landmann, & Langhans, 2002), illustrating that both CD14 and TLR-4 are essential for LPS-induced anorexia (Figure 2). LPS inhibited feeding in TLR-2 KO and corresponding WT mice similarly, confirming that TLR-2 is not essential for LPS effects. Interestingly, however, neither TLR-2 KO nor CD14 KO mice reduced food intake in response to ip muramyl dipeptide (von Meyenburg *et al.*, unpublished), which represents Gram-positive bacterial cell wall components and generally has similar effects as LPS. These results are consistent with the hypothesis that TLR-2 and CD14 are involved in the responses triggered by Gram-positive bacteria (Fitzgerald & O'Neill, 2000).

In addition to monocytes and macrophages, mast cells are presumably involved in shaping the anorexigenic response to LPS. Mast cells also express TLRs and release cytokines, histamine, and other mediators in response to bacterial products (McCurdy, Olynych, Maher, & Marshall, 2003; Varadaradjalou *et al.*, 2003). Mast cells have been implicated in a variety of inflammatory conditions and in host defense against bacterial infection because mast cell-deficient mice show impaired immune reactions and increased mortality in response to immune challenges (Malaviya, Ikeda, Ross, & Abraham, 1996). Inhibition of mast cell degranulation by cromolyn inhibits the acute phase responses to bacterial products and markedly

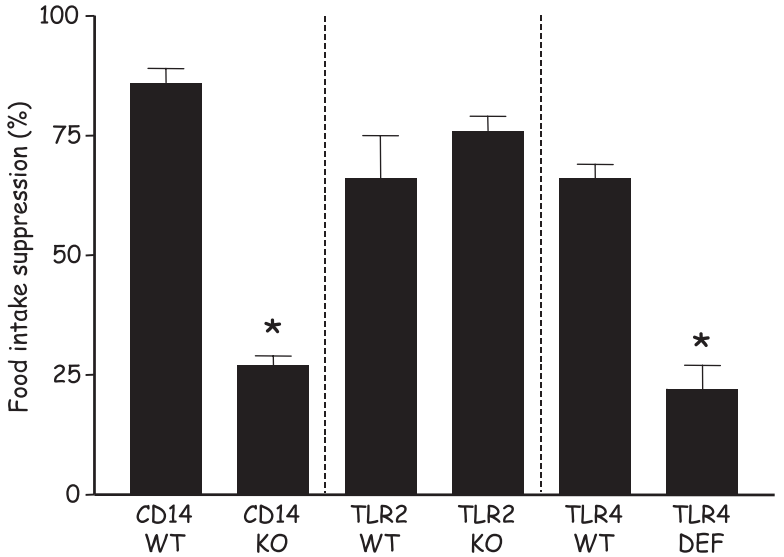

Figure 2. Intraperitoneal (ip) LPS (2 μg/mouse) nonsignificantly reduces food intake in CD14-knockout (KO) and TLR-4-deficient (DEF) mice. Each bar depicts the mean 6-hr percent food intake reduction (saline controls for each genotype = 100%) of 8–10 animals. All injections were given at dark onset. Three separate experiments were performed in which corresponding wild type (WT) and KO or DEF mice were always tested together. *Indicates that LPS reduces food intake less ($p < 0.05$ in *post hoc* tests after significant ANOVA) than in the corresponding WT animals.

attenuates LPS-induced anorexia (Asarian, Silverman, & Silver, 2003; Nava & Caputi, 1999). Therefore, mast cells and mast cell-derived mediators appear to contribute to the feeding suppressive effect of LPS; they are not indispensable for the expression of LPS-induced anorexia, however, because recent experiments revealed that LPS reduces food intake similarly in mast cell-deficient mice and in their normal counterparts (Asarian *et al.*, 2003).

Cytokines. Genetic ablation of any component of the IL-1β and TNFα cytokine systems usually does not alter the anorectic effect of peripheral LPS (Arsenijevic *et al.*, 2000; Bluthe *et al.*, 2000; Burgess *et al.*, 1998; Kozak *et al.*, 1995; Leon *et al.*, 1996). Acute pharmacologic or immunologic antagonism of the IL-1β or TNFα systems, however, attenuates the anorectic responses to LPS (Bluthe, Dantzer, & Kelley, 1992; Laye *et al.*, 2000; Porter, Arnold, & Langhans, 1998; Porter *et al.*, 2000; Swiergiel & Dunn, 1999). This finding is consistent with a role of IL-1β and TNFα in LPS-induced anorexia and suggests that the value of KO preparations for studies of complex behavioral control mechanisms is at times limited. Interference with both the IL-1 and TNFα systems has a more profound effect on LPS-induced anorexia than acute blockade of either cytokine alone and may at times be necessary to observe an effect (Burgess *et al.*, 1998; Porter *et al.*, 2000; Swiergiel & Dunn, 1999). In one study, LPS reduced food intake similarly in IL-1 receptor KO mice and WT control mice, but concomitant ICV administration of a fragment of soluble TNF receptor eliminated the feeding suppressive effect of LPS (Bluthe *et al.*, 2000). These results suggest that neither cytokine receptor alone is necessary for the feeding-suppressive and other sickness-inducing effects of LPS, but that the IL-1β and TNFα pathways can replace each other in mediating LPS-induced anorexia (Bluthe *et al.*, 2000).

IL-6 is the third macrophage-derived cytokine that is often implicated in infection-related phenomena. The results concerning the role of IL-6 in LPS-induced anorexia are somewhat inconsistent. Whereas the feeding suppressive effects of ip and icv LPS were attenuated in IL-6 KO mice in one study (Bluthe *et al.*, 2000), anorexia and cachexia due to sepsis induced by cecal ligation and puncture were not attenuated in another study (Leon, White, & Kluger, 1998). The latter finding may be due to developmental compensation, but acute antagonism of IL-6 by administration of monoclonal antibodies also failed to attenuate the suppressive effect of LPS on milk intake in mice (Swiergiel & Dunn, 1999). Together, these results do not suggest a crucial role of IL-6 in LPS-induced anorexia. Only the combined antagonism of IL-1β, TNFα, and IL-6 blocked the suppressive effect of LPS on milk intake in this experiment (Swiergiel & Dunn, 1999), again consistent with the view that macrophage-derived proinflammatory cytokines act in concert to reduce food intake.

Unlike the situation described for IL-1β, TNFα, and IL-6, genetic ablation of IFNγ-receptors or selective pharmacologic antagonism of IFNγ markedly attenuated the anorectic and hypermetabolic effects of LPS (Arsenijevic *et al.*, 2000). This finding implicates IFNγ in LPS-induced suppression of feeding and stimulation of metabolism. Exogenous IFNγ potently reduces food intake after parenteral administration (Langstein *et al.*, 1991; Plata-Salamàn, 1992). T cells and natural killer cells are the main sources of endogenous IFNγ (Billiau & Vendenbroeck, 2001), and pretreatment with Anti-Asialo GM1 antibodies, which are known to deplete natural killer cells, attenuated the anorectic effect of LPS (Arsenijevic *et al.*, 2000). This observation suggests a role for natural killer cell IFNγ production in LPS-induced anorexia. LPS stimulates IFNγ production indirectly through macrophage-derived IL-12 and IL-18 as well as TNFα (Billiau & Vendenbroeck, 2001; Doherty *et al.*, 1992). Macrophages are required for the stimulation of IFNγ production by LPS because neither natural killer cells nor T cells possess TLR-4 (Beutler, 2000). In turn, the main function of IFNγ in the cytokine network is to activate macrophages and endothelial cells, partly in synergy with macrophage-derived cytokines (Billiau & Vendenbroeck, 2001). Based on these results, it is tempting to speculate that the enhancement of proinflammatory cytokine production and action by IFNγ is necessary for the full expression of LPS-induced anorexia. Such an enhancement by IFNγ appears to be involved in the lethal effect of high LPS doses (Chang & Bistrian, 1998; Doherty *et al.*, 1992). Thus, with regard to the mediating role of cytokines in LPS-induced anorexia, the following sequence of events appears likely (Figure 3): LPS stimulates macrophages and other cytokine-producing cells to secrete an array of proinflammatory cytokines including IL-1β, IL-6, TNFα, and the natural killer cell and T cell activation factors IL-12 and IL-18. As a result, IFNγ production increases and provides the necessary positive feedback for the production of the macrophage-derived cytokines, which jointly affect eating.

Mechanisms of LPS-Induced Cytokine Production. As outlined above, stimulation of proinflammatory cytokine production by LPS ultimately results in activation of the transcription factor NFκB. Interference with NFκB production (Biswas, Ahlers, Dezube, & Pardee, 1994) is the most likely mechanism by which phosphodiesterase inhibitors such as pentoxifylline (PTX) antagonize the feeding suppressive effect of LPS (Porter *et al.*, 2000). PTX completely blocked TNFα and attenuated IL-1β production, presumably by preventing intracellular cAMP degradation (Endres *et al.*, 1991; Huizinga, Brinkman, & Verweij, 1996). Further, PTX did not influence the feeding suppressive effect of exogenous TNFα (Porter *et al.*,

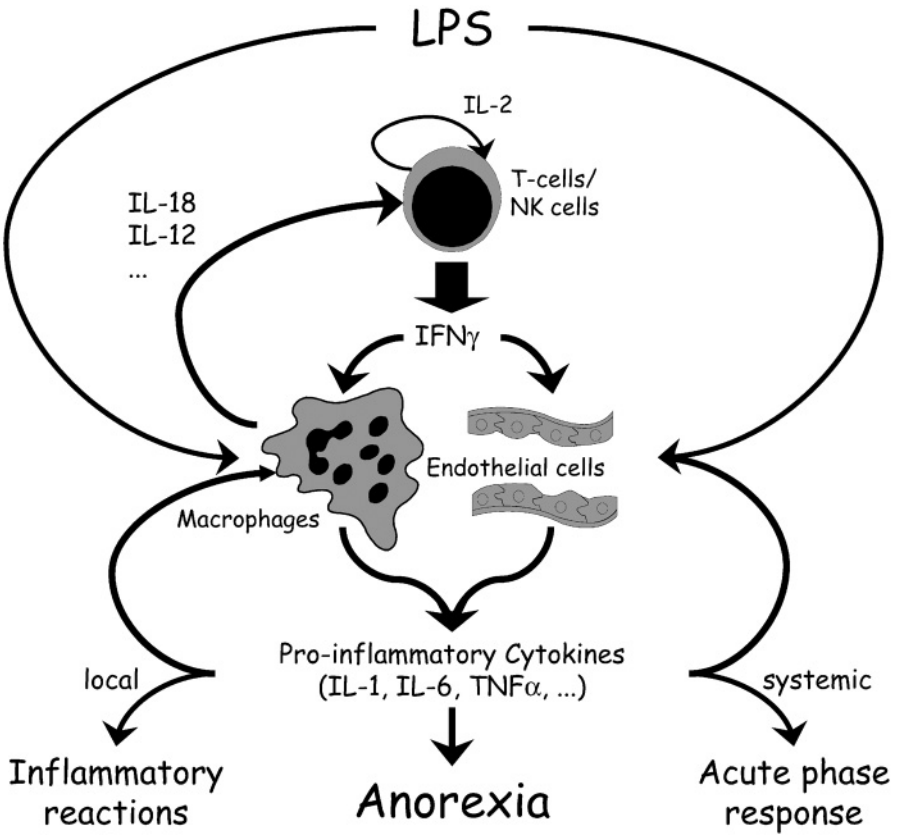

Figure 3. Proposed function of proinflammatory cytokines in bacterial lipopolysaccharides (LPS)-induced anorexia. The diagram integrates currently available data from genetic and pharmacologic antagonism studies and depicts an advanced stage of the cytokine reaction. Interferon-γ (IFNγ) appears to be important for the inherent positive feedback of the reaction and, hence, for the food intake suppression. IL = interleukin (IL-1, IL-2, IL-6, etc.), NK cells = natural killer cells, T cells = T lymphocytes, TNFα = tumor necrosis factor-α. See text for further details.

2000). This finding implicates TNFα in peripheral LPS-induced anorexia and indicates that PTX does not act downstream of TNFα production. Nonspecific phosphodiesterase inhibitors, such as PTX, have been in clinical use for many years and are known to inhibit TNFα production in response to several pathologic states (Breuille *et al.*, 1993; Dezube *et al.*, 1993; D'Hellencourt, Diaw, Cornillet, & Guenounou, 1996). PTX also has been shown to inhibit LPS-induced production of IL-18 in mice and the synergistic induction of IFNγ by combined IL-12/IL-18 treatment *in vitro* and *in vivo* (Samardzic, Jankovic, Stosic-Grujicic, Popadic, & Trajkovic, 2001). These effects might contribute to the elimination of LPS-induced anorexia by PTX.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily. PPARα usually controls intra- and extracellular lipid metabolism, whereas PPARγ triggers adipocyte differentiation and promotes lipid storage. PPARβ is ubiquitously expressed and is the most abundant of the three PPARs in many tissues except for adipose tissue (Escher *et al.*, 2001) and liver, where PPARα prevails (Everett, Galli, & Crabb, 2000). Recent findings indicate that PPARs are also involved in immune

reactions (Delerive, Fruchart, & Staels, 2001; Michalik *et al.*, 2001; Roberts-Thomson, 2000; Zhang & Young, 2002). PPARα and PPARγ ligands inhibit proinflammatory cytokine and prostanoid production (Delerive *et al.*, 2001; Jiang, Ting, & Seed, 1998; Staels *et al.*, 1998). PPAR activators presumably exert anti-inflammatory activities in various cell types by inhibiting the expression of proinflammatory genes. They do so by negatively regulating the transcription factors NFκB and activating protein-1 and by stimulating the catabolism of proinflammatory prostanoids (Delerive *et al.*, 2001; Zhang & Young, 2002). Yet, there are conflicting reports concerning the anti-inflammatory actions of PPAR ligands (Everett, Galli, & Crabb, 2000; Thieringer *et al.*, 2000). In addition, we recently observed that LPS (4 μg/mouse, ip) failed to reduce 24-hr food intake in PPARβ KO mice (Control: 3.9 ± 0.3 g, LPS: 3.7 ± 0.1 g) whereas it markedly reduced 24-hr food intake in corresponding WT mice (Control: 4.1 ± 0.4 g, LPS: 1.8 ± 0.2 g, $p < 0.01$). Under the same conditions, LPS reduced food intake similarly in PPARγ KO and WT mice. The failure of LPS to reduce food intake in PPARβ KO mice was associated with a complete elimination of the LPS-induced increase in plasma TNFα concentration which was observed in all other genotypes tested (Arsenijevic & Langhans, unpublished), suggesting that blockade of LPS-induced TNFα production in PPARβ KO mice contributed to the prevention of anorexia in this model. It is reasonable to assume that the inhibition of TNFα production in PPARβ KO mice was related to a lack of NFκB activation, but further studies are necessary to critically examine the mechanism(s) involved.

In previous experiments, pretreatment with the calcium (Ca^{++}) channel blocker verapamil inhibited the anorectic effect of ip LPS (Langhans, Harlacher, & Scharrer, 1989) but not of TNFα (Langhans, Balkowski, & Savoldelli, 1993), suggesting that a Ca^{++}-sensitive mechanism is involved in LPS-induced anorexia. Moreover, the results are consistent with the assumption that verapamil antagonizes LPS anorexia by inhibiting TNFα production. Various Ca^{++} antagonists (Ca^{++} channel blockers and dantrolene, which blocks the release of Ca^{++} from intracellular storage sites) have protective effects in cytokine-mediated pathophysiologic processes (Hotchkiss & Karl, 1994). These effects appear to be related to an inhibition of proinflammatory and an increase in anti-inflammatory cytokine production (Nemeth, Hasko, Szabo, Salzman, & Vizi, 1998; Szabo, Hasko, Nemeth, & Vizi, 1997).

In sum, various experimental manipulations that interfere with proinflammatory cytokine production antagonize the feeding suppressive effect of LPS in animal models of systemic bacterial infections. This conclusion is consistent with the putative role of these cytokines in the anorexia during bacterial infections. Some of these manipulations may be useful for therapeutic approaches to counteract the anorexia during infectious diseases.

Role of Conditioning and Motivation in the Anorexia During Disease

Conditioned aversions to the sensory properties of food, in particular to its taste, suppress eating when no other food is available. Conditioned taste aversions are the predominant cause for the anorexia that accompanies nutrient deficiencies and diseases that are directly related to the composition and/or quality of the ingested food (Langhans, 1986). A prototypic example for this kind of anorexia is

the food intake suppression that occurs in rats fed a tryptophan-deficient diet (e.g., Treneer & Bernstein, 1981). This anorexia is characterized by a reduction in meal size with unchanged meal frequency (Treneer & Bernstein, 1981), suggesting that the animals are still hungry but judge the available food unacceptable. Similarly, the tryptophan-deficient rats, but not the control rats, displayed a marked preference for a novel diet when offered a choice between their familiar diet and the novel diet. Interestingly, the tryptophan-deficient rats preferred the novel diet although it was tryptophan-free. Similar findings have first been reported for experimentally induced vitamin deficiencies (see Rozin & Kalat, 1971), and they exist for deficiencies of other essential nutrients (see Langhans, 1986). Such findings demonstrate that animals in a state of essential nutrient deficiency associate the sensory properties of the deficient diet with the negative consequences of its consumption, which they try to avoid by reducing food intake. In addition, conditioned taste aversions presumably contribute to cancer anorexia (Bernstein & Sigmundi, 1980) and to the anorexia during some parasitic infections (Keymer, Crompton, & Sahakian, 1983). Cancer therapy also can cause conditioned taste aversion and anorexia (Bernstein & Sigmundi, 1980; Bernstein, 1982).

Prompt acquisition of a taste aversion might also contribute to the anorexia after a single LPS injection because the feeding inhibition usually starts around 2 hr after LPS injection, that is, after the animals have consumed some food, which may provide the conditioned stimulus. As other LPS-triggered phenomena can be conditioned (Bull, Exton, & Husband, 1994; Exton, Bull, & King, 1995), the production of cytokines by LPS may be conditionable as well. Intraperitoneal LPS produced an aversion to the taste of saccharin solution in a standard two bottle-preference test (Langhans, Balkowski, & Savoldelli, 1991). Ingestion of a saccharin solution that had been paired with LPS previously led to an inhibition of feeding that had a time course similar to that of the anorectic effect of LPS (Exton *et al.*, 1995). These findings show that LPS-induced anorexia can be conditioned, but they do not prove that conditioning contributes to the anorectic effect of a single LPS administration in rats eating lab chow. An experiment designed to address this question yielded inconclusive results; pairing of an ip LPS injection with the subsequent presentation of a familiar diet or of a novel-tasting saccharin-flavored diet for several hours did not affect intake of the familiar diet or of the saccharin-flavored diet when the same diet was offered a few days later after 12 hr of food deprivation (Weingarten, Senn, & Langhans, 1993). When food was not withheld prior to the second feeding test, food intake was reduced in this test when the saccharin-flavored diet, but not when the familiar diet, was offered. Therefore, conditioning by LPS was sufficient to inhibit feeding only under conditions that favor associative learning, that is, when the conditioned stimulus was new and perhaps strong (saccharin-flavored diet), and when hunger was moderate (no food deprivation prior to the feeding test). These limitations suggest that conditioning does not play a crucial role in LPS-induced anorexia when animals are fed lab chow (Weingarten *et al.*, 1993), as is usually the case. This conclusion also holds for the anorexia induced by several cytokines such as IL-1β (Bauer, Weingarten, Senn, & Langhans, 1995), TNFα (Bernstein, 1996), and IFNγ (Segall & Crnic, 1990).

Lesions of the area postrema (AP) and parts of the adjacent nucleus of the solitary tract (NST) often have been shown to attenuate or eliminate anorexia due to taste aversion (Bernstein, Taylor, & Bentson, 1991). Therefore, it is interesting that an AP/NST lesion enhanced LPS-induced anorexia (Weingarten *et al.*, 1993). This experiment was performed 9 weeks after AP/NST lesioning (Weingarten *et al.*,

1993), by which time the anorexia produced by the surgery had completely disappeared and when rats were fed a familiar diet. Thus, it is unlikely that the observed enhancement of LPS-induced anorexia was related to postsurgery illness. Also, AP/NST lesion did not influence the anorectic effect of ip injected IL-1β (Bauer *et al.*, 1995) or TNFα (Bernstein *et al.*, 1991) when rats ate a familiar diet. All these results support the view that conditioned taste aversions are not the primary cause for the anorexia in response to LPS or cytokine administration, and that the AP/NST modulates the anorectic effect of LPS through some as yet unknown mechanism.

Food ingestion normally is comprised of an appetitive and a consummatory phase (Craig, 1918). The appetitive phase of ingestion involves behaviors such as detecting and approaching the food. Consummatory behaviors are more stereotyped, such as licking, chewing, and swallowing. Any physiologic or pathologic mechanism—or any manipulation—that affects food intake may do so by influencing either the appetitive or the consummatory phase of ingestion, or both. Common intake measures (e.g., recording of ingestion from a food hopper or a bottle) do not dissociate the appetitive and consummatory phases of ingestion. The appetitive phase of ingestion can be examined by recording an animal's food-motivated or food-oriented behavior. A selective recording of the consummatory phase of ingestion requires employment of the intraoral feeding test originally developed by Grill and Norgren (1978). In this test, a cannula is implanted into the oral cavity. Intraoral infusion of glucose through this cannula and simultaneous recording of the rat's behavior reveals whether the animal swallows the solution (active consumption), lets it drip out of its mouth (passive avoidance), or actively rejects it (as indicated by gaping or chin rubbing). Very often the results obtained with selective measurements of the appetitive and consummatory phases of ingestion parallel each other. There are, however, a few notable exceptions. Neuropeptide Y (NPY), for instance, has long been known to markedly stimulate food intake after icv administration (Gehlert, 1999). On the other hand, NPY does not affect intraoral fluid intake (i.e., the consummatory phase of ingestion; Seeley, Payne, & Woods, 1995; Woods *et al.*, 1998). To my knowledge, similar tests have not yet been performed systematically with immune stimuli such as LPS or cytokines. In one study (Cross, Kent, Ossenkopp, & Kavaliers, 1999), LPS reduced normal drinking of sucrose solution, but did not affect orofacial and somatic responses to brief (30 s) intraoral infusions of sucrose solution in a taste reactivity test. This finding suggests that LPS does not reduce intake by decreasing palatability; it remains to be demonstrated whether LPS also has no effect on longer term intraoral sucrose intake. It is known that the administration of LPS and cytokines affect food-motivated behavior (Bluthe *et al.*, 2000; Bret-Dibat *et al.*, 1997; Kent *et al.*, 1996; Larson, Romanoff, Dunn, & Glowa, 2002). In addition, a recent study revealed that the effects of IL-1β are situationally variable and depend on the deprivation state. Thus, IL-1β (100 ng/mouse) decreased consumption of sweetened milk to a greater extent in ad libitum fed mice than in mice that were food-restricted to maintain 85–90% of their free-feeding body weight. Furthermore, when operant responding for milk was maintained under a fixed-ratio response schedule of milk delivery, IL-1β decreased milk intake in mice trained to a high but not a low response schedule of milk delivery. These results suggest that the effects of IL-1β on food-maintained behavior depend on the level of motivation (as assessed by food restriction) and on the response cost for the milk (as assessed by ratio requirement). Thus, motivational factors appear to be capable of modulating the feeding effects of IL-1β. This interpretation also could explain the attenuation

of the feeding suppressive effects of LPS and IL-1 in restrictively fed animals with reduced body weight (Lennie, 1998; Mrosovsky, Molony, Conn, & Kluger, 1989).

Gender Differences in the Anorexia during Disease

Gender differences in immune function can have great biological significance. Sexually differentiated responses occur in molecular, cellular, physiologic, and systemic aspects of immune function in relation to acquired and innate immunity. The gender differences apparently include lifelong effects based on genetic differences or organizational effects of gonadal hormones early in development, as well as activational effects by gonadal hormones in adults (Geary, 2001). Gender differences in the susceptibility and course of various diseases are very common (Klein, 2000). Males are generally more susceptible to many infectious diseases and certain forms of cancer, whereas autoimmune diseases are more common in females (Klein, 2000), a phenomenon presumably related to the enhanced immune function in females. The greater immunoreactivity and cytokine production of females (Li, Danis, & Brooks, 1993; Lynch, Dinarello, & Cannon, 1994) presumably also accounts for their more pronounced acute phase reactions compared to males. Fever responses after administration of LPS, IL-1β, prostaglandins, or other challenges vary through the estrus cycle and during pregnancy in female rats (Martin, Malkinson, Veale, & Pittman, 1996; Mouihate, Chen, & Pittman, 1998). Endogenous estrogen appears to enhance the LPS-induced cytokine production by peripheral blood monocytes (Schwarz, Schafer, Bode, & Bode, 2000), and exogenous estrogen administration in rats increased the fever response to IL-1β (Mouihate *et al.*, 1998) and the sensitivity of Kupffer cells to LPS (Ikejima, Enomoto, Iimuro, Brenner, & Thurman, 1998). Most of these differences may involve organizational or activational effects of gonadal hormones on immune cells, which possess estrogen and androgen receptors (Olsen & Kovacs, 1996).

Furthermore, the effects of cytokines on various behaviors, including eating, differ between males and females, and this difference is also related mainly to estrogen (Geary, 2001). For example, estradiol enhances the anorectic effect of IL-1β (Butera, Doerflinger, & Roberto, 2001) and LPS (Geary, 2001). Cyclic estradiol replacement therapy in ovariectomized rats enhanced the anorectic effect of ip LPS, which was due to a decrease in meal frequency with no change in meal size (Sheahan, Asarian, Langhans, & Geary, 2002). The enhancement of this effect by estradiol resulted in an even greater decrease in meal frequency. This finding is remarkable because estradiol also decreased meal size in ovariectomized rats, as it usually does. The differential effects of estradiol on meal size and meal frequency suggest that different mechanisms mediate the physiologic effect of estradiol on normal feeding and its pathophysiologic action on LPS-anorexia. The mechanisms of these effects await further characterization.

Varma *et al.* (Varma, Chai, Meguid, & Yang, 2001) investigated sex-specific effects in cancer anorexia in rats inoculated with methylcholanthrene sarcoma cells. Although male and female rats displayed similar degrees of anorexia, the feeding patterns that produced the anorexia differed; both meal size and meal number decreased in male rats, whereas only meal number decreased in female rats. This finding suggests that the tumor affects, at least to some extent, different controls of feeding in male and female rats. Furthermore, the effect on meal number in female rats appears to be under the control of ovarian hormones because

both the onset and the course of cancer anorexia were delayed in ovariectomized rats in comparison to intact female rats. In sum, there are not enough studies on gender differences in disease-related anorexia to draw firm conclusions about the exact mechanisms involved. Yet, the results that are available suggest quantitative and qualitative gender differences in the feeding responses to various immune challenges that are worth exploring from a scientific as well as from a practical point of view.

Biological Mechanisms of the Anorexia during Disease

Cytokines in the CNS

Glia cells and neurons in various brain areas express several proinflammatory cytokines as well as their accessory proteins and receptors (Gayle, Ilyin, & Plata-Salamàn, 1997b; Vitkovic, Bockaert, & Jacque, 2000). Acute and chronic CNS diseases (e.g., meningitis, encephalitis, multiple sclerosis, Alzheimer's disease, stroke, and brain tumors) stimulate CNS cytokine production (Plata-Salamàn & Turrin, 1999; Rothwell, 1999). Intracerebroventricular administration of proinflammatory cytokines (Sonti *et al.*, 1996) presumably models the clinical features and anorexia of such diseases, although currently it is unclear where centrally produced (or icv administered) cytokines act to reduce food intake. For instance, icv administered IL-1β rapidly spreads along white matter fiber bundles and blood vessels, and may thus activate distant neurons that express the type 1 IL-1 receptor (Konsman, Tridon, & Dantzer, 2000).

Also, some types of peripheral immune stimulation increase *de novo* synthesis of proinflammatory cytokines in the brain (Gabellec, Griffais, Fillion, & Haour, 1995; Haour *et al.*, 1995; Plata-Salamàn *et al.*, 1998; Wong, Bongiorno, Rettori, McCann, & Licinio, 1997), which may be involved in centrally controlled phenomena in these conditions (Cartmell, Luheshi, & Rothwell, 1999; Gabellec *et al.*, 1995; Haour *et al.*, 1995; Kakizaki, Watanobe, Kohsaka, & Suda, 1999; Wong *et al.*, 1997). Apparently, cytokines synthesized in the brain contribute to the anorexia in some cancer models (Laviano *et al.*, 1995; Plata-Salamàn *et al.*, 1998) and to anorexia that occurs after rectal administration of TNB in the rat (Mchugh, Collins, & Weingarten, 1994). Often, however, it is questionable whether centrally produced cytokines play an exclusive role in the anorexia accompanying models of systemic disease because (1) an essential cytokine is not expressed in the brain (Arsenijevic *et al.*, 2000), (2) the spatial and temporal expression pattern of the cytokines and cytokine receptors does not fit a role in the anorexia observed in a particular model (Eriksson, Nobel, Winblad, & Schultzberg, 2000; Herkenham, Lee, & Baker, 1998; Konsman, Kelley, & Dantzer, 1999; Quan, Whiteside, & Herkenham, 1998b), and (3) only "suprapathophysiologic" peripheral stimuli reliably induce CNS cytokine synthesis (Eriksson *et al.*, 2000; Quan, Stern, Whiteside, & Herkenham, 1999; Wong *et al.*, 1997). In collaboration with C. Plata-Salamàn and his group, we found that ip injection of LPS (100 μg/kg) induced marked increases in the mRNA levels of the components of the IL-1 system (IL-1β, IL-1 receptor antagonist, IL-1 receptor 1, IL-1 receptor accessory proteins I and II) as well as of TNFα in several brain areas (mostly cerebellum and parieto-frontal cortex) as measured by RNase protection assay (Turrin *et al.*, 2001). Yet, the changes induced in the hypothalamus were comparatively small, and it is unclear whether they are causally related to the behavioral effects of this peripheral LPS dose. Recently, we confirmed the limited increase in

CNS IL-1β and TNFα in response to peripheral LPS using the sensitive TaqMan quantitative PCR technique (Lugarini *et al.*, unpublished results). Moreover, we found no increase in CNS IFNγ, which is not surprising given the fact that there are few T cells and natural killer cells in normal brain. This result is interesting, however, because IFNγ plays a major role in peripheral LPS-induced anorexia (Arsenijevic *et al.*, 2000).

Finally, peripheral immune stimulation may increase brain cytokine expression but reduce cytokine receptor expression (Haour *et al.*, 1995), and the latter effect may attenuate any net effect of centrally produced cytokines on food intake. Results from some acute studies using antagonist drugs also question a role of centrally produced cytokines in the anorectic response to peripheral LPS (Yao *et al.*, 1999). Thus, icv administration of IL-1 receptor antagonist inhibited the effect of icv but not ip injected LPS in rats (Bluthe *et al.*, 1992; Kent *et al.*, 1996). Similarly, icv IL-1 receptor antagonist blocked the depressive effect of icv IL-1 on food-motivated behavior but only slightly attenuated the effect of ip IL-1 (Kent *et al.*, 1996). The same group recently reported an attenuation of ip LPS-induced anorexia by icv administration of IL-1 receptor antagonist in mice (Laye *et al.*, 2000). The reason for this discrepancy is unclear, but species differences (e.g., mouse vs rat) and the high dose of LPS (5–10 μg/mouse) may be involved. All in all, some peripheral diseases stimulate cytokine synthesis in the brain, but centrally produced cytokines presumably do not account for all CNS controlled phenomena in the course of systemic diseases.

Cytokines in the Periphery

General Aspects.

Systemically produced cytokines can reach neuronal cytokine receptors through active or passive transport mechanisms (Banks & Kastin, 1996; Banks, Kastin, & Gutierrez, 1993) or through circumventricular organs (Banks & Kastin, 1996; Herkenham *et al.*, 1998; Ota, Katafuchi, Takaki, & Hori, 1997). The anteroventral region of the third ventricle (AV3V) that includes the organum vasculosum laminae terminalis is one site of IL-1 action on the brain because electrolytic lesions of the AV3V in several animal species decrease the febrile and plasma ACTH responses to systemic administration of IL-1β (Stitt, 1985). AV3V neurons respond to systemically administered IL-1β (Ota *et al.*, 1997), but it is unknown whether the AV3V and its hypothalamic projections play a role in the feeding suppressive effect of IL-1β. Another potential site of cytokine action on the brain, the AP, does not seem to be involved in the anorexia during infection. As mentioned above, AP lesion(s) did not alter hypothalamic *c-fos* expression in response to iv IL-1β (Ericsson, Arias, & Sawchenko, 1997), and AP/NST lesions did not change the anorectic effects of ip administered IL-1β (Bauer *et al.*, 1995) or TNFα (Bernstein, 1996). Cytokine-mediated activation of brain regions involved in control of food intake may, however, occur through neural and/or humoral pathways.

Neural Afferent Mediation.

Immune stimulation induces IL-1β immunoreactivity in dendritic cells and macrophages in connective tissue associated with the abdominal vagus (Goehler *et al.*, 1999), and vagal afferents in the rat have been shown to respond to IL-1β (Kurosawa, UvnasMoberg, Miyasaka, & Lundeberg, 1997; Niijima, 1996). Locally released cytokines (Miller, Hopkins, & Luheshi, 1997) thus may inhibit feeding by activating vagal afferents even without a concomitant increase in circulating cytokines. Subdiaphragmatic vagotomy attenuated some but

not all cytokine-induced phenomena (Dantzer, Konsman, Bluthe, & Kelley, 2000). In relation to anorexia, vagotomy in mice attenuated ip LPS- and IL-1β-induced suppression of instrumental responses to obtain food (Bret-Dibat, Bluthe, Kent, Kelley, & Dantzer, 1995). Systemic capsaicin pretreatment in rats, however, failed to block the effects of ip administered IL-1β and LPS on food-motivated behavior (Bret-Dibat *et al.*, 1997). Using a more focused surgical procedure, we observed that subdiaphragmatic vagal deafferentation, alone and in combination with celiac-superior mesenteric ganglionectomy, did not alter the anorexia after ip injection of LPS, MDP, or IL-1β in rats (Porter, Hrupka, Langhans, & Schwartz, 1998). These findings indicate that sensory visceral afferents are not necessary for the anorectic effects of these immune stimuli, at least in the rat.

Humoral Mediation by Leptin. Proinflammatory cytokines increase the expression and production of leptin in adipose tissue (Faggioni, Fantuzzi, Fuller, Dinarello, Feingold, & Grunfeld, 1998; Finck, Kelley, Dantzer, & Johnson, 1998; Grunfeld *et al.*, 1996; Kirchgessner, Uysal, Wiesbrock, Marino, & Hotamisligil, 1997; Sarraf *et al.*, 1997), and these increases correspond to the feeding suppressive effects of the cytokines (Grunfeld *et al.*, 1996; Sarraf *et al.*, 1997). Thus, leptin could be a mediator of LPS- and/or cytokine-induced anorexia. If so, then the feeding suppressive effects of LPS and cytokines might be anticipated to be weaker or absent in animals with genetic defects in the leptin system. Compared to normal control animals, LPS had a more pronounced feeding suppressive effect in *ob/ob* (leptin-deficient) mice, and caused weaker food intake suppression in *db/db* (leptin receptor-deficient) mice (Faggioni, Fuller, Moser, Feingold, & Grunfeld, 1997). We found that a single ip LPS injection (100 μg/kg) reduced food intake similarly in lean (*Fa/?*) and obese (*fa/fa*) Zucker rats (Lugarini, Hrupka, Schwartz, Plata-Salamàn, & Langhans, 1999), which have largely dysfunctional leptin receptors. High doses (500 μg or 1.0 mg/kg) of ip LPS also caused a similar initial (day 1) feeding suppression in lean and obese Zucker rats, but recovery of food intake was somewhat delayed after the highest dose (1.0 mg/kg) in obese rats. After chronic ip administration of LPS (2.45 or 9.8 μg/h) through osmotic minipumps, post-implantation recovery of food intake was similar, but body weight regain was much slower in lean than in obese rats (Lugarini *et al.*, unpublished results). This result might be related to a stimulating effect of LPS-induced leptin on metabolism in lean but not obese rats, but further experiments are necessary to test this idea. In another study, concomitant icv leptin administration prolonged the anorectic effect of an ip LPS injection; 24-hr food intake in normal Sprague Dawley rats given ip LPS alone (100 μg/kg) or together with icv leptin (0.8 μg/rat) was 93% and 70%, respectively ($P < 0.05$), of control intakes (Langhans, Lugarini, Hrupka, Schwartz, & Plata-Salamàn, 2000). The leptin dose alone did not reduce food intake under these conditions. We could also not detect a differential effect of ip IL-1β on food intake in lean and obese Zucker rats. In contrast, the anorectic effect of systemic TNFα was enhanced in obese Zucker rats in a previous study (Vasselli & Denise Casey, 1996). All in all, the available data suggest that leptin or functional leptin receptors are not necessary for the feeding suppressive effect of LPS and/or proinflammatory cytokines, but leptin may contribute in several ways. For instance, after acute supraphysiologic or chronic pathophysiologic doses of LPS, leptin may be involved in the adaptive response. Recently, a substantial decrease in circulating ghrelin in response to ip LPS has been reported (Basa *et al.*, 2003). Although it is unclear how LPS or cytokines may suppress ghrelin production in the stomach, the facts that ghrelin potently stimulates feeding (Tschöp, Smiley, & Heiman, 2000)

and that LPS suppresses feeding by reducing meal number (see above) are consistent with the idea that a decrease in ghrelin might be involved. Clearly, further studies are necessary to critically examine the cytokine–leptin interactions as well as possible contributions of other peripheral peptides to the anorexia during disease.

Central Action of Circulating Cytokines. The surface area of the blood–brain barrier (BBB) is much larger than that of the circumventricular organs (Kastin, Pan, Maness, & Banks, 1999), and efficient transport systems regulate the uptake of many substances, including cytokines, into the CNS (Banks & Kastin, 1996). In addition, proinflammatory cytokines and LPS increase BBB permeability and may promote cytokine uptake across the BBB in this way (Arsenijevic *et al.*, 2000; DeVries *et al.*, 1996) (see also below). Furthermore, circulating cytokines may influence food intake without crossing the BBB by acting on brain capillary endothelial cells (Licinio & Wong, 1997). Those cells and perivascular cells (e.g., microglia, macrophages) possess cytokine receptors (Deckert-Schluter, Bluethmann, Kaefer, Rang, & Schluter, 1999; Nadeau & Rivest, 1999; VanDam *et al.*, 1996), and peripheral administration of IL-1β or LPS leads to a rapid induction of *c-fos* mRNA in non-neural cells of the BBB (Herkenham *et al.*, 1998; Quan *et al.*, 1998b). In BBB endothelial cells, bacteria, viruses, LPS, proinflammatory cytokines, and other immune stimuli cause activation of the transcription factor NF-κB (Bierhaus, Chen, Liliensiek, & Nawroth, 2000; Laflamme, Lacroix, & Rivest, 1999) and trigger the release of neuromodulators such as prostanoids or nitric oxide (Cao, Matsumura, Yamagata, & Watanabe, 1996; Nadeau *et al.*, 1999; Rivest, 1999; VanDam *et al.*, 1996). NFκB is considered to be a major mediator of the immune response because it regulates the expression of proinflammatory cytokines, chemokines, and other immune factors (Pahl, 1999). Accordingly, endothelial cells also produce cytokines in response to various stimuli (Bierhaus *et al.*, 2000; Licinio & Wong, 1997), and this effect leads to signal amplification. In sum, in response to infectious stimuli, endothelial and perivascular cells of the BBB produce cytokines and other mediators that can jointly affect neurons involved in control of energy balance.

Several cytokines (e.g., IL-1β, TNFα, and INFγ) synergistically increase cyclooxygenase-2 (COX-2) mRNA expression in brain endothelial cells (Cao *et al.*, 1996; Kalaria, 1999; Lacroix & Rivest, 1998; Perkins & Kniss, 1997; Quan, Whiteside, & Herkenham, 1998a) and potently stimulate prostanoid production (Cao, Matsumura, Yamagata, & Watanabe, 1998; Rivest, 1999). LPS also increases COX-2 mRNA in endothelial cells and stimulates prostaglandin-E_2 (PGE_2) production (Cao, Matsumura, Yamagata, & Watanabe, 1997; Elmquist *et al.*, 1997; Lacroix & Rivest, 1998; Quan *et al.*, 1998a). Intravenous LPS-induced COX-2 like immunoreactivity in perivascular microglia and in meningeal macrophages throughout the brain (Elmquist *et al.*, 1997). LPS-induced activation of endothelial cells and stimulation of prostanoid production depends on soluble CD14 (Bierhaus *et al.*, 2000) and is independent of IL-1β (Laflamme *et al.*, 1999). The latter result might explain some of the failures of IL-1 antagonism to block peripheral LPS effects on CNS functions (e.g., Bluthe *et al.* [1992]; Burgess *et al.* [1998]).

PGE_2 is the primary prostanoid produced by BBB endothelial cells in response to LPS, IL-1β, and IL-6 (DeVries *et al.*, 1995). Nonspecific inhibitors of COX attenuated the anorectic effects of IL-1β and LPS (Langhans *et al.*, 1989, 1993; Swiergiel, Smagin, & Dunn, 1997) as well as cancer cachexia and anorexia (Cahlin *et al.*, 2000). Indomethacin also attenuated the LPS or IL-1β-induced increase in *c-fos* mRNA expression in hypothalamic paraventricular nucleus (PVN)

(Ericsson *et al.*, 1997; Lacroix & Rivest, 1997). These data suggest that prostanoids produced at or around the BBB are involved in the anorexia observed during various diseases. In our hands, the COX-2 inhibitor NS-398, but not the COX-1 inhibitor resveratrol, attenuated the anorectic effect of ip LPS (Lugarini, Hrupka, Schwartz, Plata-Salamàn, & Langhans, 2002). Concomitant with its feeding suppressive effect, ip LPS markedly increased PGE_2 concentration in the cerebrospinal fluid but not in plasma (Lugarini *et al.*, 2002). NS-398 also blocked this LPS-induced increase in cerebrospinal fluid PGE_2, whereas resveratrol had no effect (Lugarini *et al.*, 2002). Thus, cerebrospinal fluid PGE_2 may somehow be involved in the feeding suppressive effect of LPS. Subdiaphragmatic vagotomy failed to attenuate the increase in cerebrospinal fluid PGE_2 after LPS (Matsumura *et al.*, 2000), indicating that vagal connections between periphery and brain are not necessary for this response. This result is interesting because surgical elimination of subdiaphragmatic vagal afferents and all sympathetic connections between periphery and brain did not eliminate the anorectic response to ip LPS and IL-1β (Porter *et al.*, 1998). It is also important to note that COX-2 inhibition has been shown to reduce tumor growth and cachexia in some models of cancer (Cahlin *et al.*, 2000; Hussey & Tisdale, 2000). All these findings are consistent with the

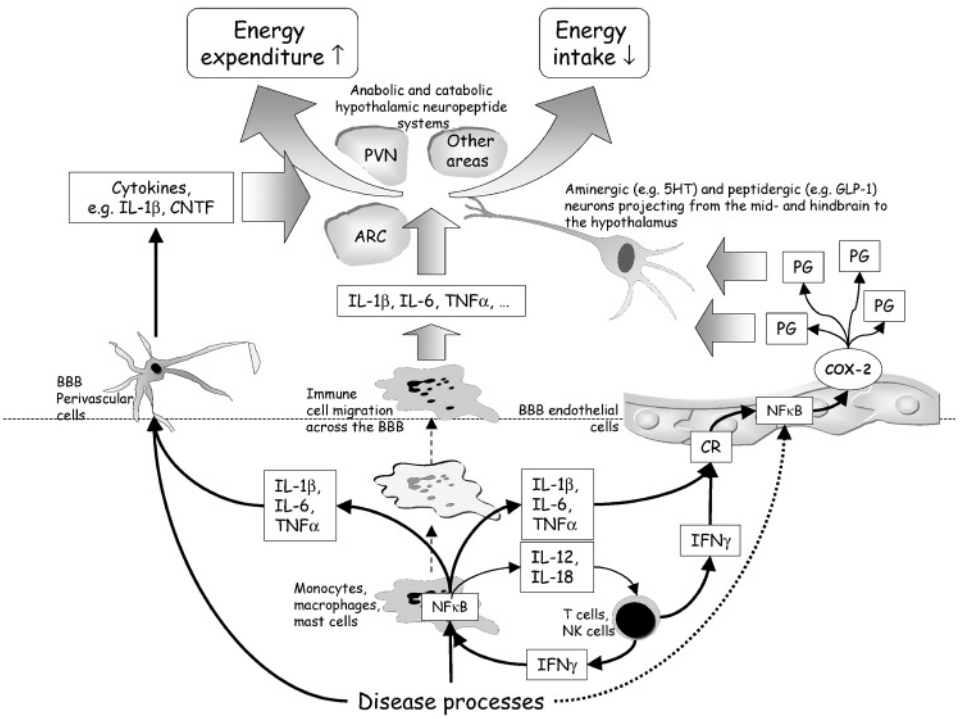

Figure 4. Proposed pathways for the effects of peripheral disease processes on hypothalamic neuropeptide systems controlling energy balance. The diagram depicts parallel—and not mutually exclusive—pathways for signal transmission across the blood brain barrier (BBB). ARC = arcuate nucleus, CNTF = ciliary neurotrophic factor, COX-2 = cyclooxygenase-2, CR = cytokine receptors, IFNγ = interferon-γ, IL = interleukin (IL-1, IL-2, IL-6, etc.), NFκB = nuclear factor kappa-beta, NK cells = natural killer cells, PG = prostaglandin, PVN = paraventricular nucleus, T cells = T lymphocytes, TNFα = tumor necrosis factor-α. See text for further details.

following sequence of events for the effects of circulating cytokines on food intake and perhaps other centrally controlled disease phenomena (Langhans & Hrupka, 2003). The synergistic action of cytokines on BBB endothelial and perivascular cells triggers the production and release of prostanoids, in particular of PGE_2. PGE_2 (and/or other prostanoids) act on prostanoid receptors on neurons that project to brain sites involved in food intake control, in particular to the PVN of the hypothalamus (Figure 4).

Migration of Immune Cells across the BBB

Alterations in BBB permeability and concomitant migration of immune cells into the brain have long been known to accompany infections of the brain as well as neurodegenerative disorders (see Mallat, Calvo, & Dobbertin [1997]; Merrill & Murphy [1997]). Peripheral immune challenges can also increase BBB permeability (Arsenijevic *et al.*, 2000). Migration of immune cells across the BBB is therefore another pathway through which disease processes located inside or outside the BBB can deliver cytokines and other mediators to specific brain areas, and influence food intake and other centrally controlled disease phenomena (Mallat *et al.*, 1997; Persidsky, 1999). Several immune or endothelial cell-derived mediators enhance BBB permeability, thus promoting cell migration into the brain. In addition to macrophages, mast cells invade the brain in response to a variety of physiologic and pathologic stimuli (Silverman, Sutherland, Wilhelm, & Silver, 2000). Intraperitoneal LPS, for instance, induces a rapid and massive mast cell infiltration in various brain areas, in particular in the thalamus and the median eminence (Asarian *et al.*, 2003). This is interesting because in one study (Nava & Caputi, 1999) icv as well as ip administration of cromolyn, an inhibitor of mast cell degranulation, attenuated the anorexia and several other phenomena induced by ip LPS (e.g., inhibition of water intake, fever, reduction in locomotor activity, and increased anxiety levels). As cromolyn does not cross the BBB, this result raises the possibility that peripheral LPS triggers mast cell migration into the brain, where subsequent degranulation delivers cytokines and other mediators to neurons involved in control of food intake. This interpretation is questionable, however, because icv cromolyn failed to inhibit ip LPS-induced anorexia in another study (Asarian *et al.*, 2003). Mast cell degranulation also enhances BBB permeability (Zhuang, Silverman, & Silver, 1996), thus enhancing the traffic of cells and molecules across this important interface. In sum, immune cell migration across the BBB (Figure 4) presumably contributes to the anorexia during disease, but its exact role remains to be established.

Central Nervous System Pathways Involved in Anorexia

Hindbrain–Hypothalamic Connections

Serotonergic Pathways. Circulating cytokines and LPS activate medullary and hypothalamic paraventricular neurons (Elmquist & Saper, 1996; Ericsson *et al.*, 1997; Lacroix & Rivest, 1997), and indomethacin blocks these effects (Ericsson *et al.*, 1997; Lacroix & Rivest, 1997). Intraperitoneal LPS and IL-1β trigger the release of PGE_2 from brainstem slices (MolinaHolgado, Borrell, & Guaza, 1998), and PGE_2 leads to activation of PVN neurons when injected into the rostral

ventrolateral medulla (Ericsson *et al.*, 1997). The latter observation is interesting because serotonin and catecholamine cell groups in the midbrain and hindbrain, but not in the PVN, possess PG EP_3 receptors and are activated by PGE_2 (Ericsson *et al.*, 1997; Nakamura, Li, Kaneko, Katoh, & Negishi, 2001). In particular, serotonergic neurons, projecting from the midbrain dorsal raphe nucleus and the hindbrain to the hypothalamus, are primary candidate pathways for the anorectic effect of circulating cytokines and LPS. Recent findings from our laboratory (Hrupka & Langhans, 2002) suggest a causal link between BBB endothelial cell-derived prostanoids and the activation of dorsal raphe nucleus serotonergic neurons in LPS-induced anorexia. Microinjection of the COX-2 inhibitor NS-398 into the dorsal raphe nucleus (1 ng/rat) markedly increased food intake in ip LPS (100 μg/kg) injected rats (Figure 5), whereas microinjection of PGE_2 into the dorsal raphe nucleus decreased food intake (Figure 6).

The dorsal raphe nucleus also contains IL-1 receptors type 1 (Cunningham *et al.*, 1992), and central as well as peripheral administration of IL-1β and TNFα increases serotonergic activity in this area (Clement *et al.*, 1997). Similar to cytokines, peripheral LPS increased the concentrations of dopamine and serotonin and its metabolite 5-hydroxyindoleacetic acid in the PVN, and these effects were blocked by IL-1ra (MohanKumar, Mohankumar, & Quadri, 1999). This finding indicates that IL-1β mediates at least some of the LPS-induced changes in PVN monoamine metabolism. Interestingly, subdiaphragmatic vagotomy did not attenuate the effect of ip LPS on PVN monoamines (MohanKumar, Mohankumar, & Quadri, 2000), suggesting that this effect, like the feeding suppressive effect of LPS, is independent of intact vagal afferents. Serotonin potently inhibits eating, presumably through the $5\text{-}HT_{1b}$ and/or $5\text{-}HT_{2c}$ receptors (Simansky, 1996). Serotonin also appears to mediate the feeding suppressive effect of rectal TNB administration in the rat (Ballinger *et al.*, 2000),

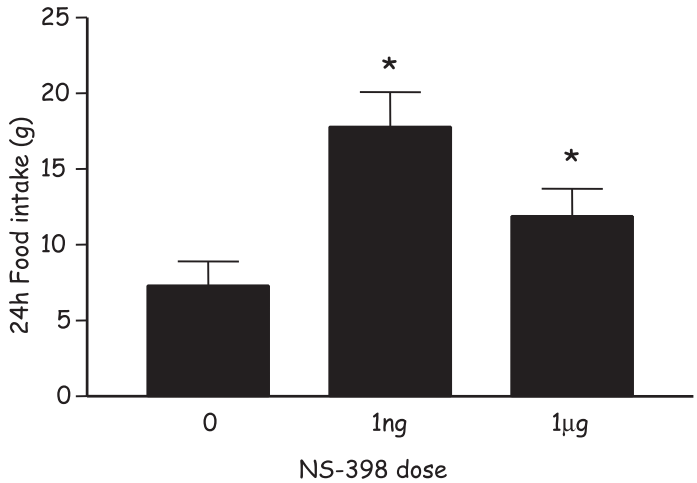

Figure 5. Microinjections of the COX-2 inhibitor NS-398 into the dorsal raphe nucleus increases food intake in LPS injected rats. Rats were implanted with 25 g guide cannulae directed toward the dorsal raphe nucleus. LPS (100 μg/kg) was injected ip and food cups were removed at 3 hr before dark onset. NS-398 was injected at dark onset (0.25 μl/2.5 min) and food intake was recorded. *Indicates significant ($p < 0.01$ in *post hoc* tests after significant ANOVA) increase in food intake compared to vehicle-injected controls. $N = 7$ for all treatments.

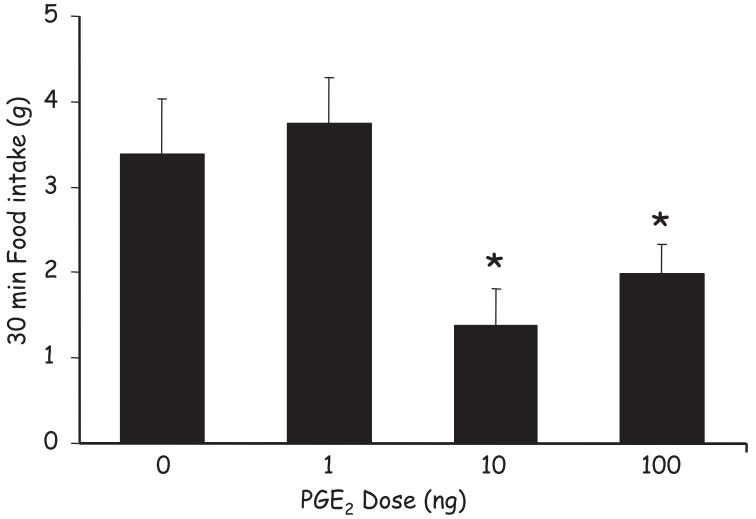

Figure 6. Microinjections of PGE_2 into the dorsal raphe nucleus reduces food intake in the rat. Rats were implanted with 25 g guide cannulae directed toward the dorsal raphe nucleus and adapted to overnight food deprivation. PGE_2 was injected at dark onset (0.25 μl/2.5 min) and 30 min food intake was recorded. *Indicates significant ($p < 0.01$ in *post hoc* tests after significant ANOVA) suppression of eating. $N = 7$ for all treatments.

which is presumably mediated by IL-1β (Mchugh *et al.*, 1994). We recently demonstrated that specific 5-HT_{2c} receptor antagonism blocks the anorexia induced by peripheral and central injection of LPS and IL-1β (von Meyenburg, Langhans, & Hrupka, 2003a, 2003b). Administration of the 5-HT_{1A} autoreceptor agonist, 8-OH-DPAT, directly into the dorsal raphe nucleus also blocked the feeding suppressive effect of peripheral LPS and IL-1β (von Meyenburg *et al.*, 2003a). Pharmacologic antagonism of other 5-HT receptors (5-HT_{1B}, 5-HT_{2A}, 5-HT_3) failed to attenuate the anorectic effects of peripheral LPS and IL-1β (von Meyenburg *et al.*, 2003b). These findings implicate the 5-HT_{2C} receptor in LPS- and IL-1β-induced anorexia and indicate that the median raphe nucleus is important for the anorectic response to peripheral LPS and IL-1β. Given the distinct differences in the meal pattern effects of icv and ip LPS (see above), the fact that 5-HT_{2c} antagonism attenuated both icv and ip LPS-induced anorexia suggests that the potentially different pathways mediating the effects of icv and ip LPS converge on the serotonergic system. All these data are consistent with the hypothesis that serotonergic neurons originating in the hindbrain play a role in mediation of cytokine-induced inhibition of feeding (Figure 4). On the other hand, Swiergiel and Dunn reported that numerous serotonin antagonists did not attenuate IL-1β-induced suppression of short-term milk intake in mice (Swiergiel & Dunn, 2000). Whether this discrepancy is due to a species difference or to methodological differences is not clear. CNS serotonin antagonism also yielded conflicting results concerning cancer anorexia (attenuation of anorexia [Laviano *et al.*, 1996], or no effect [Chance, von Meyenfeldt, & Fischer, 1983]). Thus, the central serotonergic mediation of cytokine-induced suppression of eating appears to be situationally variable and may be related to changes in serotonergic systems in various brain areas.

Peptidergic Pathways. In addition to ascending aminergic pathways, peptidergic neurons that link the hindbrain and the hypothalamic PVN may

contribute to mediation of anorexia in some diseases. Rinaman (1999) has shown that LPS activates glucagon-like peptide-1 (GLP-1) neurons, and that GLP-1 neurons project to the PVN. Also, icv administration of a GLP-1 receptor antagonist enhanced the fever-inducing effect of ip LPS (Rinaman & Comer, 2000) and attenuated the feeding suppressive effects of ip LPS (Comer & Rinaman, 2000; Rinaman & Comer, 2000), suggesting that GLP-1 is in fact involved in mediation of LPS-induced anorexia. Whether the same holds true for cytokine-induced changes awaits clarification.

Hypothalamic Neurochemistry

Cytokines can directly change the activity of hypothalamic neurons involved in the control of food intake (Katafuchi, Motomura, Baba, Ota, & Hori, 1997; Plata-Salamàn & Ffrench-Mullen, 1994). Such a direct action might be involved in the feeding suppressive effects of cytokines in some situations, in particular when cytokines are produced in the CNS (see above). Interestingly, IL-1β appears to be essential for the production of ciliary neurotrophic factor (CNTF) in response to brain injury or trauma (Herx, Rivest, & Yong, 2000). CNTF was first characterized as a trophic factor for motor neurons in the ciliary ganglion and spinal cord. Subsequently it was found to markedly reduce food intake and body weight (see Lambert *et al.* [2001]). These findings make CNTF a possible downstream mediator of central IL-1β effects on food intake and energy expenditure. Some data suggest that CNTF ultimately affects energy balance by reducing the expression and action of NPY (Xu *et al.*, 1998). A reduction of hypothalamic NPY has also been implicated in the feeding suppressive effect of IL-1β (Gayle *et al.*, 1997a) and tumors (Chance, Balasubramaniam, & Fischer, 1995; McCarthy, McKibbin, Perkins, Linton, & Williams, 1993). The observed changes in NPY expression appear to be too small to account for a substantial feeding suppressive effect. It is feasible, however, that a cytokine-induced decrease in NPY attenuates feeding that normally would occur in response to an energy deficit (Inui, 1999).

Peripheral injection of IL-1β increases hypothalamic corticotropin releasing factor (CRF) mRNA (Suda *et al.*, 1990), and IL-1β-induced anorexia was attenuated by icv administration of a CRF antagonist (Uehara, Sekiya, Takasugi, Namiki, & Arimura, 1989). This finding suggests that CRF is involved in the neurochemical mediation of IL-1β-induced anorexia. Interestingly, prostanoids appear to mediate the effect of IL-1β on hypothalamic CRF release (Watanabe, Morimoto, Sakata, & Murakami, 1990), which might provide a link between the presumed roles of prostanoids (Langhans, Savoldelli, & Weingarten, 1993; Lugarini *et al.*, 2002) and CRF (Uehara *et al.*, 1989) in the feeding suppressive effect of LPS and IL-1β. In relation to the possible role of CRF in IL-1β-induced anorexia, it is noteworthy that at least some of the feeding suppressive effect of central CRF appears to be mediated through brain oxytocin receptors (Olson, Drutarovsky, Stricker, & Verbalis, 1991). Whether central oxytocinergic neurons play a role in mediating IL-1β-induced anorexia remains to be examined.

Finally, LPS stimulates the release of αMSH (Catania, Suffredini, & Lipton, 1995). Alpha-MSH antagonizes inflammatory and acute phase reactions at various levels (cytokine production, cytokine action) in the periphery and the brain (Lipton & Catania, 1998). Alpha-MSH binds to central melanocortin receptors (MC3-R and MC4-R), and central administration of MC4-R agonists inhibits food intake, increases energy expenditure, and reduces body weight (see Tritos & Maratos-Flier [1999] and Chapter 6 by Seeley). In turn, deficiency of the MC4-R is associated with

increases in food intake and body weight (Fan, Boston, Kesterson, Hruby, & Cone, 1997). Intracerebroventricular αMSH enhanced ip LPS-induced anorexia in rats, whereas administration of the melanocortin (MC3/MC4) receptor antagonist SHU9119 attenuated it (Huang, Hruby, & Tatro, 1999). Moreover, the anorexia and cachexia induced by ip LPS or by a syngenic carcinoma were attenuated in MC4-R KO mice and also by the MC3-R/MC4-R antagonist Agouti-related peptide (Marks, Ling, & Cone, 2001). Recent studies showed that MC3-R KO mice reduced food intake and body weight in response to LPS and cytokines, and showed enhanced anorexia and tissue wasting in tumor models (Marks, Butler, Turner, Brookhart, & Cone, 2003). Together, these findings implicate specifically the central MC4-R in mediating LPS and cytokine-induced anorexia. Given the putative role of serotonin in the anorectic effects of LPS and IL-1β (see above), it is interesting to note that the melanocortin system recently has emerged as a downstream target of serotonergic neurons (Fan *et al.*, 2002; Heisler *et al.*, 2002).

In sum, several lines of evidence suggest that the pathways which mediate anorexia during disease, whether originating from central or peripheral cytokine production, ultimately converge on well-known neurotransmitter and hypothalamic neuropeptide systems that control food intake and energy balance.

Conclusions

Just 20 years ago, the loss of appetite observed in ill individuals was a well-known but enigmatic behavioral phenomenon. Since then, substantial progress in our knowledge of the mechanisms of cytokine production and action as well as the neurochemical control of eating has shed light on many aspects of the anorexia that occurs during disease. Studies comprising different levels of scientific analysis, from the molecular through the cellular to the systemic and behavioral levels, revealed several interesting features of the anorexia during disease: (1) The surprisingly extensive overlap between the intracellular mechanisms of cytokine production and the intracellular signaling cascades involved in cytokine action; (2) the existence of parallel communication lines between the immune system and the nervous system, including the pathways of signal processing at the BBB; (3) the striking similarity between the mechanisms of anorexia during disease and physiologic satiety, in particular, the fact that cytokine-induced signals converge on neurochemical pathways that are involved in the normal control of food intake and energy balance. All of these findings support the view that disease-related phenomena do not result from activation of some completely new "disease mechanisms," but rather from an occasionally substantial change of normal, physiologic processes. In addition, it is interesting to note that the intracellular mechanisms of anorexia and the peripheral catabolic mechanisms of muscle and adipose tissue breakdown are quite similar (Langhans, 2002).

What does all this tell us about possible therapeutic strategies to counteract chronic anorexia during disease? A complete understanding of the underlying mechanisms certainly will help to design well-targeted therapeutic approaches. The activation of immune cells and the stimulation of cytokine production by disease processes are accessible to pharmacologic intervention. Some inhibitors of cytokine production and/or action are in fact clinically well-established drugs with few side effects; other drugs certainly will become available. One problem of

anticytokine therapy for disease-related anorexia, however, is that it carries the risk of also antagonizing the beneficial defense functions of the cytokines. This risk does not apply to interventions aimed at blocking the neurochemical pathways that mediate disease-related anorexia, which therefore should provide fewer problems for successful pharmacological intervention where necessary.

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14

Neural Mechanisms of Anorexia

Alan G. Watts and Dawna Salter

Introduction

Defining the architecture of the neural systems responsible for motivated ingestive behaviors is a fundamental neuroscientific problem with a long history. Although we are still some way from providing detailed explanations, recent physiological and functional neuroanatomical studies are beginning to reveal much of the underlying processes. These include identifying which neural networks stimulate, inhibit, or switch particular ingestive behaviors, and how neuropeptides might function to facilitate these complex interactions.

Normal ingestive behaviors result from a complex interplay between the many and distributed neural networks that can stimulate and inhibit feeding. Much attention is currently focused on their constituent neural circuits and transmitters, the way they operate to maintain normal body composition, and how their dysfunction contributes to obesity. But also of clinical importance is determining how the pathological disruption of these neural systems contributes to anorexia, body wasting, and eating disorders. How these different networks are organized and the way they can interact dynamically to generate anorexia is the focus of this chapter.

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Neurobiology of Food and Fluid Intake, 2nd Ed., Volume 14 of *Handbook of Behavioral Neurobiology,* edited by Edward Stricker and Stephen Woods, Kluwer Academic / Plenum Publishers, New York, 2004.

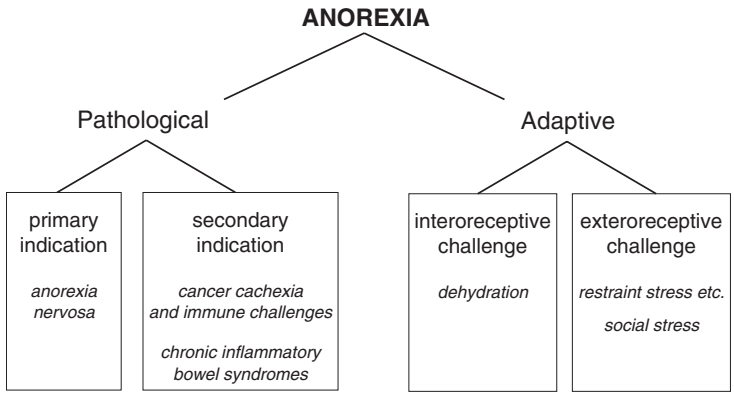

Figure 1. As a first approximation, anorexias can be divided into two broad groups: those that are associated with pathological conditions, and those that develop as adaptive responses to various challenges. Specific examples are shown in italics.

Definitions

In much of the popular press, the term "anorexia" is often used synonymously with anorexia nervosa (AN), which is the devastating condition that most often occurs in adolescent girls. More accurately, however, anorexia simply describes any loss of appetite and concomitant reduction in food intake that occurs in the presence of readily accessible food sources. Anorexia is also evident as the loss of compensatory increase in feeding after a hypocaloric challenge. It is the neural mechanisms underlying anorexia in this more general context that we address in this chapter.

Generally speaking, anorexia occurs in two broad sets of conditions (Figure 1). In the first set, anorexia is a symptom that accompanies a number of pathologies. In turn, these pathologies can be subdivided further into two groups: (1) the anorexia that is widely believed to originate psychologically, of which AN is the most prominent; (2) anorexia that is associated with disease states, of which the cachexia (disease-associated wasting) that accompanies AIDS, cancer, and other conditions is the most important and widely known. In the second set, anorexia is apparent as an adaptive behavioral response to certain homeostatic challenges. Some of these may originate externally, as is the case of anorexia that accompanies some types of stress, or it may be an adjunct to a physiological challenge such as dehydration (DE).

The mechanisms that generate these different types of anorexia are diverse and they involve a variety of different neural networks. From a systems perspective, however, it would seem reasonable to assume that they must ultimately converge upon a common set of neural circuits that is distributed throughout the brain and whose primary function is to control feeding behavior.

Anorexia in Clinically Important Settings

Anorexia Nervosa

Perhaps the most widely recognized clinical form of anorexia is AN. This condition is most commonly found in young women with 18 being the average onset age, but AN in much younger girls has been reported (Fairburn & Harrison, 2003). Less than 10% of patients with AN are males. The incidence of AN is about 0.5% of

the American female population, but its standardized mortality ratio (i.e., the observed mortality divided by expected mortality) is around 10, which is high for a psychiatric disorder, with death occurring from a variety of causes ranging from metabolic effects of negative energy balance to suicide and the complications of substance abuse (Herzog *et al.*, 1999; Nielsen *et al.*, 1998; Steinhausen, 2002). Recovery rates are not high, with less than 50% of patients showing full recovery (Steinhausen, 2002). Although it is popularly portrayed as a disease of Western culture, this conclusion has been questioned (Hoek, van Harten, van Hoeken, & Susser, 1998; Lee, 1996).

Historically, there have been many reports of anorexia across the centuries, particularly amongst young women. During the late middle ages, women such as St. Catherine of Siena expressed their Christian devotion by their virtual abstinence for food. Later in the 18th and early 19th centuries, an assortment of fasting girls made their appearance. Many of these were treated as media celebrities, and some were from families with questionable motives (Brumberg, 2000). A strong case can be made that almost none of these instances were cases of what we know today as AN. Indeed, Brumberg (2000) has argued that because of changing social and family factors, AN could only have emerged as a recognizable syndrome during the 19th century. Consequently, the first clinical reports of AN as we recognize it today were published in the early 1870s by Charles Lasegue (Vandereycken & van Deth, 1990) and Sir William Gull, with Gull providing the classic English language description (Gull, 1874). In defining the cases he came across in late Victorian England, Gull (1874) states:

> … I used the term Apepsia hysterica but … anorexia would be more correct. The want of appetite is, I believe, due to a morbid mental state … We might call the state hysterical … I prefer, however, the more general term "nervosa" (p. 25).

Defining the nature of AN reveals an extremely complex condition. Gull identified three physical symptoms: emaciation, hypothermia, and hyperactivity. Although modern diagnoses are more rigorous, these three symptoms remain prime indicators with the excessive pursuit of weight loss arising from a variety of socio-psychiatric conditions being preeminent. Interestingly, it has been suggested that true anorexia may not even be present in AN, and that eating is constrained by other factors (Fairburn & Harrison, 2003). The following are generally accepted indicators of AN (Fairburn & Harrison, 2003): overevaluation of shape and weight, that is, judging self-worth largely, or exclusively, in terms of shape and weight; active maintenance of an unduly low bodyweight, for example, body-mass index $\leq 17.5\ kg/m^2$; and amenorrhea in postmenarcheal females who are not taking an oral contraceptive.

A vast number of studies have attempted to identify causative mechanisms of AN; many are based on socio-psychiatric origins, some have concentrated on neuroendocrine and metabolic agents, while others have attempted to identify alterations in neurotransmitter systems (see Fairburn & Harrison [2003], for review). However, no single agent has emerged, and full understanding of the neural mechanisms underlying AN remains frustratingly elusive. Steinhausen (2002) describes current etiological models of AN as "emphasizing its multifactorial origin, coupled with multiple determinants and risk factors and their interactions within a developmental framework" (p. 1284).

Anorexia in Disease States

The contribution of anorexia to malnutrition complicates the prognosis of many clinical conditions. These include HIV infection (Kotler, 2000; Macallan, 1999),

cancer (DeWys, Begg, Lavin, Band, Bennett, Bertino *et al.*, 1980; Herndon *et al.*, 1998; Mani, Todd, Katz, & Poo, 1995), end-stage renal disease (Stenvinkel, 1999), chronic pulmonary disease (including tuberculosis) and cardiac disorders (Takabatake *et al.*, 1999; Witte & Clark, 2002), and chronic inflammatory bowel disease (e.g., Crohn's disease and ulcerative colitis; Ballinger, Kelly, Hallyburton, Besser, & Farthing, 1998; Rigaud *et al.*, 1994). Anorexia is a major contributor to cachexia, a condition of advanced protein calorie malnutrition that leads to invasive, expensive nutrition support therapy, iatrogenic infections, poor responses to therapy, and mortality. In fact, involuntary weight loss is an independently reliable prognostic factor in patients with AIDS, obstructive pulmonary disease, and cancer, particularly gastric and lung (Herndon *et al.*, 1998; Kotler, 2000; Plata-Salaman, 1996; Persson, Johansson, Sjoder, & Glimelius, 2002; Prescott *et al.*, 2002; Wheeler *et al.*, 1998). Finally, anorexia is increasingly being recognized as a frequent and important symptom in geriatric medicine (Wallace & Schwartz, 1997).

Individuals with disease often have difficulty obtaining adequate nutritional intake because of gastric obstructions, nausea, constipation, pain, or depression. However, even if these conditions are controlled, patients may continue restricting food intake (Rossi Fanelli & Laviano, 2002) and patients often report reduced appetite, early satiety, mouth dryness, or taste changes (Grosvenor, Bulcavage, & Chlebowski, 1989). Additionally, weight loss can be accompanied by hypermetabolism (Bosaeus, Daneryd, Svanberg, & Lundholm, 2001; Creutzberg, Schols, Bothmer-Quaedvlieg, & Wouters, 1998), yet these patients do not compensate by increasing their food intake. Numerous studies report weight loss as either the first indicator of disease or as affecting patients very early in disease development. In one study, at least a 10% body weight loss reportedly constituted the initial AIDS diagnosis in nearly 14% of patients (Melnick *et al.*, 1994). Forty-six percent of patients recently diagnosed with small cell lung, breast, or ovarian cancer reported unintentional weight loss of at least 5% in the previous 3 months, despite being ambulatory and in good physical condition (Ovesen, Hannibal, & Mortensen, 1993). Indeed, anywhere from 40% to 50% of cancer patients present significant weight loss at diagnosis (Andreyev, Norman, Oates, & Cunningham, 1998; Boldys, Marek, Wanczura, Matusik, & Nowak, 2003; Bosaues *et al.*, 2001; DeWys *et al.*, 1980). Weight loss is an objective and easily measured parameter for estimating the presence of anorexia because studies have shown that patients with weight loss had reduced energy intake while those who had not lost weight had normal amounts of intake (Grosvenor *et al.*, 1989). Although weight loss, decreased food intake, and anorexia are clearly related, they are not necessarily causal. Anorexia often cannot be defined or diagnosed simply by measuring the degree of weight loss. Anorexia does not provide an obvious clinical reason for reduced food intake, other than a self-reported lost desire to eat. Indeed it may exist some time before the patients are no longer capable of eating enough to meet their caloric needs and begin to lose weight. In fact, 43% of patients with gastric cancer report anorexia, yet only 11% have a history of weight loss (Boldys *et al.*, 2003).

Patients with life altering diseases often experience psychological distress at the uncertainties involved with diagnosis, treatment, and final outcome of the disease. Undoubtedly, this psychological state can affect appetite and food intake. However, the fact that decreased food intake and weight loss are already present at diagnosis in a substantial percentage of patients suggests anorexia, although certainly multifactorial, is primarily a physiological maladaptation of the normal feeding response to weight loss.

Part of the adaptive response to certain types of stress includes reduced blood flow and gastrointestinal activity, which facilitate the switch toward more appropriate activities such as defensive or avoidance behaviors. Anorexia may in this case be a component of the adaptive response to a temporary imposition. However, if the stressor is maintained for longer periods, then the consequences of anorexia may become a threat to survival. We discuss two examples where anorexia develops, at least initially, as part of an overall adaptive response.

Stress

Daily bouts of restraint or immobilization are the stressors used most widely to investigate this type of anorexia (Marti *et al.*, 1994; Valles, Marti, Garcia, & Armario, 2000), although repeated tail shock has also been reported to reduce food intake (Ottenweller, Natelson, Pitman, & Drastal, 1989). Hypophagia in these circumstances may be macronutrient selective (Wang, 2002). Social stress including repeated defeat (Berton, Aguerre, Sarrieau, Mormede, & Chaouloff, 1998) and crowding (Armario, Ortiz, & Balasch, 1984) generate anorexia and lead to reduced body weight. Although these models have been used most frequently to investigate the role of the serotonergic neurotransmission (Berton *et al.*, 1998; Grignaschi, Mantelli, & Samanin, 1993; Shimizu, Hori, Ogino, Kawanishi, & Hayashi, 2000; Wang, 2002) and the CRH peptide family (Samarghandian, Ohata, Yamauchi, & Shibasaki, 2003; Smagin, Howell, Redmann, Ryan, & Harris, 1999; Weninger, Muglia, Jacobson, & Majzoub, 1999) in generating anorexia, the underlying mechanisms remain unclear.

Dehydration

For many years it has been known that as animals and humans become dehydrated they develop a progressive anorexia (Adolph, 1947; Dicker & Nunn, 1957; Engel, 1988). The resulting reduction in food intake can be considered an adaptive behavioral response that slows digestion and allows water to be redistributed from the gut into the extracellular compartment in an attempt to maintain fluid balance. In laboratory rats eating conventional chow, reduced feeding also lessens the input of osmoles that can exacerbate DE. In this manner, DE-anorexia belongs to the physiologically adaptive set of anorexias (Figure 1).

The utility of the DE-anorexic rat as a model for investigating neural mechanisms of anorexia derives from some very simple properties that effectively constrain potential effector mechanisms. Thus, anorexia develops within 2 days of drinking hypertonic saline solution, and compensatory feeding develops within minutes when the rat is again provided with water. DE can be invoked experimentally by replacing drinking water with hypertonic saline (Watts, 1999). The sensory signals and transduction systems responsible for DE-anorexia are restricted to those derived from cellular DE and perhaps the taste of salt in the water. Conversely, DE-anorexia is rapidly reversed by drinking water, which is a readily identifiable and controllable stimulus (Watts, 1999). Together, these factors permit us to investigate with relative ease and precision those types of interactions at the network level that turn ingestive behaviors on and off.

It is important to note that investigating neural mechanisms in DE-anorexic rats is not going to reveal how specific signals initiate particular types of pathological anorexias; clearly these are going to be different in each case—for example, cytokines for some types of anorexia, complex psychiatric processes for others (although disturbed fluid balance has been discussed as a complication in AN; Lowinger, Griffiths, Beumont, Scicluna, & Touyz, 1999). What we suggest is that there are neural networks that can inhibit feeding behavior in the face of normal signals of starvation, and that the DE-anorexic rat is an excellent model for investigating where they are located and how they are organized. It seems reasonable to assume that increased activity in inhibitory networks must ultimately affect a core set of hypothalamic/hindbrain circuits that control the complex dynamics of feeding behavior. During different types of anorexia there must be a variety of mechanisms that can suppress normal hypothalamic and hindbrain responses to those signals generated by the negative energy balance accompanying anorexia.

Behavioral Characteristics of Dehydration-Anorexia. By comparing the amount of food eaten as animals begin to drink hypertonic saline to that when water is available, we determined that anorexia develops during the second night of treatment. This is the point when the amount of food eaten first falls outside the normal range (Figure 2A; Watts, 1999). Nocturnal food intake continues to fall as saline consumption continues, until by the fifth night, food intake may be reduced by as much as 75% from controls (Watts, 1999). Interestingly, the small amount of spontaneous diurnal feeding—normally around 10–12% of total intake—remains unchanged during the time hypertonic saline is consumed (Watts, 1999).

A significant and compelling feature of DE-anorexic rats from an experimental perspective is that feeding begins less than 10 min after animals again begin to drink

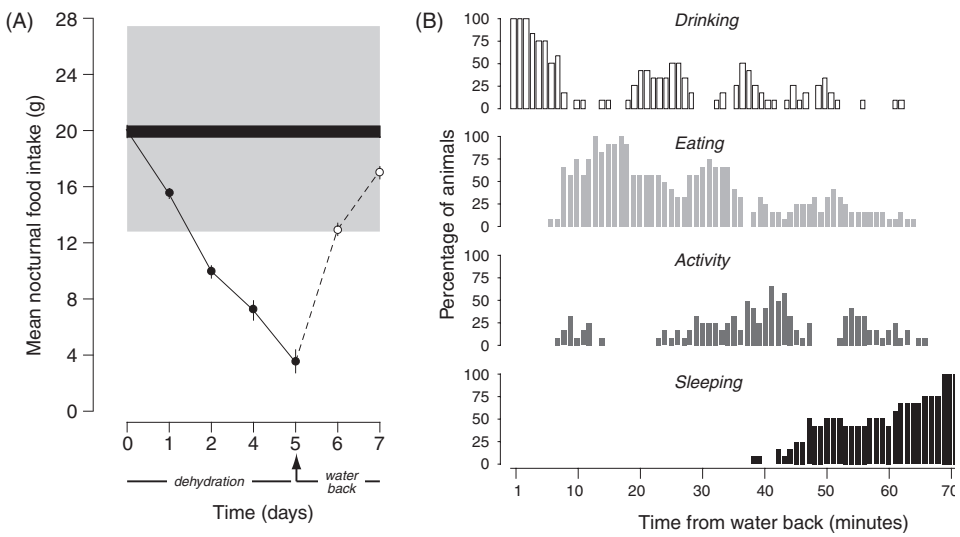

Figure 2. (A). The mean nocturnal food intake of rats given 2.5% saline to drink on day 0. The horizontal black line and the gray box represent the mean food intake and the two standard deviation range of control animals. The mean food intake of dehydrated rats moves outside range on day 2 and quickly normalizes once water is returned. (B) The behavior of animals following the return of water. Animals begin to eat within 10 min of the resumption of drinking water. Data in both panels are adapted from Watts (1999).

water (Figure 2B; Watts, 1999). This response is highly reproducible, temporally precise, behaviorally ordered, and very robust—within the first hour of drinking water, rats consume more food than in the previous 12 hr. Knowing that anorexia in dehydrated rats is precisely terminated by a simply evoked stimulus allows us to distinguish between "cause and effect" by comparing the neural responses to drinking water in the absence or presence of food.

A graphic illustration of what is perhaps an equivalent human experience comes from a firsthand account by Frank Worsley of Ernest Shackleton's ill-fated Antarctic expedition in 1916 (Worsley, 1977). These men endured many days in the southern Atlantic in small boats that were adequately supplied with food but had virtually no fresh water. He describes the feeling after their raging thirst had been relieved with frozen seawater:

> Chipping the salt off the ice with their knives, the men joyfully sucked until, parched tongues and mouths being moistened, they remembered their hunger … What a glorious sensation it was to feel fresh water trickling down my burning throat. Then we ate. (p. 82).

Responses of the Dehydrated Rat to Metabolic Challenges. The mechanisms controlling spontaneous nocturnal feeding are not the only ones that are suppressed during DE. We have recently investigated how DE affects the compensatory actions occurring in response to two metabolic challenges commonly used to investigate the neural mechanisms that control feeding.

Overnight Fasting. Measuring the compensatory feeding response to an overnight fast is a simple and widely used index of hunger. When DE animals are tested in this manner they exhibit a significant suppression of the compensatory feeding response. Thus, DE-anorexic animals that are tested 3 days or 5 days into the DE showed a significant and progressively lower feeding response than either ad lib fed or their pair-fed control groups (Salter & Watts, 2003). The degree of suppression was correlated with how long they had been drinking saline.

2-Deoxyglucose. Cellular oxidation of glucose can be inhibited using the noncompetitive inhibitors 2-deoxyglucose or 5-thioglucose. When animals are given these inhibitors either intravenously or directly into brain, they initiate three distinct compensatory motor responses: increased glucocorticoid secretion that is mediated by CRH neurons in the hypothalamic paraventricular nucleus (PVH), elevated plasma glucose concentrations, and initiation of feeding (Ritter, Bugarith, & Dinh, 2001). These responses are mediated by glucose receptors in the hindbrain that are able to transduce the cellular responses to glucoprivation into neural signals (Ritter, Dinh, & Zhang, 2000). In turn, these receptors engage ascending catecholaminergic projections to activate hypothalamic mechanisms in the case of feeding and glucocorticoid secretion, and descending catecholaminergic projections that mediate sympathoadrenal responses that increase blood glucose (Ritter *et al.*, 2001; Ritter, Watts, Dinh, Sanchez-Watts, & Padrow, 2003).

If DE-anorexic animals are challenged in this way, their hyperglycemic and glucocorticoid responses are indistinguishable from those of controls. However, their feeding responses are almost totally abolished (Salter & Watts, 2003). But the fact that these animals do eat soon after they are given water suggests that circuits stimulating food intake can be quickly released by the appropriate stimulus (Salter & Watts, 2003).

Similar responses have also been reported in rats with activity-based anorexia (ABA) (Aravich, Stanley, & Doerries, 1995) and, perhaps most interestingly, in

patients with AN. Here, intravenous infusions of 2-deoxyglucose or insulin in control patients increase hunger ratings within minutes. But patients with AN report no increase in their hunger ratings following these challenges relative to saline infusions, even though their blood glucose and cortisol responses were the same as those of controls (Nakai & Koh, 2001; Nakai *et al.*, 1987a).

Collectively, these results suggest that in certain types of anorexia, the ability of neural circuits to generate feeding-related motor programs in response to metabolic challenges are inhibited. The fact that in experimental and clinical settings both hyperglycemic and glucocorticoid responses to these metabolic challenges remain intact suggests that the sensory transduction systems engaged by 2-deoxyglucose are not affected by the processes that inhibit feeding or the desire to eat.

Organization of Neural Systems that Control Motivated Behaviors

Although the mechanisms responsible for the broad range of anorexias are poorly understood, it would seem reasonable to assume that they must at some point converge upon the neural networks responsible for controlling ingestive behaviors. Much work over the past 20 years has shown the pivotal role played by a hypothalamic network containing the arcuate–paraventricular–lateral hypothalamic (ARH–PVH–LHA) areas in energy balance. However, appreciation is growing that these cell groups are but one set of components within a wider core network of cortical, subcortical, hypothalamic, and hindbrain circuits that interact to control feeding behavior (Grill & Kaplan, 2002; Saper, Chou, & Elmquist, 2002; Watts, 2000). Although the nature of these interactions is being clarified (Elmquist, Elias, & Saper, 1999; Sawchenko, 1998; Swanson, 2000), our understanding of how hypothalamic or indeed any other neural circuit is specifically involved with anorexia is emerging even more slowly (Watts, 2000, 2001). For example, we don't know which of the neural circuits activated during stress are responsible for reducing food intake. How are metabolite driven feeding mechanisms inhibited during DE-anorexia and AN? What neural mechanisms might be recruited to overcome this inhibition? Answers to these questions would greatly enhance our understanding of anorexia.

To begin considering how neural mechanisms operate during anorexia, it is useful at this point to outline a general organizational model that we have begun to use for exploring how the brain controls motivated behaviors in general (Watts, 2000; Watts & Swanson, 2002). Using this type of model as a guide allows us to consider neural networks that encompass the whole brain as we develop strategies for understanding the specific neural mechanisms of anorexia. The danger of not taking such a broad approach is that we can become too narrowly focused on specific signals, neuropeptides, or cell groups in our efforts to understand these complex behaviors. For example, while it is likely that hypothalamic mechanisms participate in some, perhaps even all, aspects of anorexia, other mechanisms—particularly in those pathologies such as AN—probably also involve networks that control reward and arousal (Bergh & Södersten, 1996; Bergh, Brodin, Lindberg, & Sodersten, 2002). Indeed it seems inconceivable that conditions where there are profound and sometimes catastrophic changes in an animal's appreciation of food do not involve alterations to the mechanisms underlying these complex brain functions. Similarly, given the primary role of the hindbrain in processing sensory information from the

gut and for directly controlling oropharyngeal motor function and meal size (Grill & Kaplan, 2001, 2002), hindbrain neural circuits likely are heavily implicated in generating anorexia.

With these issues in mind, we have developed an organizational model by merging our current understanding of rat brain circuitry (e.g., Swanson, 2000; Watts, 2001) with what is essentially a Hullerian incentive model of motivation, where certain behaviors are selected at a particular time to reduce the level of associated drive states (for further discussion, see Berridge & Robinson,1998; Bindra, 1978; Toates, 1986). Although the way the brain is organized to control motivated behaviors is undoubtedly far more complex than is accounted for by this rather simplistic schema, it does have the advantage of supporting relatively simple and experimentally testable hypotheses for addressing the neuroanatomical organization of motivational systems in general, and how they function during anorexia.

Model

Incentive models of behavior derive from the notion that at any one time the level (or intensity) of each drive state—and hence the probability of a particular behavior being expressed—is dependent upon the result of integrating four sets of inputs that project to those parts of the brain responsible for selecting the appropriate behavioral action (Figure 3). These inputs are: information from systems that control behavioral state; interosensory information that encodes internal state (e.g., hydration state, plasma glucose, leptin, gastric distension, etc.); inputs that carry information from the neural processing of the classic sensory modalities (i.e., exterosensory information); and modulatory hormonal inputs such as the gonadal steroids that facilitate sexual behavior. The integration of these four input sets within the brain is central to selecting the appropriate motor action for the ongoing behavior, as well as inhibiting inappropriate actions. A model of this type is consistent with the long-held idea that at some point there are separate neural mechanisms available for stimulating and inhibiting the specific motor actions that constitute the full repertoire of ingestive behaviors.

This model (Figure 3) posits that at any one time a particular motivated behavior can be initiated when any one of these four sets of inputs predominates. For example, feeding can occur at one time because of strong signals encoding negative energy balance (interosensory information), but at another because a highly desirable food object is encountered in the environment (exterosensory

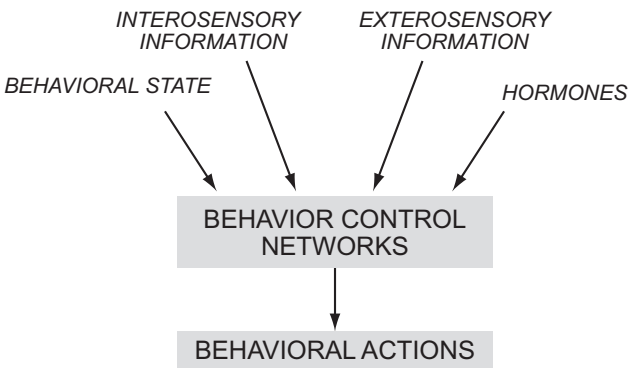

Figure 3. The four classes of inputs that influence motivated behavioral actions.

information), and yet at another time because of anticipatory signals generated from the circadian timing system (behavioral state). Along these same lines, it is possible that anorexia develops in some instances because the way the incentive value of food is represented in the brain is changed in a manner that suppresses the strong interosensory signals encoding the developing negative energy balance. As we shall discuss, much more is known about the nature of the many potential anorexigenic signals than either the neural networks they influence or how these networks interact to reduce appetite.

Organization

At the simplest level, we consider that five broad-ranging functional neural systems are concerned with generating motivated behaviors. They are responsible for:

1. the transduction of sensory signals and their central processing,
2. controlling behavioral state (arousal, circadian timing, and attention),
3. the processing of information associated with the neural representation of sensory objects,
4. motor control, and
5. hormonal modulation and feedback.

These systems are represented at the simplest level in Figure 4, without reference to anatomical locus.

The notion of drive and the idea that particular behaviors are selected to reduce the level of specific drive states have together been very influential if somewhat controversial concepts in neuroscience. From the perspective of delineating neural systems, it is useful to think of drives as being dynamic properties within different sets of neural networks, each of which is concerned with regulating a specific motivated behavior. In this way, drives are properties of behaviorally specific networks within the motor control module of Figure 4. Drive states are determined by the inputs from sensory processing, arousal state control, and object representation

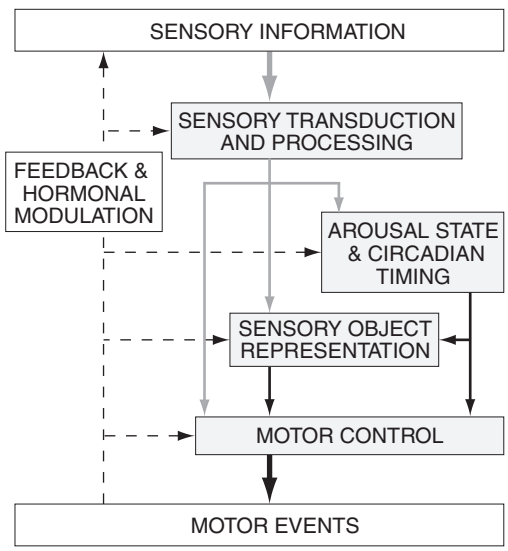

Figure 4. A schematic representation of the neural systems and their interactions involved with controlling motivated behaviors. Sensory inputs are shown in gray, central neural connections in black, and hormonal and feedback signals as dashed lines.

systems. The values of drive states within these networks are altered by these inputs in a way that increases or decreases the probability of a particular behavior being expressed at any one time.

Figure 5 expands the scheme shown in Figure 4 to illustrate specific components within the object representational and motor control networks. It shows that there are four principal inputs that can activate motor systems. The most complex motor control processes are those that generate anticipatory behaviors. In some instances, information from systems controlling arousal state—for example, circadian timing—provides the predominant signals (input 1, Figure 5). But this type of anticipatory control often derives from interactions between processed sensory information and those forebrain systems concerned with encoding object representation, particularly learning and memory, reward, and spatial orientation and navigation. The integrated output of these regions then regulates motor control systems (input 2, Figure 5). However, increased drives for motivated behaviors also can be produced by hormones or internally generated deficit signals (e.g., the thirst arising from DE, or the hunger from starvation) that access motor control networks more directly (input 3, Figure 5).

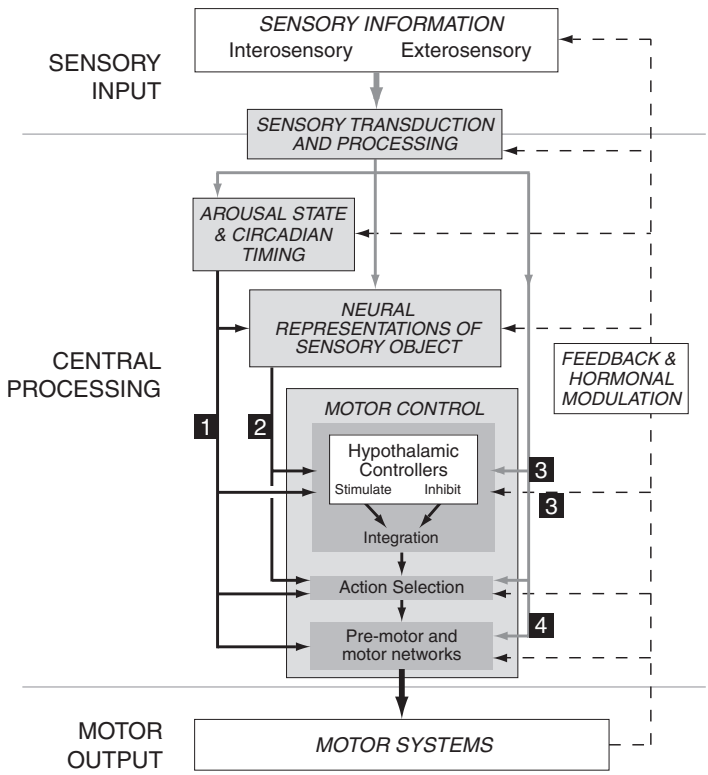

Figure 5. Motor control networks are organized at three levels: drive networks that can either stimulate, or inhibit behaviors; action selection networks that integrate the outputs of drive networks with those of other systems; and executive pre-motor and motor neuron networks. The generation of motivated behavioral actions by motor control networks can be initiated by four different sets of inputs. (1) from systems controlling arousal state and circadian timing; (2) from systems that generate representations of sensory objects; (3) directly from modulatory hormone and the sensory signals that encode physiological deficits; (4) sensory signals that generate reflex actions by interacting directly with pre-motor and motor neuron networks. Sensory inputs are shown in gray, central neural connections in black, and hormonal and feedback signals as dashed lines.

Collectively, inputs 1, 2, and 3 to the motor control networks can be thought of as being "drive-determining" interactions. Mechanisms then integrate outputs from different drive networks to select the behavioral action most appropriate for reducing the drive state with the highest value, thereby initiating the appropriate procurement phase.

Finally, simple reflex actions are generated by direct sensory inputs to the premotor and motor networks with little higher order processing (input 4, Figure 5). However, although these reflex actions lack any motivated character, they make important contributions to the consummatory phase of motivated behaviors.

The Transduction and Processing of Sensory Information. Neural systems that control motivated behaviors are regulated by a host of sensory inputs, which are defined either as interosensory signals encoding internal state, or exterosensory inputs that encode features of the goal object such as smell, taste, temperature, tactile properties, and appearance. Each of these sensory modalities has specific receptors, transduction mechanisms, and "labeled line" access to central processing networks located throughout the brain. Although important sensory processing occurs within the telencephalon, particularly sensory cortex, the initial sensory processing that occurs sub-cortically has important implications for controlling motivated behaviors; for example, altered sensitivity to the taste of sodium occurs in the hindbrain of hyponatremic animals and is an important adjunct to increased sodium appetite (Contreras, 1977; Contreras & Frank, 1979).

Some sensory signals directly access drive networks, as typified by the drinking initiated by increasing plasma osmolality or angiotensin II (A-II), or the deficit-induced feeding activated by adiposity signals (primarily leptin and insulin) that have direct hypothalamic actions (Elmquist *et al.*, 1999; Obici, Feng, Karkanias, Baskin, & Rossetti, 2002). In both these cases however, it is not clear whether the outcome of processing the deficit signal in the hypothalamus requires close interaction with the object representation networks. The fact that hypothalamic regions involved with this type of sensory transduction project directly to regions concerned with ingestive motor control (Elmquist *et al.*, 1999; Saper *et al.*, 2002; Swanson, 1987) suggests that they may not.

Circadian Timing and Arousal State Control. Parts of the brain provide critical circadian timing information and control arousal state that enable motor command networks to generate anticipatory behaviors. The circadian timing system originates in the hypothalamic suprachiasmatic nucleus (SCH), which generates the signal that entrains virtually all neural activity within limits determined by the prevailing photoperiod. Catecholamine cell groups in the hindbrain (e.g., the locus coeruleus), histaminergic neurons in the tuberomammillary nucleus, the ventrolateral preoptic nucleus, and the recently identified hypocretin/orexin neurons in the LHA supply information that is of critical importance for controlling arousal state.

Neural Representation of Sensory Objects. Those systems that generate neural representations of sensory objects are important for controlling ingestive behaviors. These include: learning and memory mechanisms in the telencephalon and cerebellum; reward/aversion systems in the midbrain ventral tegmentum, parts of the basal forebrain (particularly the nucleus accumbens), amygdala, and parts of the cortex, particularly prefrontal regions; and systems in the hippocampus and

parts of the parietal cortex responsible for allocentric and egocentric spatial representation. A great deal of exterosensory information is processed by these networks, parts of which collectively assign what has been called "incentive value" to a particular goal object. Neural pathways mediating the interactions between the object representation and motor networks are not fully understood, but sets of bidirectional connections between the hypothalamus and cortical structures such as the prefrontal cortex and hippocampus, together with subcortical regions such as the amygdala, septal nuclei, bed nuclei of the stria terminalis, and basal ganglia are all likely to be critical for the integrative operations that designate and coordinate these aspects of motivated behaviors (Petrovich *et al.*, 2002; Risold *et al.*, 1997; Saper, 1985; Swanson & Petrovich, 1998).

Motor Control. Broadly speaking, motor control systems operate at three hierarchically organized levels (Figure 5):

1. *Hypothalamic Controllers*, each of which contains sets of drive networks whose output is integrated by nuclei in a hypothalamic behavior control column that set up and coordinate the specific motor events of a particular behavior (Swanson, 2000).
2. *Action Selection* occurs in regions that receive inputs from the hypothalamic controllers and are concerned with planning, selecting, and maintaining the sequences of motor actions appropriate for that behavior.
3. *Action Execution* is accomplished by pre-motor/motor neuron networks that directly control the activity of effector systems such as striate and smooth muscle and the pituitary gland.

This hierarchical organization is consistent with the incentive/drive-reduction model outlined earlier, and requires that the selection of the motor programs appropriate for a particular aspect of ingestive behavior be biased by the integrated output from different hypothalamic controllers.

Hypothalamic Controllers. Of paramount importance to the overall expression of all motivated behaviors are the dynamic interactions between the separate hypothalamic controllers that control different behaviors. For example, the effects of negative energy balance are not limited only to increasing the drive to eat; rather they also reduce reproductive capacity (Schneider, Zhou, & Blum, 2000). Similarly, as we have discussed, DE leads to severe anorexia in addition to increasing the drive to drink (Watts, 2001). This cross-behavioral coordination (Schneider & Watts, 2002) is part of the mechanism that not only selects those motor actions with the highest behavioral priority, but also suppresses those actions that may interfere with the expression of that behavior or may further exacerbate any physiologically challenged components. This coordination most likely involves sensory and hormonal modulation acting together with the divergent neuroanatomical outputs of individual hypothalamic control networks.

Action Selection. Those parts of the brain concerned with the planning, selection, and moment-to-moment execution of particular motor actions include parts of the motor cortex, basal ganglia, midbrain, and hindbrain. Like the object representational systems, these regions at a systems level are generally behaviorally nonspecific. Although they express topography with regard to the mapping of

particular motor actions, they do not seem to be organized in the behaviorally specific manner of the drive networks. To organize the appropriate behavior, regions controlling action selection must receive the integrated outputs of the drive networks. Although they are not well understood, complex sets of projections from the hypothalamus are most likely involved with this function.

Action Execution. α-Motor neurons in the cranial nerve nuclei and the ventral horn of the spinal cord control the entire striate musculature and hence the expression of all behavior. In turn, sets of pre-motor networks directly control oscillatory and the more complex patterns of motor neuron firing. Simple rhythmic movement patterns develop from an interaction between oscillatory rhythm generators, which directly involve the motor neurons, and networks of pre-motor central pattern generators located somewhat more distally in the spinal cord and hindbrain (Rossignol, 1996). A critical feature of these pattern generators is that they are capable of producing rhythmic output without sensory input (Arshavsky, Deliagina, & Orlovsky, 1997). In turn, pattern generator output is modulated further by afferents from those parts of the appropriate command networks in the diencephalon and telencephalon. These often highly varied inputs provide the critical drive and contextual information that select the most appropriate motor program at any particular time.

Hormonal Modulation, Feedback, and Satiety. Hormones have been known for many years as critical modulators of motivated behaviors that influence a variety of neural structures at all brain levels (Figures 4 and 5). In this manner, because they are not encoding specific aspects of internal state, they are not feedback signals, but act more as permissive factors. Steroid hormones, particularly gonadal steroids, are important signals of this type. Similarly, the adrenal corticosteroids cortisol (corticosterone in the rat) and aldosterone play similar modulating roles in feeding and sodium appetite (Dallman *et al.*, 2003; Ma, McEwen, Sakai, & Schulkin, 1993).

Feedback is a critical feature of behavioral motor control, and sensory signals encoding the magnitude and consequences of generated motor actions are able to control the length of a motivated behavioral episode such as feeding. For example, post-absorptive humoral feedback (e.g., increasing levels CCK or peptide $YY_{3\text{-}36}$ in the blood) and interosensory signals (e.g., those derived from gastric distension, oropharyngeal metering) lead to the termination of ingestive behaviors and the subsequent behavioral refractoriness that is satiety, a term we can define as a decreased desire to continue the interaction with behavioral goal object, in this case food.

Satiety involves sets of specific signals that are generated by the consequences of feeding. In turn, these sensory signals interact with sets of neural networks and lead to a termination of the behavior and the accompanying feeling of fullness. For feeding, these signals include humoral factors such as CCK, ghrelin, and peptide $YY_{3\text{-}36}$ (Ariyasu *et al.*, 2001; Batterham *et al.*, 2002; Neary, Small, & Bloom, 2003), as well as the neural signals generated by gastric and intestinal distension that are conveyed to the hindbrain by the vagus nerve (Schwartz, 2000). However, they also may involve changes in the incentive value of the current goal object. In the case of ingestive behaviors, this can be confined to specific tastes, perhaps involving the processing of the reward value of sensory information in the orbitofrontal cortex (Rolls, 1999).

Together, these five sets of neural networks interact dynamically to control the full spectrum of expression of ingestive behavior patterns. In this section, we will examine what is known about how the various constituents of these five sets can be adapted and altered to generate anorexia.

The Transduction and Processing of Sensory Information in Anorexia

Both exterosensory and interosensory information play a critical role in controlling the motor programs for the appropriate feeding responses. Exterosensory inputs provide gustatory, olfactory, visual, and tactile information about the food object. Interosensory information provides essential information about the levels of energy stores, gastrointestinal status, fluid balance, etc. In turn, the central nervous system integrates and processes this information to determine whether to initiate, continue, or terminate a feeding episode.

Given the nature of anorexia, it seems quite likely that the way sensory information is processed by the brain can play a key role in its development. In this manner anorexia may develop as a direct consequence of a particular sensory signal, as is the case of cytokines (see below). Alternatively, anorexia may result from a change in the way sensory systems process the signals derived from a particular food stimulus, which then leads to a change in hedonic value. An example of this second state is the global reduction in taste responsiveness and subjective appetite reported by cancer patients or in tumor-bearing rats (DeWys & Walters, 1975; Smith, Barker, Schork, & Kluger, 1994; Trant, Serin, & Douglass, 1982), and in AN (Nozoe *et al.*, 1996) or Crohn's disease (Bannerman *et al.*, 2001). Of course, the problem here is to determine whether the changes in sensory processing proceed, and therefore might be considered causal, or whether they are the consequence of other mechanisms associated with the anorexia.

Another problem with determining causal relationships of this type occurs when we consider the anorexia that can accompany certain aversive events. Some stimuli lead to a visceral sickness that is modeled by conditioned taste aversion (CTA), where animals quickly learn to avoid a particular ingested substance because of its association with the sickness generated by a second conditioning stimulus. CTA is not the same as anorexia, although there is likely some mechanistic convergence within the neural pathways, particularly in the hindbrain, that are ultimately responsible for controlling feeding (Reilly, 1999; Watts, 2001). Furthermore, it has been suggested that learned food aversion underlies some aspects of cancer-associated cachexia in humans (Bernstein, 1999; Bernstein & Sigmundi, 1980), although this is not so clear in animal studies (Levine & Emery, 1987).

The problem of trying to dissociate anorexia from aversion and visceral illness is illustrated by considering the mechanisms of action of two agents. First, glucagon-like-peptide 1 (GLP-1), which is not only a strong anorectic agent when injected into the brain (McMahon & Wellman, 1997; Tang-Christensen *et al.*, 1996), but also will generate CTA and responses similar to visceral illness (Rinaman, 1999; Turton *et al.*, 1996; van Dijk *et al.*, 1996). Kinzig, D'Alessio, & Seeley, (2002) have recently shown that different neural mechanisms may be responsible for the anorexic and visceral sickness effects of GLP-1, with hypothalamic and hindbrain sites responsible for the anorexia, and the central nucleus of the amygdala more concerned with

the CTA effects. Second, systemic injections of lithium chloride lead to anorexia and CTA. However, lesions of the area postrema eliminate the CTA but not anorexia (Curtis, Sved, Verbalis, & Stricker, 1994). Both of these examples highlight the importance of determining whether reduced feeding is associated with visceral illness or derives from centrally generated inhibition.

A variety of interosensory inputs can alter the function of motor systems that control ingestive behaviors (Figure 5). These include the hormones that are classically associated with energy balance, metabolites, and a variety of circulating factors that are important in disease states. We will discuss two sets of interosensory signals in this category that have been intensely studied as causative agents in anorexia: physiological signals generated as a consequence of negative energy balance, and cytokines.

Energy Balance during Anorexia. Anorexia involves reduced food intake which, if maintained for a significant period, leads to a negative energy balance where expenditure exceeds intake. Evolution has provided animals with a series of adaptive processes that allow them to respond to this situation with a coordinated set of motor responses that both control ongoing energy expenditure and increase energy intake through feeding. As part of this process, a range of physiological signals is generated that are normally interpreted by the brain in a manner that increases the drive to eat. This scheme raises a number of questions that should be considered as we think about the neural mechanisms of anorexia. Does the anorexic brain see the same physiological signals as does a normal brain during negative energy balance? If so, why are they often interpreted in a manner that can be quite different from the normal brain? Which signals and responses are associated with the consequence of anorexia—negative energy balance—and which signals can be explored as potential causative agents?

When considering the potential role of metabolic signals in neural mechanisms of anorexia, it is important to differentiate between chronic anorexia conditions (e.g., AN, cancer cachexia, AIDS), where there is a significant and persistent drop in the body mass index (BMI), and shorter term pathologies that show more acute anorexia, where a reduction in BMI may not be as marked. Furthermore, some conditions—cancer-associated cachexia, for example—are often complicated by the presence of cytokines or other factors that may compromise energy metabolism (Plata-Salaman, 2000). These complicating factors can make it difficult to determine the way metabolic signals are interpreted by the brain and their significance for altering neural mechanisms. In other types of chronic anorexia, the underlying state of energy metabolism is similar to that seen during starvation and may simply reflect reduced caloric intake. In AN, for example, most studies show that metabolic signal dynamics do not deviate significantly from the classic profile seen as negative energy balance develops. Thus, serum leptin and insulin levels are reduced, while serum ghrelin and glucocorticoid levels are elevated (Casper, 1996; Gniuli, Liverani, Capristo, Greco, & Mingrone, 2001; Polito *et al.*, 2000; Tolle *et al.*, 2003). Similarly, carbohydrate metabolism in AN appears to follow the classic homeostatic response to caloric restriction and negative energy balance (Casper, 1996; Gniuli *et al.*, 2001), although there are reports of some abnormalities in glucose metabolism (Gniuli *et al.*, 2001). Many of these variables normalize upon refeeding and recovery, strongly suggesting that they are responding normally to the negative energy balance seen with AN and are not causative agents (Casper, 1996; Wallace *et al.*, 2002), although metabolic disturbances continue to be implicated by some in the etiology of this condition (Naisberg, Modai, & Weizman, 2001).

Because of the role of glucocorticoids in the etiology of clinical depression (Parker, Schatzberg, & Lyons, 2003) and their ability to alter neuronal function on a widespread scale (De Kloet, Vreugdenhil, Oitzl, & Joels, 1998), much attention has been focused on the role of glucocorticoids in anorexia. Although hypercortisolemia is consistently found in AN (Licinio, Wong, & Gold, 1996), the underlying mechanisms that elevate blood glucocorticoid are unclear. Increased cortisol half-life is evident in AN, and reduced cortisol clearance is reportedly less than half of that seen in control patients (Boyar *et al.*, 1977). It seems reasonable to assume that this altered glucocorticoid metabolism is partly related to the reduced thyroid hormone activity that accompanies AN (Douyon & Schteingart, 2002; Nedvidkova, Papezova, Haluzik, & Schreiber, 2000), as seems to be the case in food restricted rats (Woodward, Hervey, Oakey, & Whitaker, 1991). Since hypercortisolemia in AN is not always accompanied by increased ACTH secretion, and ACTH responses to CRH are blunted in patients with AN (Brambilla *et al.*, 1993; Licinio *et al.*, 1996), it is unclear how hypothalamic CRH neuroendocrine mechanisms function in this condition. Although there are reports of elevated levels of CRH in the CSF of patients with AN, there is no direct evidence that this finding is a consequence of altered hypothalamic neuroendocrine CRH neuronal activity. When interpreting CRH levels in CSF, it must be noted that there are numerous and widespread groups of CRH neurons in the brain (Swanson *et al.*, 1983), some of which increase their activity with elevated glucocorticoids (Makino, Gold, & Schulkin, 1994; Swanson & Simmons, 1989; Watts & Sanchez-Watts, 1995).

Cytokines. Inflammatory or infectious agents stimulate macrophages and monocytes to produce cytokines. Cytokines orchestrate the host's inflammation response through the production of acute-phase proteins and the mobilization of lymphocytes, while increasing blood flow to improve vascular permeability (Gabay & Kushner, 1999) and aiding in eradication of pathogens. The role of cytokines and the mechanisms they engage to reduce food intake are subjects of intense investigation because of their importance to clinical medicine and possibly eating disorders (Corcos *et al.*, 2003; also see Chapter 13 by Langhans in this volume). Elevated cytokine levels elicit neuroendocrine and metabolic responses leading to fever, somnolence, and anorexia (Gabay & Kushner, 1999). In fact, the levels of circulating cytokines are tightly correlated with anorexia and its accompanying weight loss in many circumstances (Aleman *et al.*, 2002; Ballinger *et al.*, 1998; Plata-Salaman, 1999; Scott, McMillan, Crilly, McArdle, & Milroy, 1996; Takabatake *et al.*, 1999; Verbon *et al.*, 1999). The fact that there is accumulating evidence implicating a specific neuropeptide system, the melanocortins (MC), in cytokine-associated anorexia is of considerable interest when considering underlying neural mechanisms.

How brain cytokines are regulated during peripheral inflammation or infection is not clear. Prostaglandins and cytokines themselves are produced and released by microglia and/or the endothelial cells of the circumventricular organs or vasculature of the brain, or cytokines may be transported across the blood brain barrier (Maness, Kastin, & Banks, 1998), or by neural signaling provided by either vagal or sympathetic afferents (Goldbach, Roth, & Zeisberger, 1997). Two proinflammatory cytokines, tumor-necrosis factor-α (TNF-α) and interleukin-1 (IL-1), induce both their own expression and that of other cytokines including IL-6, IL-11, ciliary neurotrophin factor (CNTF), and interferon-γ. Both TNF-α and IL-1 have been proposed as mediators in the anorexic response to disease. Thus, chronic

administration of TNF-α or IL-1 upregulates leptin gene expression and stimulates peripheral leptin release (Kirchgessner, Uysal, Wiesbrock, Marino, & Hotamisligil, 1997), and produces a dose-dependant increase in serum leptin levels in both experimental animals and in humans (Grunfeld *et al.*, 1996; Sarraf *et al.*, 1997; Zumbach *et al.*, 1997).

In this manner, one potential mechanism of how cytokines affect food intake is a pathological augmentation of the negative feedback system normally engaged by leptin, which reduces feeding, body weight, and masks the compensatory mechanisms that normally should be initiated by these changes. However, clinical conditions where an inflammatory response is present with both weight loss and increased circulating leptin are surprisingly rare. In Crohn's disease, an inflammatory bowel disease associated with elevated proinflammatory cytokines (MacDermott, 1996), patients tend to have higher circulating leptin than matched controls (Bannerman *et al.*, 2001). Patients suffering from chronic renal failure also appear to have elevated blood leptin levels, which may contribute to the anorexia that often accompanies uremia (Bergstrom, 1999). In this case, elevated blood leptin levels may be due to the chronic inflammation that is a common feature of renal failure, or may be a consequence of the fact that ordinary low-flux dialysis membranes are not able to clear circulating leptin (Merabet *et al.*, 1997; Sharma *et al.*, 1997).

On the other hand, weight-losing patients with chronic obstructive pulmonary disorder show both increased circulating levels of TNF and TNF production (de Godoy, Donahoe, Calhoun, Mancino, & Rogers, 1996; Di Francia, Barbier, Mege, & Orehek, 1994). However, serum leptin levels in these patients were not elevated over those in healthy controls (Takabatake *et al.*, 1999). Further, circulating leptin concentrations were not elevated in weight-losing patients with gastrointestinal or lung cancer (Simons, Schols, Campfield, Wouters, & Saris, 1997; Wallace, Sattar, & McMillan, 1998). In fact, in patients with advanced stage non-small cell lung cancer, leptin levels were inversely related to the intensity of the inflammatory response (Aleman *et al.*, 2002). Severely wasted patients with AIDS or chronic inflammatory bowel disease also show increased levels of proinflammatory factors but no elevation of leptin (Ballinger *et al.*, 1998). Even severe inflammation resulting from sepsis did not elevate leptin levels in humans (Carlson, Saeed, Little, & Irving, 1999). Thus, even intense acute inflammatory responses that produce high levels of cytokines do not appear to result in high serum leptin levels. Therefore, it is unlikely that anorexia in these circumstances is due to a dysregulation in leptin production or release.

With regard to potential mechanisms, it is of considerable interest that the anorexic effects of cytokines and leptin may engage the same catabolic effector pathways in the hypothalamus. The leptin receptor is a member of the class 1 cytokine receptor family known to signal through the Janus kinase/signal transducer and activator of transcription (STAT) pathway (Tartaglia *et al.*, 1995). The STATs, once activated, dimerize and translocate to the nucleus where they affect gene expression (Darnell, 1997). A peripheral injection of either leptin or TNF-α stimulates STAT3 tyrosine phosphorylation in the hypothalamus, and injection of both leads to a synergistic increase in phosphorylation that is 24-fold greater than if either is injected alone (Rizk, Stammsen, Preibisch, & Eckel, 2001), suggesting the strong possibility of common signaling pathways. In fact, the glycoprotein 130 signal-transducing subunit of the IL-6 type receptors is a homolog of the leptin receptor (Baumann *et al.*, 1996). Evidence supports this hypothesis in experimental animals. For example, intracerebroventricular injection of IL-1 induces anorexia

and may elicit some of the same central effects on feeding pathways as that seen after leptin injection (Gayle, Ilyin, & Plata-Salaman, 1997). CNTF also potently elicits decreased food intakes and body weight when injected either systemically or into the brain, and can engage at least some of the same neural signaling pathways that are activated by leptin (Henderson *et al.*, 1994; Pu, Dhillon, Moldawer, Kalra, & Kalra, 2000; Xu *et al.*, 1998). Whether or not this occurs in human inflammatory disease states is not known, although one post-mortem study examining the brains of humans after illness associated anorexia did not show abnormal responses to the circulating levels of leptin within putative hypothalamic feeding pathways (Goldstone, Unmehopa, Bloom, & Swaab, 2002).

Circadian Timing, Arousal State, and Anorexia

Virtually nothing is known about whether disturbance of the circadian timing system is a contributory factor to any type of anorexia. Disruptions of some variables that exhibit daily rhythms have been reported, although it is not clear whether these are a consequence of timekeeping problems or are secondary effects related to other aspects of the disease. For example, melatonin secretion, which is driven by a photoperiod-dependent signal from the SCH, is reportedly disrupted in patients with AN in some (Brambilla *et al.*, 1988; Pacchierotti, Iapichino, Bossini, Pieraccini, & Castrogiovanni, 2001) but not all cases (Mortola, Laughlin, & Yen, 1993). The fact that gonadal steroids directly influence melatonin synthesis and secretion means that it is difficult to determine whether circulating melatonin in patients with AN reflect altered signals from the circadian timing system or are a consequence of reduced reproductive function (Pacchierotti *et al.*, 2001). The presence of altered circadian timekeeping is not consistent with an electroencephalographic analysis of sleep patterns in patients with AN (Nobili *et al.*, 1999). These workers found that the timing of sleep onset and termination in AN were the same as in controls. However, sleep in AN was more fragmentary and exhibited an altered distribution of sleep stages throughout the night.

Although little is known about how networks responsible for controlling arousal state are involved with anorexia, it seems likely that they may contribute to those conditions where increased physical activity is reported as a potential triggering mechanism (Beumont, Arthur, Russell, & Touyz, 1994; Hulley & Hill, 2001; Smith, 1980). The finding that patients with AN exhibit hyperactivity was one of the symptoms mentioned in the first report of the disease (Gull, 1874), and has been consistently noted as an "early and enduring feature of AN" (Kron, Katz, Gorzynski, & Weiner, 1978). As a consequence of this observation, one animal model that has received significant attention is ABA. This phenomenon occurs in rodents that are allowed to feed only during a restricted period—usually around 90 min—and have access to a running wheel (Routtenberg & Kuznesof, 1967). In these circumstances, animals develop anorexia and progressive weight loss, and can die if the regime is maintained. Interestingly, ABA develops only when animals run in a wheel and not when they run on a circular track (Koh, Lett, & Grant, 2000). Sherwin (1998) has noted in a comprehensive review that rodents are strongly motivated to wheel-run, which may be a more rewarding endeavor than the more general activity involved with track running (Lett, Grant, Byrne, & Koh, 2000). One explanation for ABA proposes that conflicts between competing drives—including circadian timing (Beneke, Schulte, & vander Tuig, 1995; Dwyer & Boakes, 1997; Morse *et al.*, 1995)—occur when

adaptation to new schedules is required (Dwyer & Boakes, 1997). However, this explanation is not supported by other studies (Lett, Grant, Smith, & Koh, 2001) leaving the mechanisms responsible for ABA unclear. Although ABA has been suggested as a good model for investigating AN (e.g., Epling, Pierce, & Stefan, 1983; Lambert, 1993; Rieg, 1996; Smith, 1989), others disagree (Beneke *et al.*, 1995). Indeed, rodent ABA would seem better suited for investigating the specific relationship between hyperactivity and caloric intake rather than the human complexities of AN (Koh *et al.*, 2000).

Anorexia and the Neural Representation of Sensory Objects

The importance of the complex neural processes underlying memory, reward, and egocentric perception for controlling ingestive behaviors has long been appreciated. However, it is only quite recently that a rigorous neural systems approach has been applied to the process of integrating hypothalamic and hindbrain networks responsible for feeding with those telencephalic regions involved with the neural representation of sensory objects and cognition (e.g., Saper *et al.*, 2002; Sawchenko, 1998; Watts, 2000, 2001). For example, recent work has shown that interactions among the nucleus accumbens, amygdala, and hypothalamus have been complex and very important influences on the context in which ingestive behaviors are expressed (Baldo, Sadeghian, Basso, & Kelley, 2002; Kelley *et al.*, 2002; Petrovich *et al.*, 2002; Stratford & Kelley, 1999). The fact that we are only just beginning to unravel both the neuroanatomical complexity of these neural networks as well as the mechanisms they use to influence ingestive behaviors is perhaps not surprising that virtually nothing is known about how systems in the cortex, amygdala, and ventral striatum are involved with the development of anorexia (Watts, 2001). However, given the undoubted influence these cognitive systems have on ingestive behaviors in humans, exploring this whole area would seem to be one that will provide a wealth of information about the neural mechanisms of anorexia.

Anorexia and Motor Control

The neuroanatomical revolution of the past 30 years has replaced the idea of isolated motor control "centers" with a scheme in which sets of more widely distributed but highly interconnected networks direct the motor responses that make up ingestive behaviors. However, in accord with Stellar's original idea (Stellar, 1954), these individual control networks are thought to stimulate, inhibit, or disinhibit the motor events associated with behavior (Swanson, 2000; Watts, 2001; Watts & Swanson, 2002). Conceptually, it is easy to see how dysfunctions within this type of motor control network could lead to anorexia. Either an abnormally extended upregulation of inhibitory mechanisms or a pathological downregulation of stimulatory mechanisms would each lead to a reduction in food intake. There are many data in the literature supporting the idea that both mechanisms are involved with mediating different types of anorexia.

The core hypothalamic structures that are critical for controlling energy balance have been identified and extensively studied during the past 25 years. Based on lesion studies and, more recently, leptin responsiveness, together with neuropeptide physiology, pharmacology, and genetic manipulation, the field currently recognizes a group of nuclei in the hypothalamus, including the LHA, PVH, ARH, dorsomedial (DMH), and possibly the ventromedial (VMH) nuclei, as containing

neurons that are important for controlling energy balance (Watts, 2001). In this section, we will review the data implicating various components of the hypothalamic motor control column (Swanson, 2000) in generating anorexia.

The Arcuate Nucleus—Neuropeptide Y and the Melanocortins. The ARH is a narrow and elongated periventricular cell group located immediately dorsolateral to the median eminence at the base of the third ventricle. Because of its position it has long been implicated in neuroendocrine control, and some of its neurons release chemical signals into the hypophysial portal vasculature to control anterior pituitary function. These include β-endorphin released from pro-opiomelanocortin (POMC) neurons that are implicated in controlling gonadotrophin secretion (Herbison, 1998; Kalra *et al.*, 1997), and some tuberoinfundibular dopaminergic neurons that regulate prolactin secretion. With this in mind, it is interesting to note that some forms of anorexia are accompanied by anoestrus or amenorrhea that may involve altered ARH function (Schneider & Watts, 2002).

ARH neurons synthesize at least five peptides that are implicated in the control of feeding. Two of them—NPY and agouti related protein (AgRP)—are orexigenic and co-localized in one population of leptin-sensitive ARH neurons, while three others—α-melanocyte-stimulating hormone (α-MSH, a peptide synthesized from the POMC gene), cocaine- and amphetamine-regulated transcript (CART), and neurotensin—are anorexigenic. CART and α-MSH are also co-localized in another population of leptin-sensitive ARH neurons. The chemical phenotype of neurons that contain neurotensin is unclear, although some growth hormone-releasing hormone neurons both within and lateral to the ARH co-express neurotensin (Sawchenko, Swanson, Rivier, & Vale, 1985). It is also worth noting that virtually all ARH neurons are GABAergic (Bowers, Cullinan, & Herman 1998, Ovesjo, Gamstedt, Collin, & Meister *et al.*, 2001) and that any changes in peptide release from ARH neurons may well be accompanied by changes in the release of this inhibitory transmitter.

NPY is a powerful orexigenic peptide and many studies have implicated NPY projections from the ARH to the PVH and LHA as being pivotal for increasing eating in response to food deprivation (Bivens, Thomas, & Stanley, 1998; Elmquist *et al.*, 1999; Kalra *et al.*, 1991; Stanley, Magdalin, Seirafi, Thomas, & Leibowitz, 1993). Since NPY mRNA in the ARH is downregulated by increased leptin (Ahima *et al.*, 1999; Woods, Seeley, Porte, & Schwartz, 1998), one mechanism by which appetite is augmented by starvation is to increase NPY synthesis and release as a result of falling plasma leptin concentrations (Ahima *et al.*, 1996, 1999; Woods *et al.*, 1998). Conversely, it is conceivable that pathological downregulation of the ARH NPY system may inappropriately reduce feeding. The role of NPY in anorexia has been extensively discussed (Inui, 1999), but considering the different circumstances in which anorexia is evoked, it is clearly not useful to consider it a single condition that is initiated by a single mechanism. Despite body wasting being the common outcome, the mechanism underlying the anorexia of AIDS is likely quite different from that underlying AN.

In an effort to understand these different mechanisms one way that might be helpful is to classify anorexias—at least as a first approximation—to differentiate them into two groups based on what we know about their hypothalamic NPY profiles and how they respond to exogenous NPY administration. This then makes NPY a powerful experimental tool to probe the substrates of anorexia because neurons that express this peptide constitute one of the best-characterized feeding systems.

Anorexias that Remain Sensitive to NPY. In some anorexias, increased circulating leptin follows the administration of some cytokines, including IL-1, TNF, and leukemia inhibitory factor (Sarraf *et al.*, 1997). But with other cytokines such as CNTF, anorexia develops with reduced leptin levels (Lambert *et al.*, 2001, Pu *et al.*, 2000). However, when present in experimental animals, all these anorexias have reduced hypothalamic NPY gene expression and release, and they all respond to exogenous NPY administration with increased food intake (Inui, 1999; Pu *et al.*, 2000; Turrin, Flynn, & Plata-Salaman, 1999). This profile suggests that in these cases anorexia involves a pathological augmentation of a negative feedback system that ordinarily decreases NPY activity. This change in feedback sensitivity could result from increased leptin levels, or from mechanisms that interfere with leptin signaling in NPY neurons (Inui, 1999; Lambert *et al.*, 2001; Xu *et al.*, 1998).

Anorexias with Reduced Sensitivity to NPY. The neural circuits and mechanisms implicated in a second group of anorexias include those that accompany certain cancers, acute colitis, AIDS, and those of psychological (e.g., stress) and possibly psychiatric origin. Some experimental animals with tumors show significantly decreased circulating leptin and a concomitant increase in NPY gene expression in the ARH (Chance, Sheriff, Moore, Peng, & Balasubramaniam, 1998; Wisse, Frayo, Schwartz, & Cummings, 2001). An identical profile has been reported with the anorexia that accompanies experimental colitis (Ballinger, Williams, Corder, El-Haj, & Farthing, 2001), in DE-anorexia (O'Shea & Gundlach, 1995, Watts, Sanchez-Watts, & Kelly, 1999), and in the anorexia following psychological stress (Makino, Asaba, Nishiyama, & Hashimoto, 1999; Baker, Smith, & Gold, 2000). Critically, in contrast to the first group, all these anorexias show significantly reduced feeding responses to exogenous NPY administration (Ballinger *et al.*, 2001; Chance *et al.*, 1996; Inui,1999). Interestingly, AIDS and AN patients have reduced circulating leptin (Ballinger *et al.*, 1998; Kaye, Klump, Frank, & Strober, 2000; Polito *et al.*, 2000). This means that if the NPY gene in the human ARH responds to leptin as it does in experimental animals, then low circulating levels of leptin should increase NPY gene expression in this critical cell group. Although patients with AIDS or AN both have elevated NPY levels in their CSF (Kaye *et al.*, 2000; Malessa *et al.*, 1996), examining NPY levels in the human ARH obviously requires postmortem analysis, and has yet to be examined directly in these illnesses. Of great interest, however, are reports that increases in NPY expression are found in the ARH region of patients who died following extended premorbid illnesses that are accompanied by body wasting (Corder, Pralong, Muller, & Gaillard, 1990; Goldstone *et al.*, 2002).

Considered together, the profile of this group of anorexias is consistent with the idea that reduced appetite is not the consequence of reduced NPY "drive" from the ARH. On the contrary, it would seem that there is elevated activity in these circuits, and that a significant component of these types of anorexia is an inhibition of those leptin-sensitive NPY mechanisms that usually stimulate eating. Although the nature of these mechanisms is currently unknown, the fact that in some instances these anorexias are ameliorated by manipulating MC or CRH receptors (Malessa *et al.*, 1996; Smagin *et al.*, 1999; Wisse *et al.*, 2001) suggests that circuits containing these peptides contribute to an inhibition of the ARH NPY system. However, it remains unknown whether the inhibitory circuits are the same for all anorexias, where they are located in the brain, and how they interact at a systems level with the neurons that stimulate feeding.

Melanocortins. The MCs are a peptide family derived from the precursor peptide POMC, and includes adrenocorticotropic hormone and α-MSH. α-MSH is synthesized in POMC neurons located in the ARH adjacent to NPY neurons (Baskin, Hahn, & Schwartz, 1999) and in neurons in the hindbrain (Bronstein, Shafer, Watson, & Akil, 1992). Unlike NPY, which increases feeding when injected into the hypothalamus, α-MSH or MC3/4 receptor agonist administration leads to anorexia and weight loss (Fan, Boston, Kesterson, Hruby, & Cone, 1997; Giraudo, Billington, & Levine, 1998; McMinn, Wilkinson, Havel, Woods, & Schwartz, 2000). This effect appears to be the result of reduced meal size (Williams, Grill, Weiss, Baird, & Kaplan, 2002), and involves amplified post-ingestive feedback inhibitory mechanisms in the hindbrain (Williams et al., 2002). Food deprivation and subsequent weight loss reduce POMC mRNA in the ARH (Schwartz et al., 1997) while leptin administration stimulates POMC gene expression (Thornton, Cheung, Clifton, & Steiner, 1997). α-MSH is an agonist of the MC3/4 receptors, which are found quite widely in the brain and spinal cord, with high levels in the PVH, the perifornical (pf) area of the LHA, and the dorsal vagal complex (Cowley *et al.*, 1999; Kishi *et al.*, 2003; Mountjoy, Mortrud, Low, Simerly, & Cone, 1994). Interestingly, AgRP is an endogenous antagonist for MC3/4 receptors that will stimulate food intake over long periods (Kim *et al.*, 2000; Rossi *et al.*, 1998).

There is a growing and convincing body of evidence implicating the MC signaling system in some types of clinically important anorexias. Although there is one report of increased polymorphisms in the AgRP gene in patients with AN (Vink *et al.*, 2001), the majority of the evidence implicates the MC system in cytokine-associated anorexias. Peripheral administration of lipopolysaccharide (LPS), a component of the outer membrane of gram-negative bacteria, is often used experimentally to mimic inflammation and induce anorexia in rats (O'Reilly, Vander, & Kluger, 1988). Central administration of α-MSH into LPS-injected rats enhanced the LPS-induced suppression of food intake (Huang, Hruby, & Tatro, 1999), while central administration of SHU 9119 (a MC3/4 receptor antagonist) reversed this effect. Of significant clinical interest is the observation that SHU 9119 will reduce cytokine and tumor-associated anorexias in rats (Huang *et al.*, 1999; Lawrence & Rothwell, 2001; Wisse *et al.*, 2001). Furthermore, blocking the MC system by administering the endogenous antagonist, AgRP, or deleting the MC-4 receptor gene ameliorates anorexia in response to both LPS and tumor growth in mice (Marks *et al.*, 2001). If POMC mRNA levels are decreased in the ARH by food restriction, the anorexia produced by acute inflammation is significantly reduced when compared to controls (Lennie, Wortman, & Seeley, 2001). The reduced anorexia is interpreted as being a consequence of reduced activity in the MC signaling system rather than of elevated circulating cytokines, which are not altered in this model.

Two final points should be noted when interpreting these data. First, although they support a role for ARH peptidergic systems in cytokine-associated anorexia, Reyes and Sawchenko (2002) suggest that the way this cell group functions in these circumstances is quite complex. Based on the fact that ARH lesions exacerbate IL-1 anorexia, they argue that the net output of this cell group actually restrains cytokine-associated anorexia. They make the important point that it is not possible to predict the net effects of ARH on food intake as the simple outcome of the opposing actions of POMC and NPY neurons (Reyes & Sawchenko, 2002). Second, the ARH is not the only site of POMC biosynthesis. Results from Grill and associates (Grill, Ginsberg, Seeley, & Kaplan, 1998; Grill & Kaplan, 2002; Williams, Kalpan, & Grill, 2000; Williams *et al.*, 2002) show that pharmacological manipulation of

hindbrain MC receptors has a profound impact on feeding. These findings highlight the need to consider a broad neural network approach for understanding anorexia (Grill & Kaplan, 2001; Watts, 2000).

The Paraventricular Nucleus and Anorexia. The PVH is a critical hypothalamic cell group that regulates many of the motor functions associated with energy balance. These functions are controlled by two sets of PVH neurons: neuroendocrine neurons that project to the neurohypophysis and regulate endocrine function, and neurons that have descending projections that control a range of behavioral and autonomic motor actions.

Neuroendocrine responses are mediated by CRH motor neurons that ultimately control glucocorticoid secretion (Watts, 1996), along with groups of thyrotropin-releasing hormone (TRH) and somatostatin neuroendocrine neurons that are pivotally placed to regulate endocrine metabolism (Swanson, 1987). These neuroendocrine neurons affect anterior pituitary function by way of projections to the median eminence. PVH neurons also influence a wide range of autonomic processes associated with energy metabolism including gastrointestinal activity and sympathetic activation. Finally, the PVH is heavily implicated in controlling feeding behavior, most notably because of the strong feeding responses elicited by targeted injections of either NPY or norepinephrine (Leibowitz, 1978; Stanley & Leibowitz, 1985). These autonomic and behavioral motor events are mediated by PVH neurons that project caudally to the periaqueductal gray, parabrachial nucleus, dorsal vagal complex, and to pre-ganglionic neurons in the dorsal, and ventrolateral medulla and intermediolateral column of the spinal cord. The peptides expressed by these descending PVH neurons include CRH, oxytocin, dynorphin, and vasopressin (Hallbeck, Larhammar, & Blomqvist, 2001; Sawchenko & Swanson, 1982).

To regulate these diverse motor functions, PVH neurons receive an array of afferents, including ascending, predominantly monoaminergic inputs that relay vagally mediated information from the viscera critical for coordinating feeding responses with peripheral requirements, and leptin- and insulin-related viscerosensory information from the ARH and DMH nuclei (Baskin *et al.*, 1999; Elmquist, Ahima, Elias, Flier, & Saper, 1998; Sawchenko & Swanson, 1983). In addition, CRH and orexin-containing neurons in the LHA provide afferents into the PVH that can encode information relating to energy balance and arousal state (Backberg, Herrieu, Wilson, & Meister, 2002; Champagne, Beaulieu, & Drolet, 1998; Marcus *et al.*, 2001; Peyron *et al.*, 1998; Watts *et al.*, 1999). Finally, telencephalic influences from the cortex and amygdala are channeled to the PVH mostly by way of the bed nucleus of the stria terminalis (BST; Dong, Petrovich, Watts, & Swanson, 2001; Herman & Cullinan, 1997; Swanson, 2000).

Commensurate with this central position in the motor networks controlling ingestive behaviors, some studies have implicated the PVH as a key component for generating anorexia. Although it is not clear how this outcome is achieved, one way would be for PVH neurons to integrate information from a range of sources and then produce signals to activate an appropriate and coordinate set of motor response (Cowley *et al.*, 1999).In this respect, inputs from the ARH are well placed to encode information relating to circulating hormones such as leptin and insulin (Elmquist *et al.*, 1999), catecholaminergic afferents are important for relaying information relating to glucose metabolism (Ritter *et al.*, 2001, 2003), while LHA and BST afferents are well placed to bring information concerning more complex aspects of feeding such as current incentive value (Watts & Swanson, 2002). One way in which

these different systems might function to generate anorexia is illustrated by the behavior of neuropeptidergic circuits involving the PVH during DE-anorexia.

Neuropeptide gene expression patterns in DE animals show that ARH neurons respond to negative energy balance in a manner indistinguishable from food-restricted animals; NPY mRNA levels increase while POMC mRNA levels are reduced (Watts *et al.*, 1999). This pattern of gene expression is typical of that seen during negative energy balance, and is thought to be a contributor to the robust compensatory feeding seen after starvation. However, compensatory feeding does not occur during many types of anorexia, presumably because of the presence of inhibitory signals from other sources that are present in anorexic animals and are able to mask the increased activity from the ARH. We have suggested that increased release of CRH from neurons in the LHA and part of the BST may serve this function (Watts, 2001). It is interesting to note in this regard that CRH can inhibit NPY-induced feeding (Heinrichs, Menzaghi, Pich, Hauger, & Koob, 1993; Menzaghi, Heinrichs, Pich, Tilders, & Koob, 1993).

Although we do not yet know which neurons in the PVH might integrate this information to inhibit feeding, those oxytocin-containing neurons that project to the hindbrain are well positioned for this purpose. Oxytocin is an anorexic neuropeptide when injected icv, an effect that is blocked by pre-administration of its antagonist (Arletti, Benelli, & Bertolini, 1989; Olson *et al.*, 1991a). The downstream anorectic actions of oxytocin may involve GLP-1 neurons in the hindbrain (Rinaman & Rothe, 2002).

Central oxytocinergic systems are strongly implicated in both CRH-mediated and DE-anorexias. Thus, the oxytocin antagonist [d(CH2)5,Tyr(Me)2, Orn8] vasotocin abolished the icv CRH suppression of food intake (Olson, Drutarosky, Stricker, & Verbalis, 1991c), and acute DE (Olson, Drutarosky, Stricker, & Verbalis, 1991b). Critically, increased oxytocin gene expression occurs after DE in the non-neuroendocrine PVH oxytocin neurons that project to the medulla and spinal cord (Pretel & Piekut, 1989), and these neurons show increased Fos expression after DE (Salter & Watts, 2002). Collectively, these data raise the possibility that CRH inhibits food intake by stimulating the activity of the non-neuroendocrine oxytocin neurons in those parts of the PVH that provide descending projections (Olson *et al.*, 1991c; Pretel & Piekut, 1989; Sawchenko & Swanson, 1982).

Interestingly, Stricker and Verbalis (1987) have proposed that dynamic patterns of peripheral oxytocin secretion reflect the activity of central oxytocinergic neurons. Thus, after various treatments (CCK, DE, lithium chloride, or icv CRH administration) in which central oxytocin has been implicated in reducing food intake and in modifying gastric and intestinal motility, parallel changes in plasma oxytocin concentrations occur that correlate very strongly with the intensity of the motor act (Bruhn, Sutton, Plotsky, & Vale, 1986; McCann, Verbalis, & Stricker, 1989; Olson *et al.*, 1991b; Plotsky *et al.*, 1985; Verbalis, McHale, Gardiner, & Stricker, 1986). Central but not peripheral administration of oxytocin mimics many of these effects showing that the peptide must be acting in the brain (Stricker & Verbalis, 1987). Finally, the fact that NPY-induced eating is augmented by pretreatment of rats with ethanol (Blackburn, Stricker, & Verbalis, 1994), which reduces oxytocin secretion, is consistent with the idea that oxytocin acts to restrain feeding in a number of different circumstances.

The Perifornical Part of the LHA. The LHA has long been associated with the central control of motivated behaviors. It is a large and heterogeneous

collection of neurons that has diverse and complex connections throughout the brain (see Sawchenko, 1998; Watts, 2000; Watts & Swanson, 2002 for reviews). Interest in the LHA as a substrate of ingestive behaviors has been invigorated recently by the identification of two peptides that apparently stimulate feeding and are synthesized only in LHA neurons: melanin-concentrating hormone (MCH) and hypocretin/orexin (orexin), both of which have extensive projections throughout the brain (Bittencourt *et al.*, 1992; Peyron *et al.*, 1998; Shimada, Tritos, Lowell, Flier, & Maratos-Flier, 1998; Willie, Chemelli, Sinton, & Yanagisawa, 2001).

The LHA as a whole has proved very difficult to parcellate. However, one region—its pf part—has consistently been shown by lesion and microinjection studies to be intimately involved in controlling ingestive behaviors. The LHApf is the most sensitive site in the brain for the orexigenic actions of NPY (Stanley *et al.*, 1993), and it receives NPY and AgRP projections from the ARH (Elmquist *et al.*, 1999). Furthermore, glutamate mechanisms in the LHApf regulate feeding but not other behaviors (Khan *et al.*, 1999; Stanley, Willett, Donias, Dee, & Duva, 1996). Excitotoxic lesions in those parts of the LHA most closely related to feeding cause mild rather than catastrophic hypophagia, and do not impede compensatory responses following food deprivation (Winn, 1995). However, they markedly attenuate compensatory feeding responses to 2-deoxyglucose treatment (Winn, 1995). The LHApf receives a strong projection from the fusiform nucleus of the BST (Dong *et al.*, 2001; Kelly & Watts, 1996), and from the subfornical organ and the median preoptic nucleus, both of which are critical for regulating drinking behavior and for the increases in CRH gene expression seen in the LHApf following DE (Kelly & Watts, 1996; Swanson & Lind, 1986). The LHApf has strong projections to the periaqueductal gray, parabrachial nucleus, dorsal medulla (Kelly & Watts, 1998; Moga *et al.*, 1990; Swanson, 1987), and to the PVH (Larsen, Hay-Schmidt, & Mikkelsen, 1994; Swanson, 1987; Watts *et al.*, 1999). Collectively, these data emphasize that the LHApf may act to coordinate signals derived from internal state variables (e.g., those originating in the ARH) with those mechanisms originating in the telencephalon that are responsible for motivated anticipatory action (Sawchenko, 1998; Watts & Sanchez-Watts, 2000; Winn, 1995).

The LHApf and DE-Anorexia. A potential role of the LHA in mediating anorexia has been most extensively studied in DE-anorexia. Before anorexia develops to any degree in DE animals, there are increased levels of CRH and neurotensin in a population of LHApf neurons (Watts, 1992, 1999) that project to the PVH and the parabrachial nucleus (Champagne *et al.*, 1998; Kelly & Watts, 1998; Watts *et al.*, 1999). These neurons are distinct from those expressing MCH and orexin (Sanchez-Watts, Kay-Nishiyama, & Watts, 2000), and they express Fos-ir throughout DE and after animals have drunk water (Watts & Sanchez-Watts, 2000). Interestingly, the orexin but not MCH neurons in this same region express Fos-ir only after DE animals have drunk water (Watts & Sanchez-Watts, 2000), emphasizing that there are functional differences between these three populations of neurons.

From these data we have proposed that the LHApf contains at least two distinct populations of neurons that have different functions during the development and reversal of DE-anorexia. First, CRH/NT neurons are activated by DE and contribute to the anorexia by way of their projections to the PVH and possibly parabrachial nucleus. Second, although MCH and orexin neurons can both stimulate feeding, only orexin neurons are activated during the reversal of anorexia (Watts & Sanchez-Watts, 2000). We hypothesize that these orexigenic actions may

be inhibited as a result of CRH projections from the fusiform nucleus of the BST, but possibly also the LHApf.

Feedback, Satiety, and Anorexia

A broad range of neural and humoral feedback signals provide a wealth of information to the brain about the status of ongoing feeding activities. These signals contribute toward satiety and interact with mechanisms that inhibit feeding. They are used both in the short term to control meal size and duration and in the longer term, where they can influence the size of energy stores, adiposity levels, and body weight. With this in mind, it is easy to conceive how anorexia can occur as a consequence of the increased activity of these satiety processes. Although the anorexia associated with aging appears to be altered sensitivity to satiety signals, in this instance CCK (MacIntosh *et al.*, 2001), it seems that most other anorexias are not simply the result of increased active of satiety signals or increased sensitivity of their receptor systems. For example, ghrelin and CCK levels are abnormally low in patients with AN (Baranowska *et al.*, 2000; Tolle *et al.*, 2003), and these normalize upon recovery. The role of feedback and satiety mechanisms and how they interact with other mechanisms that can inhibit feeding, particularly in the hindbrain, would seem to be a fruitful target for future work.

Conclusion

Studying the neural mechanisms that underlie anorexia presents an important opportunity for approaching two significant problems. First, this work should delineate how ingestive behaviors are controlled at a neural systems level. Because there is clear evidence for distinct circuits that are responsible for inhibiting and stimulating feeding behavior, experimental anorexias like those associated with DE, locomotor activity, or cytokines can be used as tools to probe the organization of the neural networks that control feeding. Second, as we have seen, anorexia is a significant complication to many clinical conditions. Although determining the etiologies of clinically important anorexias using animal models presents a significant challenge, we are making significant progress toward understanding how feeding is compromised in some instances, for example, cancer cachexia, (Lechan & Tatro, 2001). But understanding the mechanisms responsible for AN is proving to be particularly challenging. Perhaps this stems from the fact that AN is a wholly human disease, perhaps even a disease of our time. In this way it is, like schizophrenia, difficult and perhaps even impossible to approach in its entirety using experimental animal models. This is particularly apparent if we consider that most studies attempt to use the rat or mouse to model AN. These animals, when compared to humans, have a less complex cortical organization and quite different social structures making comparisons difficult. But despite these problems, the significant clinical and social cost of anorexia should provide us with adequate motivation to increase our understanding of the neural bases of anorexia.

Acknowledgments

We would like to acknowledge the generous support of project grants from NINDS (NS 29728) and NIMH (MH 66168) for work performed in the authors' laboratory.

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Comparative Studies

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15

Studies of Food Intake: Lessons from Nontraditionally Studied Species

Timothy J. Bartness and Gregory E. Demas

Introduction

A reasonable question for a reader to ask is "Why should I care about the controls of food intake in animals other than rats, mice, or humans and how can researchers justify studying species other than these three?" Indeed, one of the authors (TJB) posed this question to his first postdoctoral advisor when asked to help solve the riddle of a lack of increased food intake after a fast by Syrian golden hamsters (*Mesocricetus auratus*)—a response primarily shared with other hamster species (see below). To continue in this vein, it would seem that further study of laboratory rats and mice is most warranted based on the shear volume of accumulated knowledge on the controls of food intake for these species. With the advent of gene knockouts and knock-ins that have largely been accomplished in mice, a strong case could be made for further narrowing of our research species to laboratory mice as a means of understanding human food intake.

One answer to the initially posed question is that, despite the considerable knowledge already achieved from food intake studies of laboratory rats and mice, and from humans, many or most of the fundamental problems in ingestive behavior

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Neurobiology of Food and Fluid Intake, 2nd Ed., Volume 14 of *Handbook of Behavioral Neurobiology*, edited by Edward Stricker and Stephen Woods, Kluwer Academic / Plenum Publishers, New York, 2004.

have not been satisfactorily solved. Perhaps new approaches using more ecologically relevant animal species might yield insights into solving the puzzles of ingestive behavior—and this is precisely the underlying premise of this review. Therefore, we hope that the reader may find some useful lessons learned via the study of "experiments of nature" (i.e., species-specific food intake behaviors that have been shaped by evolution, that occur in the real world and many of which can be conveniently studied in the laboratory). For example, a negative consequence of sustained exposure to a calorically dense high fat diet (HFD) is overeating (Ramirez & Friedman, 1990), consequent obesity, and its associated health problems (Satcher, 2001). Some animals have evolved to be naturally resistant to the almost reflexive overeating triggered by HFD exposure for many species including humans (Blundell, Lawton, Cotton, & Macdiarmid, 1996). Perhaps by studying the physiological mechanisms underlying food intake in these less-studied species that have been shaped by evolutionary forces, we might gain greater insight into the functional and adaptive significance of resistance to dietary obesity. Similarly, by studying animals that go through self-imposed fasts for weeks or months at a time, even in the presence of food (see below), we might gain insight into mechanisms underlying satiety.

With that focus in mind, note that this chapter will not be a 'Noah's Ark' of food intake across the wide spectrum of animal species, or even covering any one taxonomic order such as *Rodentia*—there are ~1,700 species of rodents (Vaughan, 1978). Thus, given space constraints, any comparative study of food intake in rodents must focus on only a relatively small number of species and largely ignore the others. We have attempted, however, to highlight some selected examples of the feeding responses of nontraditionally studied species that may allow us to identify and isolate specific physiological states, ingestive responses, and/or environmental conditions controlling food intake that are so often inextricably intertwined in laboratory rat and mouse eating behavior. In some cases, experimenters explicitly chose animals as potential models of human food intake/physiology (e.g., HFD feeding by Syrian hamsters), whereas other species were chosen based on the commercial value of the species (e.g., chickens, pigs, and sheep). Still others were studied as part of behavioral ecological experiments, the focus of which often is to better understand the behavior and physiology of the particular species (e.g., penguins). Nevertheless, in retrospect, it appears that at least some of these species-specific responses lend themselves well as models to study long-standing problems in the field of ingestive behavior.

We divided the chapter into sections that focus on contemporary issues in the study of food intake, including: (1) responses to fasting and satiety as viewed from self-imposed reductions in food intake, (2) metabolic control of food intake, (3) peptidergic control of food intake, (4) responses to HFDs, and (5) appetitive ingestive behaviors (foraging/hoarding).

Responses to Fasting, Voluntary Fasting/Reductions in Food intake and Satiety

Shortfalls in food are common in nature and strategies to cope with energy deficits are diverse across animal species (e.g., mobilization of triglyceride or glycogen, decreases in energy expenditure, use of external energy stores; for review

see Le Maho, 1989). In addition, although one might envision that the subsequent feeding response to a fast is a highly conserved behavior across most, if not all species, this is not the case. For example, hamster species differ in that they do not overeat after a fast as mentioned above (Bartness, 1997; Bartness & Clein, 1994; Borer, Rowland, Mirow, Borer, & Kelch, 1979; Day, Mintz, & Bartness, 1999; Rowland, 1982; Silverman & Zucker, 1976; Simek & Petrasek, 1974; Wong & Jones, 1985). Because absence of a post-fast hyperphagia by hamsters has been most thoroughly studied from a mechanistic standpoint, we will take an in-depth look into this phenomenon and attempt to answer the seemingly perplexing questions: Why don't hamsters overeat after a fast and how is this an adaptive response? We will first look at the less studied, but fascinating cases of prolonged involuntary fasting.

Researchers studying mechanisms of food satiation in laboratory rats and mice typically fast animals and then, just before refeeding, administer a naturally occurring chemical found in the CNS (e.g., neuropeptides such as cocaine- and amphetamine-regulated transcript [CART] (Kristensen *et al.*, 1998; Lambert *et al.*, 1998) or in the periphery (e.g., cholecystokinin [CCK] (Gibbs & Smith, 1982; Waldbillig & Bartness, 1982)) in an attempt to inhibit the post-fast hyperphagia. What was made abundantly clear in the insightful review by Mrosovsky and Sherry on animal anorexias (Mrosovsky & Sherry, 1980) is that several species exhibit prolonged period of fasting *voluntarily*. Naturally occurring reduced food intake can take one of several forms including complete inhibition of feeding for months (e.g., marmots [*Marmota monax*, Kortner & Heldmaier, 1995]; artic ground squirrels [Galster & Morrison, 1976]; emperor penguins [*Aptenodytes forsteri*, Dewasmes, Le Maho, Cornet, & Groscolas, 1980]) or reduced food intake (e.g., golden-mantled ground squirrels [*Citellus lateralis*, Barnes & Mrosovsky, 1974; Zucker & Boshes, 1982]). Perhaps these impressive voluntary fasts by various penguin species, some occurring for several months (Castellini, Costa, & Huntley, 1987; Cherel, Leloup, & Le Maho, 1988; Cherel *et al.*, 1988; Le Maho, Delclitte, & Chatonnet, 1976; Nordoy & Blix, 1991), might be useful to explore in our attempts to understand the systems engaged in the termination of a meal. Unfortunately, information on the feeding responses after voluntary fasting is severely limited because few studies have measured food intake in these species (for review see Davis, 1976)

In order to understand voluntary food intake reductions, it is important to determine the metabolic consequences of food deprivation because it is generally thought that alterations in fuel oxidation *per se*, a metabolic by-product of these oxidation changes, or the resulting modifications of brain neurotransmitter systems, are the factors responsible for triggering post fast increases in food intake. To this end, fasting has been classically parceled into four phases indicated by the predominant status of lipid, carbohydrate and most importantly, protein metabolism (for review see Newsholme & Leech, 1983, and for a schematized view of the many interrelations between lipid, protein, and carbohydrate utilization during fasting in birds, see Figure 1). Briefly, Phase I (i.e., the post-absorptive period), starts when food has been fully absorbed by the intestine after a meal, the duration of which varies with the caloric content of the meal among other factors. For most animals, this lasts for several hours. Relatively quickly once absorption ends, mobilization of liver and muscle glycogen begins in an apparent attempt to counter the slow decline in blood glucose concentrations after their post-meal peak. Because glycogen stores are rapidly depleted with fasting (Newsholme & Leech, 1983), this source of energy is not even sustainable for relatively short fasts (e.g., Syrian hamsters and laboratory rats

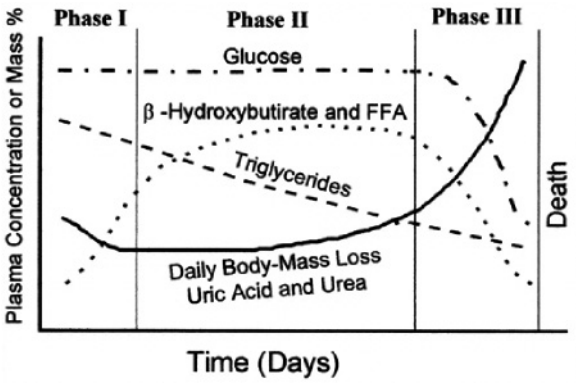

Figure 1. Stylized view of the interrelations among several metabolic measures and body mass during fasting in bird species, especially penguins and gulls (from Alonso-Alvarez & Ferrer, 2001).

[Rowland, 1984] and certainly not for fasts lasting for months [emperor penguins; Groscolas & Clement, 1976]). Some protein breakdown also occurs during this short phase and toward its end, body mass declines more rapidly. Phase II (i.e., early starvation) is characterized by continued and accelerated release of liver glucose into the circulation and mobilization of white adipose tissue (WAT) triglyceride stores for oxidation of their fatty acids by muscle and kidney. This utilization of fatty acids, in turn, spares glucose for use by brain and other tissues. Some gluconeogenesis (the formation of glucose from amino acids resulting from protein breakdown) is stimulated in the liver in Phase II, but this occurs very late in the phase using amino acids derived from protein catabolism and is relatively insignificant (typically this phase is known for its protein sparing). Body mass steadily declines, but at a slower rate than in Phase I. In Phase III (i.e., intermediate starvation), the gluconeogenesis begun late in Phase II begins to diminish, likely due to fulfillment of the mounting energetic needs by the conversion of the adipose-derived fatty acids to ketone bodies in the liver for use by the brain and other tissues. Despite diminished use of amino acids for gluconeogenesis, a critically high rate of protein breakdown occurs, ending the protein sparing of the earlier phases. Body mass loss during this phase is again rapid and impressive. Phase IV (i.e., prolonged starvation) is known for its steady-state rates of carbohydrate, lipid and protein metabolism, and ultimately death (Newsholme & Leech, 1983).

In the typical laboratory rat and mouse fasting–refeeding experiments alluded to above, the animals are fasted overnight or else for 24–48 hr; thus Phase II, and perhaps at the longer durations, early Phase III, is reached before food access is reinstated. With resumption of free-feeding, food intake in rats is increased and, up to a point, is positively correlated with the duration of the fast (Baker, 1955; Lawrence & Mason, 1955). After short fasts, a similar relation holds for subsequent food intake by mice (Ross & Smith, 1953); however, because they are prone to fasting-induced torpor, an energy saving response (Gavrilova *et al.*, 1999; Webb, Jagot, & Jakobson, 1982; Webb & Skinner, 1996), fasted-refed mice do not eat as much as would be expected given their high metabolic rates when fed ad libitum. This is most likely because the caloric deficit is lessened by the torpor-induced decrease in energy expenditure and the post-torpor decrease in locomotor activity.

In stark contrast to most animals across many taxa, all species of hamsters tested to date show no post-fast hyperphagia (i.e., Syrian [*M. auratus*, Silverman &

Zucker, 1976]; Turkish [*Mesocricetus brandti*, Rowland, 1982]; and Siberian [*Phodopus sungorus*, Bartness & Clein, 1994]). Substantial efforts have been made to understand this apparent "maladaptive response" to refeeding after starvation. Interestingly, clear documentation of post-fast increases in food intake in humans is incredulously missing. In a recent unpublished study of Mormons who fast once a month (Fast Saturday) for about a day, upon refeeding they do not initially increase their food intake the next day (Sunday), but rather increase their food intake the following weekend (Plunkett, 2002). How such a delayed post-fast hyperphagia might occur mechanistically is unknown.

The lack of post-fast hyperphagia in hamsters was shown initially when Syrian hamsters (*M. auratus*) were schedule-fed such that their food availability was limited each day. When access to food was restricted to only 1 hr per day, the hamsters never overate as do similarly treated laboratory rats (Simek & Petrasek, 1974), and consequently had progressive losses in body mass and fat resulting in death (Simek & Petrasek, 1974). Using a less severe test of fasting–refeeding responses, Silverman and Zucker (1976) fasted Syrian hamsters or laboratory rats for 24 hr every other day (intermittent feeding/fasting); the rats, but not the hamsters, compensated for the lost calories due to these alternating days of fasting by overeating during the days of ad libitum food access. Indeed, 80–100% of the hamsters died with this fasting/feeding regimen across several experiments, whereas all of the rats thrived (Silverman & Zucker, 1976). Syrian hamsters continue to progressively lose body mass and never overeat even after 6–20 weeks of this restricted feeding schedule (Simek, 1974, 1975, 1980; Simek & Petrasek, 1974).

Detailed analysis of food intake after fasts of varying lengths by Syrian hamsters suggests they do not cope with fasts longer than 12 hr well, or with body mass losses greater than 20% of body weight, becoming "debilitated" (Borer *et al.*, 1979). The pattern of food intake upon refeeding by fasted Syrian hamsters is not different from that of ad libitum fed hamsters with fasts of 5–12 hr (Borer *et al.*, 1979), but the latency to eat after a fast is significantly decreased (DiBattista, 1983). The type of food offered after a fast does not affect this post-fast normophagia, even if the starved hamsters are allowed to self-select their diet from foods varying in caloric density and macronutrient composition (Day *et al.*, 1999; DiBattista, 1987).

It might be argued that restricting food intake to a few hours a day, or every other day, may be contrived and that hamsters in nature would not experience this type of food availability and therefore this is the reason they do not adapt to this feeding schedule. The artificial nature of this type of manipulation, however, may be more apparent than real. That is, animals in the wild do have restrictions on foraging for food and consequently eating because of the presence of predators outside their burrows, as well as inclement weather. Why traditional laboratory animals, such as laboratory rats, are able to adapt to this feeding schedule, whereas nontraditional animals, such as hamsters, are not able to do so has remained a puzzle, although some insights have occurred (see later). Before looking at the behavioral and metabolic responses to fasting by hamsters, laboratory rats and other species more closely, note that the failure to increase food intake by hamsters during refeeding does not preclude recovery of the lost body and lipid mass. Given ample time between fasts (clearly not every other day as in the intermittent fasting models above), Syrian (Borer, Allen, Smalley, Lundell, & Stockton, 1985; Borer *et al.*, 1979; Granneman & Wade, 1982) and Siberian (Day *et al.*, 1999; Wood & Bartness, 1996) hamsters will regain fasting/food restriction-induced body and

lipid mass losses, but do so through decreased energy expenditure/increased efficiency in the utilization of their non-elevated food intakes (Borer *et al.*, 1985).

Silverman and Zucker (1976), and others (Rowland, 1982), have shown that the failure to exhibit a post-fast hyperphagia by Syrian hamsters is not a consequence of being physically unable to overeat. That is, diluted liquid or solid food triggers increases in food intake by Syrian hamsters (Rowland, 1982; Silverman & Zucker, 1976), as do lesions of certain brain areas (i.e., ventromedial hypothalamus [VMH]/paraventricular hypothalamic nucleus [PVN]; (Rowland *et al.*, 1986)), cold exposure (Bartness, Ruby, & Wade, 1984; Simek, 1980), and exercise (Bartness *et al.*, 1984; Browne & Borer, 1978; Shapiro, Borer, Fig, & Vinik, 1987; Tsai, Bach, & Borer, 1981; Tsai, Rosenberg, & Borer, 1982). Silverman and Zucker (1976) speculated that hamsters may have evolved a different strategy to counteract shortfalls in foragable food—building food caches (food hoards; for review see Bartness & Day, 2003) thereby rendering the effects of decreased food availability less costly. In addition, it should be noted that there is an increase in food hoarding accompanying the post-fast increases in food intake by laboratory rats, if they are permitted to do so (Baker, 1955), and they increase both food intake and food hoard size even with restricted access to food (Borker & Gogate, 1981). Food hoarding by laboratory rats is, at best, a secondary priority in that they eat first and then hoard (Day & Bartness, 2003); moreover, it seems that rats do not hoard food in their natural environment (Calhoun, 1962; Lore & Flannelly, 1978; Pisano & Storer, 1948; Takahhashi & Lore, 1980; Whishaw & Whishaw, 1996). In addition, unlike hamsters that possess specialized sublingual pouches to facilitate hoarding, rats lack such specializations. Fasted Syrian (Lea & Tarpy, 1986; Schneider & Buckley, 1993; Wong & Jones, 1985) and Siberian (*P. sungorus*, Bartness, 1997; Bartness & Clein, 1994; Day *et al.*, 1999; Wood & Bartness, 1996) hamsters markedly increase their food hoarding

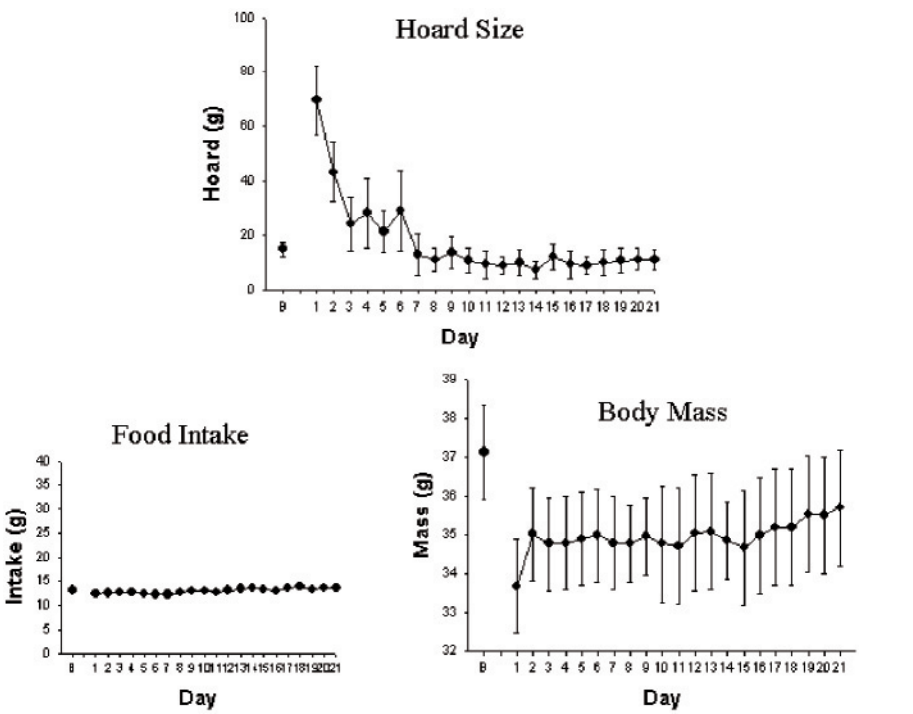

Figure 2. Stimulation of food hoarding, but not food intake by Siberian hamsters after a fast (from Day *et al.*, 1999).

during refeeding, but do not increase their food intake (Figure 2; see below for in-depth discussion).

Perhaps the failure of hamsters to increase food intake after a fast is due to a markedly different physiological metabolic response to starvation. The fasting-induced increases in circulating fatty acids, ketones, and decreases in circulating glucose, insulin, and leptin, as well as liver glycogen by Syrian hamsters (Borer *et al.*, 1979; Mercer, Lawrence, & Atkinson, 1996; Rowland, 1984; Schneider, Blum, & Wade, 2000; Simek & Petrasek, 1974) are similar to those of laboratory rats (e.g., Owens, Thompson, Shah, & DiGirolamo, 1979; Rowland, 1984), however. The lack of a post-fast hyperphagia might likewise be due to differences in the control of gene expression (and subsequent protein synthesis) for neuropeptides thought to be involved in food intake in laboratory rats and mice. Fasting-induced changes in gene expression are similar between laboratory rats and hamsters (of several species) after food deprivation, however. Specifically, food deprivation increases hypothalamic neuropeptide Y (NPY), the long form of the leptin receptor (Ob-Rb), and agouti-related protein (AgRP) gene expression while decreasing CART gene expression by hamsters (Mercer *et al.*, 1995, 1996, 2001; Mercer, Lawrence, Moar, Atkinson, & Barrett, 1997; Mercer, Moar, Ross, & Morgan, 2000; Reddy, Cronin, Ford, & Ebling, 1999).

Likewise, the lack of a post-fast hyperphagia by hamsters could be due to the presence or absence of a dominant environmental factor such as the photoperiod (daylength) or food type. The absence of a post-fast hyperphagia occurs in either long "summer-like" days or short "winter-like" days by Syrian (Granneman & Wade, 1982) and Siberian hamsters (Wood & Bartness, 1996), however. In addition, if Syrian (DiBattista, 1987) or Siberian (Day *et al.*, 1999) hamsters are given a choice of foods, significant post-fast increases in food intake also do not occur, although fat intake increases briefly 6 hr post-fast (DiBattista, 1987). As discussed below, Silverman and Zucker's speculation (Silverman & Zucker, 1976) that hamsters may respond to fasts by increasing food hoarding, rather than food intake, was correct and by increasing hoarding, these animals can accomplish the same goal as animals that overeat—acquiring food energy for current and subsequent needs. In other words, food hoarding and food intake appear to be alternate strategies for energy storage triggered by food shortages and utilized to various degrees in a species-specific manner.

Voluntary fasting seems extremely interesting in light of the tendency for humans in Western and Westernized civilizations, and nonhuman animals in the laboratory, to overeat amidst their caloric wealth. How prevalent are these voluntary fasts? Many species exhibit complete fasts, especially during certain reproductive states (e.g., egg brooding by penguin species (Pinshow, Fedak, Battles, & Schmidt-Nielsen, 1976; Williams, Siegfried, Burger, & Berruti, 1977), post-weaning by seal pups (Castellini *et al.*, 1987), molting of scales by snakes or feathers by penguins (Williams *et al.*, 1977), and hibernation by yellow-bellied marmots [*Marmota flaviventris*, Florant, Tokuyama, & Rintoul, 1989]). The fasting associated with egg brooding and molting of penguins could be viewed simply as two behaviors that are incompatible with feeding. That is, feeding during brooding requires the penguins to leave their nest areas and consequently to leave their eggs unprotected from the elements to travel often long distances to the sea to catch fish (Williams, 1995). Feeding during molting is precluded because the penguins lose all their plumage, and consequently thermal insulation, leaving nothing to combat the icy water temperatures in the Antarctic where they exclusively feed (Groscolas & Cherel, 1992). Both the brooding/ molting and hibernation associated fasts also could be viewed as a consequence of reduced food availability. This seems somewhat unlikely because, although there is a reduction in food during the winter, hibernating mammals often

have food stores in their hibernacula, especially seeds (for review see Davis & Finnie, 1975; Morton, 1975), but even in the presence of food it is not necessarily consumed (e.g., alpine marmots, Kortner & Heldmaier, 1995). If one views the obligatory cessation of food intake during hibernation as a self-imposed fast, then post-fast (arousal) intakes of garden dormice (*Eliomys quercinus*) should be increased over pre-fast (prehibernation), but they are not for the first 24 or 48 hr post-fast (Amid, Cartan, Atgie, & Nibbelink). Hibernating Turkish hamsters also do not increase their food intake over prehibernation levels during arousals when they typically feed, regardless of the length of the hibernation bout (Bartness and Goldman, unpublished observations). Perhaps this fixed rate of feeding by hamsters and dormice after a hibernation bout may help them regulate the utilization of energy stored in the form of a food cache (see later).

The ability of the avian and mammalian species discussed above to thwart the adverse consequences of absent or reduced energy intake, especially in harsh environments such as the Artic, Antarctic, and Turkish steppes, is their principal reliance on stored energy as body fat and secondarily as carcass protein. Although the topic is too complex for a thorough discussion here, for brooding and molting penguins as well as for hibernating small mammals, energy-saving responses are triggered such as huddling for the former (Prevost, 1962) and depressed body temperature/metabolic rate for the latter. An example of the importance of such energy saving responses is the fasted and cold-exposed barn owl (*Tyto alba*) that does not huddle and has relatively limited abilities to depress body temperature/metabolic rates (Thouzeau, Duchamp, & Handrich, 1999). The inability to engage in these physiological/behavioral energy saving responses apparently underlies the high mortality rate of barn owls under these conditions (Thouzeau *et al.*, 1999).

Some fasted penguins have full stomachs—a remarkable combination of conditions. For example, King penguin parents (*Aptenodytes pantagonica*) take turns incubating the eggs so that one parent can go to sea to feed, while the eggs are warmed by the other parent. Upon return from the sea with a full stomach, this parent incubates the egg holding the food in its stomach for as long as 20 days while its mate goes off to sea to eat; the food can then be regurgitated to feed the chick upon its birth (Gauthier-Klerc, LeMaho, Clerquin, Drault, & Handrich, 2000). Digestion/composition of the food during that time is unknown, but its purpose appears to be the first meal for the hatched chicks (for review see Groscolas & Robin, 2001). Clearly, the inhibition of gastric emptying in the face of a prolonged total fast is an impressive feat and its study may lead to an understanding of potent satiety factors. Finally, it is not within our space limitations to discuss the other side of this coin in depth—resumption of feeding after voluntary fasts (see peptidergic control of feeding below for some treatment of this issue). It seems clear that, because no fasting male emperor penguin has been found starved to death, at some point if its mate does not return punctually, the egg is abandoned for a trek to the sea to feed (Groscolas & Robin, 2001). It has been postulated (Groscolas & Robin, 2001) and tested recently (Bernard, Mioskowski, & Groscolas, 2002) that a decrease in the utilization of lipid fuels triggers the resumption of food intake and this, and related topics, are considered below in the section on the metabolic controls of food intake.

What triggers the complete or partial inhibition of food intake in animals that voluntarily fast or reduce their food intake? This question, of course, can be reduced to one of the questions plaguing the field of ingestive behavior—"What makes animals stop eating a meal?" We are not going to address fully this complicated and unresolved question here, except in the few studies focusing on satiety or

anorexia in these naturally occurring examples. If one takes the view that alterations in brain and/or peripheral peptides underlie changes in food intake, then there is a plethora of peptides that could contribute to these inhibitions of food intake (see Table 1 for a list of factors affecting food intake [Schneider & Watts, 2002]). One candidate hormone is pancreatic insulin (for review see Baskin *et al.*, 1999). Specifically, in the yellow-bellied marmot that ceases food intake during the hibernation season, it has been hypothesized that peripherally released insulin could be responsible for this anorexia (Florant, Richardson, Mahan, Singer, & Woods, 1991). Thus, circulating insulin could reach brain insulin and inhibit food intake; however, insulin does not readily enter the brain at times when peripheral levels are high in this species and therefore seems unlikely to participate in this process (Florant *et al.*, 1991). Another well-studied and recognized inhibitor of food intake is peripherally- and centrally-released CCK (for review see Morley *et al.*, 1985; Reidelberger, 1994; Smith, 1996). Siberian hamsters that reduce, but do not eliminate, food intake in winter (Bartness, Morley, & Levine, 1986, 1995; Drazen, Demas, & Nelson, 2001; Wade & Bartness, 1984a) are more responsive to the inhibition of food intake by CCK during short "winter-like" days when their food intake is naturally low, than in long "summer-like" days (Bartness *et al.*, 1986). This leaves open the possibility of an involvement of CCK in winter reductions in food intake for other species as well. Another, nonmutually exclusive hypothesis is that peripheral metabolic signals indicating enlarged adipose tissue lipid fuel stores may lead to a decrease or complete inhibition of food intake during these periods of natural anorexia. Later, with the waning of these signals and/or the emergence of signals reflecting protein catabolism, the resumption of normal levels of food intake may be triggered. There has been much speculation about the identity of this metabolic signal, including the possibility that it is leptin, the peripheral factor that is widely believed to inform the brain of the size of adipose tissue lipid fuel stores (Friedman, 1998). Chronic peripheral leptin infusions block both the prehibernatory decrease in food intake and body weight by arctic ground squirrels (Ormseth, Nicolson, Pelleymounter, & Boyer, 1996), as well as the post-hibernatory increase in fattening (Boyer *et al.*, 1997), two periods of naturally occurring increases in lipid energy stores. This effect of leptin could originate peripherally through the often neglected peripheral metabolic effects of this peptide (for review see Harris, 2000; Schneider & Wade, 1999). Such changes in lipid and/or carbohydrate metabolism could, in turn trigger physiological responses within the brain. Alternatively, these effects of leptin could act *directly* to affect the central sites containing leptin receptors implicated in the control of food intake and body fat (e.g., hypothalamic sites such as the arcuate nucleus [Elmquist, 2001] or brainstem sites such as the dorsal vagal complex [Grill *et al.*, 2002]). Finally, leptin could signal the size of lipid stores by stimulating putative leptin receptors located on the sensory nerves (Fishman & Dark, 1987) that innervate WAT (Niijima, 1998, 1999). Although sensory innervation of WAT has traditionally been ignored, reliable data exist in support of such innervation (Fishman & Dark, 1987; Niijima, 1998, 1999). Whether the effects of chronic peripheral leptin on prehibernatory food intake in arctic ground squirrels discussed above are a physiological reality or a pharmacological curiosity requires considerably more study.

There are several similarities between these nonhuman animal species and humans in terms of some, but not all, of the physiological responses engaged during fasting. For example, extremely malnourished humans near death increase their energy expenditure (Rigaud, Hassid, Meulemans, Poupard, & Boulier, 2000),

Table 1. Some Central Peptides that Affect Food Intake[a]

CNS peptides that stimulate food intake
Agouti-related protein
β-Endorphin
Galanin
Melanin-concentrating hormone
Moltin
Neuropeptide Y
CNS peptides that inhibit food intake
α-Melanocyte stimulating hormone
Bombesin-like peptides
Cocaine- and amphetamine-regulated transcript
Cholecystokinin
Corticotropin-releasing hormone
Glucagon-like peptide
Insulin
Urocortin
Vasopressin

[a]Adapted from Schneider & Watts (2002).

reminiscent of the increases in energy expenditure and protein mobilization by the brooding-associated fasts of male king penguins (*Aptenodytes patagonicus*) when their mates are tardy in their return from feeding in the sea (Cherel, Charrassin, & Challet, 1994). For these humans, it appears that increases in catabolism of their remaining protein energy stores (albeit very small ones given their body mass index of <10) may trigger the increases in energy expenditure, although a potential mechanism remains to be elucidated. This finding is quite different from that of fasted healthy humans (Keys, Brozek, Henschel, Mickelson, & Taylor, 1950), or of human anorexia nervosa patients who are otherwise in generally good health (Melchoir, Rigaud, Rozen, Malon, & Apfelbaum, 1989), although both of these populations are generally not in as dire metabolic straights as the dying humans (Rigaud *et al.*, 2000). Some insight into the "terminal spurt" of increased energy expenditure by dying humans might be gained from further study of the prolonged fasts of male king penguins (Cherel *et al.*, 1994).

Metabolic Control of Food Intake

One segment of research on the control of food intake has focused on alterations in utilizable metabolic fuels as triggers for the stimulation or inhibition of food intake. As with most of the research on food intake, it has been largely conducted using laboratory rats and to a lesser extent, mice. The underlying assumption is that a change in the utilization of one (usually) or more metabolic fuels is sensed either by peripheral (e.g., liver) and/or central receptors to trigger food intake so as to offset the alterations in metabolic fuel utilization. Specifically, some hypotheses have centered on key metabolic fuels such as carbohydrates or carbohydrate-related fuels (glucose, glycogen), lipids or lipid-related fuels (free fatty acids [FFA], glycerol, ketones) or proteins or protein-related fuels (amino acids) (for review see Friedman, 1995; LeMagnen, 1984; Mayer, 1953; Scheurink & Nolan, 1996). Others have posited that it is not decreases or increases in the utilization of a specific

metabolic fuel *per se* that is important, rather it is their ultimate metabolic impact such as alterations in adenosine triphosphate (ATP; Even & Nicolaidis, 1985; Friedman, 1995) that control food intake. Experimental tests of these hypotheses have been conducted using specific metabolic fuel utilization blockers alone or in combination. Thus, to produce decreases in glucose utilization (glucoprivation) so as to increase food intake, several compounds have been administered to laboratory rats, including the glucose analogs 2-deoxy-D-glucose (2DG; Berthoud & Mogenson, 1977; Naito *et al.*, 1973; Smith & Epstein, 1969; Stricker, Rowland, Saller, & Friedman, 1977) or 5-thio-D-glucose (5TG; Flynn & Grill, 1985; Slusser & Ritter, 1980) that compete for enzymes of the glycolytic pathway where glucose ultimately feeds into the tricarboxcylic acid cycle (a.k.a. Krebs cycle). To produce decreases in lipid fuel (fatty acid) utilization so as to increase food intake, blockers of key enzymes in fatty acid transport and their ultimate conversion to ATP (methyl palmoxirate [MP; Friedman, Ramirez, Bowden, & Tordoff, 1990; Friedman & Tordoff, 1986] or mercaptoacetate [MA; Ritter, & Taylor, 1989; Scharrer & Langhans, 1986]) have been used effectively with laboratory rats, especially if they are deriving most of their calories from dietary fat. Alternatively, production of ATP can be blocked to stimulate food intake by laboratory rats, regardless of whether the carbon fragments entering the tricarboxcylic acid cycle originate from carbohydrate, lipid or protein sources such as after treatment with 2,5-anhydro-D-mannitol (2′5′AM; Rawson, Blum, Osbakken, & Friedman, 1994; Rawson & Friedman, 1994; Rawson & Ulrich, 1996) or L-ethionine (Rawson, Ulrich, & Friedman, 1994). Finally, peripheral administration of insulin can be used to clear all circulating metabolic fuels from the blood to storage and thereby increase food intake in laboratory rats (e.g., Gil & Friedman, 1982). These manipulations of metabolic fuels do not stimulate food intake universally, however (for review see Bartness, 1990). Once again, hamster species are a notable exception. Specifically, injections of 2DG or 5TG (Bartness *et al.* 1995; DiBattista, 1982; Ritter & Balch, 1978; Rowland, 1978; Sclafani & Eisenstadt, 1980; Stamper, Dark, & Zucker, 1999) do not stimulate food intake as they do in laboratory rats (Berthoud & Mogenson, 1977; Ritter & Slusser, 1980; Smith & Epstein, 1969; Stricker & Rowland, 1978). Furthermore, injections of short-acting insulin produce small or no increases in feeding in hamsters or Mongolian gerbils (*Meriones unguiculatus*), but long-lasting insulin does increase food intake ([Bartness & Clein, 1994; DiBattista, 1983, 1984; Ritter & Balch, 1978; Rowland, 1978; Wade, Schneider, & Friedman, 1991]; cf., [Bartness & Clein, 1994); laboratory rats increase food intake after administration of either insulin form (Flynn & Grill, 1983; Gil & Friedman, 1982; Rowland & Bartness, 1982). Although the physiological mechanisms for the differential effects of long versus short-lasting insulin are not known, one possibility is that long-term insulin triggers hypoglycemia, that hamsters respond to by increasing food intake, whereas short-term insulin fails to induce hypoglycemia. Although this idea is intriguing, it remains to be tested. Hamster species are not the only ones that do not increase their food intake after alterations in metabolic fuel utilization or storage. For example, Shaw's jirds (*Meriones shawi*) do not increase their food intake after peripheral injections of 2DG or insulin (Demas & Bartness, 1999), nor do deer mice (*Peromyscus maniculatus*, Rowland, Watkins, & Carlton, 1985) or Mongolian gerbils (Rowland, 1978) after peripherally injected 2DG, but unlike hamsters all increase food intake after a fast (Demas & Bartness, 1999; Rowland *et al.*, 1985; Wong & Jones, 1985). Thus, no clear predictive relation exists among the metabolic fuel utilization/storage-induced or fasting-induced increases in food intake and a failure to respond to 2DG (Table 2).

Table 2. Some Nontraditional Animal Models of Food Intake (FI)

Use	Response
Seasonality	
House mice (*Mus musculus*)	No seasonal/photoperiodic changes in FI or body mass
Norway rats (*Rattus norvegicus*)	No seasonal/photoperiodic changes in FI or body mass
Siberian hamsters (*Phodopus sungorus*)	Decreased FI and adiposity in short days
Syrian hamsters (*Mesocricetus auratus*)	Increased FI and adiposity in short days
Arctic ground squirrels (*Citellus undulates*)	Increased FI during autumnal pre-hibernation
Sheep (*Ovis aries*)	Increased FI in summer or long days
Involuntary fasting/refeeding	
House mice (*M. musculus*)	Post-fast hyperphagia
Norway rats (*R. norvegicus*)	Post-fast hyperphagia
Siberian hamsters (*P. sungorus*)	No post-fast hyperphagia
Syrian hamsters (*M. auratus*)	No post-fast hyperphagia
Shaw's jird (*Meriones shawi*)	Post-fast hyperphagia
Mongolian gerbils (*Meriones unguiculatus*)	Post-fast hyperphagia
Deer mice (*Peromyscus maniculatus*)	Post-fast hyperphagia
Voluntary fasting	
House mice (*M. musculus*)	No voluntary fasting
Norway rats (*R. norvegicus*)	No voluntary fasting
Marmots (*Marmota monax*)	Hibernation-induced fasting
Ground squirrels (*Citella lateralis*)	Hibernation-induced fasting
Garden dormice (*Eliomys quercinus*)	Hibernation-induced fasting
Turkish hamsters (*Mesocricetus brandti*)	Hibernation-induced fasting
Emperor penguins (*Aptenodytes forsteri*)	Fast during egg brooding or molt
King penguins (*Aptenodytes pantagonica*)	Fast during egg brooding
Elephant seals (*Mirounga angustirostris*)	Fast during post-weaning
Food hoarding	
House mice (*M. musculus*)	Decreased post-fast hoarding
Norway rats (*R. norvegicus*)	Increased post-fast hoarding
Siberian hamsters (*P. sungorus*)	Increased post-fast hoarding
Syrian hamsters (*M. auratus*)	Increased post-fast hoarding
Jirds (*M. shawi*)	Decreased post-fast hoarding
Metabolic control of feeding	
House mice (*M. musculus*)	Increased FI after 2DG or MA
Norway rats (*R. norvegicus*)	Increased FI after 2DG, 5TG, insulin, MA, or MP
Mongolian gerbils (*M. unguiculatus*)	No increases in FI after 2DG or insulin
Shaw's jirds (*M. shawi*)	No increase in FI after 2DG or insulin
Deer mice (*P. maniculatus*)	No increase in FI after 2DG or insulin; no MA-induced changes in FI
Siberian hamsters (*P. sungorus*)	No 2DG induced feeding, MA-induced decreases in FI, no effect of MP
Syrian hamsters (*M. auratus*)	No 2DG induced feeding; no effect of MP on FI
Peptidergic control of feeding	
Leptin	
House mice (*M. musculus*)	Decreased FI and body mass
Norway rats (*R. norvegicus*)	Decreased FI and body mass
Siberian hamsters (*P. sungorus*)	Decreased or no effect on FI in long or short days
Rhesus monkeys (*Macaca mulatta*)	Decreased FI after icv, but not peripheral injections
Domestic chickens (*Gallus domesticus*)	Dose-related decrease in FI

TABLE 2. (*Continued*)

Use	Response
Neuropeptide Y (*NPY*)	
House mice (*M. musculus*)	Increased FI after icv injections
Norway rats (*R. norvegicus*)	Increased FI after icv injections
Syrian hamsters (*M. auratus*)	Increased FI after icv infusions
Siberian hamsters (*P. sungorus*)	Increased FI after icv infusions
Sheep (*O. aries*)	Increased FI after icv infusions
Domestic chickens (*G. domesticus*)	Increased FI in adults, but not 2-day-old chicks
White-crowned sparrows (*Zonotrichia leucophrys*)	Greater increase in FI in short than long days
Goldfish (*Carassius auratus*)	Increased FI after icv infusions
Cholecystokinin (CCK)	
House mice (*M. musculus*)	Decreased FI after icv or peripheral injections
Norway rats (*R. norvegicus*)	Decreased FI after icv or peripheral injections
Siberian hamsters	Decreased FI after peripheral injections
Syrian hamsters	Decreased FI after icv injections
Sheep (*O. aries*)	Decreased FI after icv injections; CCK receptor agonist increases FI
Pigs (*Sus scrofa*)	Decreased FI and motivation to eat after peripheral injections
Domestic chickens (*G. domesticus*)	Decreased FI after iv or icv injections
Goldfish (*C. auratus*)	Decreased FI after icv or peripheral injections
High fat diets (HFD)	
House mice (*M. musculus*)	Increased body fat
Norway rats (*R. norvegicus*)	Increased body fat
Siberian hamsters (*P. sungorus*)	Slight or no effect on caloric intake; no effect on body fat
Syrian hamsters (*M. auratus*)	obesity without overeating
Meadow voles (*Microtus pennsylvanicus*)	No effect on body fat
Shaw's jirds (*M. shawi*)	No effect on FI, body mass or carcass lipid content
Mongolian gerbils (*M. unguiculatus*)	Decrease caloric intake
Bank voles (*Clethrionomys glareolus*)	No effect on body fat

Note: References for each response are contained within this review.
MA = mercaptoacetate; MP = methyl palmoxirate; 2DG = 2-deoxy-D-glucose; icv = intracerebroventricular; iv = intravenous.

This lack of an increase in food intake after treatment with glucose utilization blockers by hamsters also applies to the blockade of lipid fuels, specifically FFAs. Whereas MA increases food intake in deer mice (Stamper & Dark, 1997) and laboratory rats (Scharrer & Langhans, 1986), it *decreases* (Stamper *et al.*, 1999) or does not affect (K. Boss-Williams and T. Bartness, unpublished observations) food intake in Siberian hamsters. Similarly, whereas MP increases food intake in laboratory rats (Friedman & Tordoff, 1986), it does not do so in Syrian (Lazzarini, Schneider, & Wade, 1988; Schneider, Lazzarini, Friedman, & Wade, 1988) or Siberian (Bartness & Clein, 1994) hamsters.

One argument made for the inability of some species, especially hamsters, to respond to glucoprivation or lipoprivation is that they can effortlessly switch between utilization of lipid and carbohydrate fuels. For example, Syrian hamsters can maintain continued estrous cyclicity (a response sensitive to alterations in metabolic fuels) following blockade either glucoprivation (2DG) or lipoprivation (MP) (Schneider & Wade, 1989). Combined treatment with 2DG and MP, however,

renders animals acyclic, suggesting that either of these metabolic pathways can be utilized to maintain normal estrous cycles in Syrian hamsters (Schneider & Wade, 1989). Thus, perhaps the combination of glucoprivation (2DG) with lipoprivation (MP), which stimulates food intake in laboratory rats, even when doses of both drugs each are below the threshold dose to stimulate food intake (Friedman & Tordoff, 1986), might increase food intake in hamsters. The combined treatment of 2DG and MP does not stimulate food intake by Syrian (Lazzarini *et al.*, 1988) or Siberian hamsters (Bartness & Clein, 1994), however, nor does a combined treatment of 2DG and MA (another fatty acid utilization blocker similar in effect to MP) in deer mice (Stamper & Dark, 1997).

The inability of these manipulations of metabolic fuels to increase food intake by Syrian and Siberian hamsters is not because these treatments are ineffective in blocking fuel utilization in these species. Thus, 2DG triggers increases in circulating glucose and/or ketone bodies in Syrian hamsters and Mongolian gerbils (Angel & Taranger, 1991; Ritter & Balch, 1978; Rowland, 1978, 1983), whereas 5TG triggers increases in circulating glucose and FFAs in Syrian hamsters (DiBattista, 1982). Short- or long-acting insulin produces profound decreases in glucose concentrations in Syrian (DiBattista, 1983; Ritter & Balch, 1978; Rowland, 1978, 1983) or Siberian hamsters (Bartness, McGriff, & Maharaj, 1991; Bartness *et al.*, 1995) to levels that would cause coma in laboratory rats (e.g., 30–50 mg/dl) yet the animals are conscious and mobile. Thus, the appropriate changes in circulating metabolic fuels appear to be generated by these blockers of fuel utilization, but these animals do not have the same sensing or subsequent response systems to trigger increases in food intake as do laboratory rats and mice. Perhaps when metabolic fuel utilization is blocked in these species, other energy-related responses are generated such as savings of energy expenditure (torpor is induced by 2DG in Syrian and Siberian hamsters) or increases in energy acquisition (food hoarding, see below).

Peptidergic Control of Food Intake

As with other aspects of food intake, the role of peptides in the control of food intake has primarily been accomplished using laboratory rats and mice, but there is a growing body of research on other species. Unfortunately, there has been no successful attempt to integrate the effects of peptides on these nontraditionally studied species, perhaps because such a task seems daunting. Thus, unlike the other topics within this chapter, this section is divided into the effects of several well-studied peptides with subdivisions for single or related species. One point is clear, that there are striking similarities across species with respect to the ability of these peptides to stimulate or inhibit food intake. Therefore, we will make note of these consistencies across species, as well as pointing out the few exceptions to these generalities. Finally, in keeping with the overall theme of this review, we will incorporate possible functional significance of the effects of the peptides on feeding in light of the animal's behavioral ecology when possible.

Leptin

Leptin is a peptide hormone primarily derived from WAT and belongs to the cytokine family, the discovery of which in 1994 quickly led to its attribution as a primary conveyor of body fat levels to the brain (Campfield, Smith, Guisez, Devos, &

Burn, 1995; Halaas *et al.*, 1995; Pelleymounter *et al.*, 1995). Specifically, it was initially hypothesized that as body fat level increases, leptin secretion by the expanding adipose depot increases and impacts key forebrain (arcuate nucleus, Schwartz *et al.*, 1997; Wang *et al.*, 1997) and hindbrain sites (dorsal vagal complex, Grill *et al.*, 2002) thought to be involved in the regulation of energy balance (Table 3). Stimulation of these brain areas by leptin, in turn, would trigger corrective measures opposing the increases in adiposity (e.g., decreases in food intake, Pelleymounter *et al.*, 1995; Weigle *et al.*, 1995). With additional study, the role of leptin was expanded to include involvement in reproduction, and immune and stress responses among others (for review see Harris, 2000). Since the initial observations, excitement about the role of leptin in the control of food intake and body fat levels, indirectly and directly, has diminished somewhat because of the growing number of exceptions to the original notion of its role as a feedback signal of body fat levels to the brain. For example, exogenous leptin does not always reliably decrease food intake in genetically normal laboratory mice, especially when given peripherally (its natural origin) and when physiological circulating concentrations are achieved (e.g., Harris *et al.*, 1998). In addition, leptin may not be necessary for the regulation of total body fat. For example, the induction of a lipid deficit by surgical removal of body fat (lipectomy) results in complete or nearly complete compensatory increases in fat pad mass of the non-excised lipid depots in many species, including laboratory rats and mice (Faust, Johnson, & Hirsch, 1977; Liebelt, Ichinoe, & Nicholson, 1965; Schemmel, Mickelsen, Pierce, Johnson, & Schirmer, 1971), Siberian and Syrian hamsters (Hamilton & Wade, 1988; Mauer & Bartness, 1994, 1996, 1997), and ground squirrels (Dark, Forger, Stern, & Zucker, 1985). Lipectomy of mice with alterations in leptin synthesis (*ob/ob* mice) and expression of functional leptin receptors (*db/db* mice) results in fat pad mass compensation that is complete or nearly complete (Chlouverakis, 1974; Harris, Hausman, & Bartness, 2002) despite these alterations of leptin synthesis or signal reception that render the peptide nonfunctional in these animals. Nevertheless, leptin may play a role in regulating energy expensive physiological processes such as reproduction (e.g., Ahima *et al.*, 1996).

Leptin: Effects on Food Intake by Siberian and Syrian Hamsters

In virtually all species undergoing seasonal fluctuations in body fat, there are concomitant fluctuations in circulating leptin. Because both leptin and its receptor may be components of a body fat feedback mechanism in mammals (Berthoud, 2002), leptin also may be involved in seasonal control of body fat. Although a wide variety of mammalian species undergo seasonal cycles of body fat, the vast majority of research on the role of leptin in these seasonal responses has focused on Siberian and Syrian hamsters (Bartness & Wade, 1985). For example, serum leptin concentration correlates positively with body fat in Siberian hamsters over their yearly cycle (Drazen, Kriegsfeld, Schneider, & Nelson, 2000; Horton, Buxton, Losee-Olson, & Turek, 2000). Moreover, for this species, WAT leptin gene expression, circulating leptin concentrations, and leptin receptor gene expression all are reduced in short "winter-like" daylengths compared with long "summer-like" daylengths in accordance with short day-induced decreases in body fat (Demas, Bowers, Bartness, & Gettys, 2002; Drazen *et al.*, 2000; Klingenspor, Dickopp, Heldmaier, & Klaus, 1997; Mercer, Moar, Ross, & Morgan, 2000). Given that reduced leptin receptor gene expression contributes to a decrease in sensitivity to leptin, reduced gene expression

Table 3. Comparative Studies of the Effects of Exogenous Leptin on Food Intake

Species	Leptin treatment	Food intake	Reference
Sheep (*Ovis aries*)	20 μg/hr icv	↓	Henry *et al.* (1999)
	4 μg/hr for 3 days icv	↓	Henry *et al.* (2001)
Pigs (*Sus scrofa*)	10, 50, 100 μg icv	↓	Barb *et al.* (1998)
Syrian hamsters (*Mesocricetus auratus*)	1 μg/day icv	↓	Schneider *et al.* (1999)
	5 mg/day ip	↓	Schneider *et al.* (1998)
Siberian hamsters (*Phodopus sungorus*)	15 g/day for 7 days osmotic minipump	NC	Rousseau *et al.* (2002)
	osmotic minipump	NC (LDs), ↑(SDs)	Drazen *et al.* (2001)
	ip injections	↓LDs and SDs	Klingenspor *et al.* (2000)
	15 g/day for 14 days osmotic minipump	NC (LDs and SDs)	Atcha *et al.* (2000)
Ground squirrels (*Spermophilus lateralis*)	1 mg/m1 3 weeks osmotic minipump	↓	Boyer *et al.* (1997)
Rhesus monkeys (*Macaca mulatto*)	500 ng, 2 μg, 22 μg icv	↓	Ramsey *et al.* (1998)
	1 or 3 μg/kg sc	↓	Tang-Christensen *et al.* (1999)
Gerbils (*Psammomys obesus*)	ip injections 7–14 days	↓	Walder *et al.* (1999)
	ip injections 7d	↓	Sanigorski *et al.* (2000)
Beagle dogs (*Canis familiaris*)	0.05–μg/kg/day sc		LeBel *et al.* (1999)
Chickens (*Gallus domesticus*)	10 μg icv	↓	Denbow *et al.* (2000)
	0.2, 1, 5 μg icv	↓	Bungo *et al.* (1999)
	C4S recombinant leptin	↓	Dridi *et al.* (2000)

in short days may reduce leptin sensitivity in short-day hamsters and indeed, this seems to be the case in Siberian hamsters (Mercer *et al.*, 2000). Note, however, that the dogma associated with the regulation of body fat by leptin states that when body fat levels decrease, the decrease in leptin triggers increases in food intake. The data above support the first portion of this dogma (i.e., short-day-induced decreases in body fat are associated with decreases in leptin gene expression by white fat [Klingenspor *et al.*, 1997] and circulating leptin concentrations [Drazen *et al.*, 2000; Horton *et al.*, 2000; Klingenspor, Niggeman, & Heldmaier, 2000]); however, food intake is *decreased* in short photoperiods *not* increased, especially when body fat is at its seasonal nadir (e.g., Wade & Bartness, 1984).

At least one of the energy-related short-day-induced changes by Siberian hamsters is reversed by peripheral chronic administration of leptin—the increase in food intake occurring when these animals are switched from short to long days is blocked by exogenous leptin (Drazen *et al.*, 2001). Unlike other species, however, such as standard strains of laboratory rats and mice, leptin administration did not affect food intake when Siberian hamsters were at the body and lipid mass peaks in long days (Drazen *et al.*, 2001). This result contrasts with the findings of two earlier studies of Siberian hamsters where peripheral leptin injections decreased food intake to the same extent in both long and short days, but reduced body and fat

pad mass to a greater extent in short days (Atcha *et al.*, 2000; Klingenspor *et al.*, 2000). The exact reasons for these discrepancies are unknown, but in part may be due to differences in leptin administration, as well as other methodological considerations (for discussion see Drazen *et al.*, 2001). The most reliable effect of leptin on food intake in rats and mice is when it is given intracerebroventricularly (icv; *vide infra*) ad to our knowledge, this has not been done in Siberian hamsters. Although Siberian hamsters do not increase food intake after a fast, release from a less than complete food restriction can stimulate food intake (Fine & Bartness, 1996; Rousseau *et al.*, 2002) and chronic peripheral leptin administration does not block this increase, nor does it have any effect on body or lipid mass in these animals (Rousseau *et al.*, 2002). Despite the varied leptin-induced responses across these experiments, there is the tendency for leptin to act differentially between the photoperiods to affect energy balance and food intake. Therefore, seasonal changes in circulating leptin concentrations, coupled with changes in leptin sensitivity, may serve as part of an adaptive mechanism for increasing the odds of winter survival when food availability is decreased and adipose tissue stores are at their nadir (for review see Rousseau, Atcha, & Loudon, 2003).

Fewer data are available on the effects of leptin on food intake *per se* by Syrian hamsters and instead the work to date has focused on its metabolic effects in this species. Fasted-refed Syrian hamsters do not increase their food intake, as do rats (*vide infra*). Fasted laboratory rats have suppressed circulating leptin concentrations (Frederich *et al.*, 1995; Moinat, Deng, & Muzzin, 1995) and consequent activation of the central NPY system thought to be involved with the post-fast increase in food intake of laboratory rats (Sahu, Kalra, & Kalra, 1988; Stanley, Magdalin, Seirafi, Nguyen, & Leibowitz, 1992; White & Kershaw, 1989). Prolonged fasting also inhibits leptin gene expression by WAT in Syrian hamsters (e.g., 48 hr) with partial restoration following refeeding despite the absence of a post-fast hypophagia (Mercer *et al.*, 1996). Unlike fasted laboratory rats, however, hypothalamic NPY mRNA expression is not increased (Mercer *et al.*, 1996). Therefore, it may be that the failure of fasting to stimulate NPY gene expression by Syrian hamsters likely reflects differences in leptin signaling between laboratory rats and Syrian hamsters, and is not due to any general inability of leptin to affect energy-sensitive brain sites *per se* (Mercer *et al.*, 1996). This view is buttressed by the series of elegant studies of Schneider and colleagues demonstrating a role of leptin in the regulation of peripheral fuel metabolism. Specifically, prolonged fasting inhibits estrous cyclicity in female Syrian hamsters (Schneider & Wade, 1989) and exogenous peripheral leptin treatment given during the fast counteracts this fasting-induced inhibition of estrous cyclicity. The possibility that this effect of leptin was sensitive to metabolic fuel utilization was supported when each leptin treatment was preceded by giving metabolic fuel blockers such as 2DG (to block glucose oxidation; Schneider *et al.*, 1998). That is, 2DG treatment blocked the ability of leptin to reinstate estrous cycles that cease due to fasting. Thus, the ability of leptin to affect reproductive status in these experiments appears to rely on sufficient metabolic fuels for oxidation in this species (Schneider *et al.*, 1998).

Leptin: Effects on Food Intake by Ground Squirrels

Another seasonally breeding animal with annual cycles of body fat is the ground squirrel. Although the seasonal rhythms in ground squirrels can be modified by changes in the photoperiod and the pineal hormone melatonin (Hiebert

et al., 2000), the photoperiod is not the primary coordinator of these seasonal energy and reproductive annual cycles, as in hamster species. Instead, the underlying timing mechanism is an endogenous circannual clock, the location of which is unknown. Chronic peripheral infusions of mouse recombinant leptin given to arctic ground squirrels (*Citellus undulates*) inhibit the increase in body fat that occur in the fall during the prehibernatory period (Ormseth *et al.*, 1996). This effect of leptin on body fat likely is caused by decreases in food intake and not increased energy expenditure because resting metabolic rate, body temperature, and locomotor activity are not affected by leptin (Boyer *et al.*, 1997). The ability of exogenous leptin to inhibit food intake likely represents a pharmacological effect of the peptide because leptin was administered when food intake is naturally increasing and the greatly expanding WAT mass likely results in increased endogenous circulating leptin concentrations. Thus, leptin was given on a background of naturally occurring elevated concentrations of native peptide. That food intake naturally *increases* when circulating leptin concentrations are high suggests that there is a seasonally induced insensitivity to leptin (Rousseau *et al.*, 2003) in arctic ground squirrels, as in Siberian hamsters (Atcha *et al.*, 2000; Klingenspor *et al.*, 2000), woodchucks (*M. monax*, Concannon, Levac, Rawson, Tennant, & Bensadoun, 2001), and sheep (Marie, Findlay, Thomas, & Adam, 2001).

Leptin: Effects on Food Intake by Sheep

Sheep (*Ovis aries*) have annual cycles of reproduction, body/lipid mass, and food intake and although these seasonal rhythms are modified by the photoperiod, they have an unidentified circannual clock as their underlying basis, similar to ground squirrels and marmots. The effects of leptin on body fat and food intake have been extensively studied in sheep, likely because of their commercial importance (for review see Invartsen & Boisclaire, 2001). The seasonal peak in food intake of female sheep occurs in late-summer/early autumn when animals are reproductively inactive, and they reach a seasonal nadir during the spring when active breeding occurs (Clark, 2001). Exogenous icv leptin infusions inhibit food intake by sheep, but do not affect pituitary hormone secretion such that circulating levels of luteinizing hormone, follicle-stimulating hormone, prolactin, and growth hormone are normal. This suggests that the leptin-induced inhibition of food intake is not due to indirect effects on the output of pituitary hormones. As occurs in laboratory rats (Korner, Savontaus, Chua, Leibel, & Wardlaw, 2001), fasting increases arcuate nucleus NPY gene expression of sheep, an effect that decreases with leptin treatment (Henry *et al.*, 1999). Thus, it is likely that the effects of leptin on food intake are mediated, at least in part, by the central NPY system. The ability of leptin to decrease food intake is apparently coupled to available metabolic fuels as suggested above for its effects on Syrian hamster reproductive status, because food-restricted ewes given icv infusions of leptin do not inhibit their food intake (Henry, Goding, Tilbrook, Dunshea, & Clarke, 2001).

Leptin: Effects on Food Intake by Nonhuman Primates

As with most mammalian species studied to date, leptin decreases food intake in nonhuman primates and this effect is dependent on the route of administration. For example, icv injections of leptin decrease food intake in a dose-dependent manner in male rhesus monkeys (*Macaca mulatta*, Ramsey, Kemnitz, Colman, Cunningham, & Swick, 1998), but energy expenditure is not affected by leptin treatment. By contrast, peripheral (iv) injections of leptin do not affect food intake,

despite 100-fold increases in serum leptin concentrations (Ramsey *et al.*, 1998). Similarly, central administration of leptin increases food intake by ~50% 24 hr after administration, but peripheral injections of leptin (subcutaneous) yielding physiological circulating concentrations of the peptide do not (Tang-Christensen, Havel, Jacobs, Larsen, & Cameron, 1999). The inability of peripherally administered injections to inhibit food intake when given so as to yield physiological concentrations of the peptide in blood, but for central injections to do so, is reminiscent of the effects on nongenetically obese mice discussed above (Harris *et al.*, 1998) and casts doubt on a physiologically important role of the peptide on food intake in nonprimates. It may be, however, that the transport of leptin across the blood–brain barrier under *ad libitum* feeding conditions may limit access of the peptide to its central receptors. There is, however, the typical relation between serum leptin concentrations, WAT leptin gene expression and the level of body fat (Bodkin, Nicolson, Ortmeyer, & Hansen, 1996; Chen, Ono, Yoshida, & Yoshikawa, 2002; Hotta *et al.*, 1996), but this relation may be of more importance under times of decreased lipid fuel stores, rather than when lipid stores are in balance with energy intake or are in surplus as suggested more generally (Ahima *et al.*, 1996).

Leptin: Effects on Food Intake by Birds

Although not widely studied across species of birds, the effects of leptin have been tested in agriculturally important avian species such as domestic chickens (*Gallus domesticus*). In an initial study, mouse leptin (which shares 97% homology with chicken leptin) given icv to male broiler or male Single-Comb White Leghorn chicks did not decrease food intake at doses effective in rodents, suggesting that mouse leptin does not bind to chicken leptin receptors or that the chicken brain does not have leptin receptors (Bungo *et al.*, 1999). This latter possibility has been ruled out because a chicken leptin receptor has been discovered that is expressed in hypothalamus, along with peripheral sites such as the pancreas, and has high homology to the mammalian leptin receptor (Taouis *et al.*, 2001). Indeed, recombinant chicken leptin markedly inhibits food intake in chickens (Denbow *et al.*, 2000; Taouis *et al.*, 2001). This decrease in food intake is dose-related by icv-injected chicken leptin in both broiler and Single-Comb White Leghorn-type chickens and is a behaviorally specific effect in that water intake was not decreased (Denbow *et al.*, 2000). Food intake in chickens can be reduced by systemic leptin treatment, whereas it is not as effective, or not effective, in doing so in other species (*vide infra*). Thus, both intravenous or intraperitoneal injections of chicken leptin, ovine leptin, or a recombinant chicken leptin (C4S) reduce food intake in starved 9-day-old broiler chicks or 5-week-old layer chickens (Dridi *et al.*, 2000).

Collectively, the comparative analysis of the effects of leptin on food intake across a wide range of species discussed above suggests that leptin acts as a satiety factor in virtually all species studied to date. As with the case of most peptide hormones, the ability of leptin to inhibit feeding is largely dose-dependent, supporting the idea that food intake in these species is generally negatively correlated with total body fat, and thus, circulating leptin concentrations. Lastly, the ability to inhibit food intake with relatively small amount of centrally administered leptin supports the idea that leptin acts centrally at specific brain cites to regulate feeding, but the frequent inability of peripherally administered leptin that creates physiologically relevant circulating concentrations of the peptide questions the role of leptin in everyday food consumption.

Neuropeptide Y (NPY)

NPY appears to be a key neuropeptide in the regulation of energy balance and reproduction (for review see Broberger & Hokfelt, 2001; Kalra, Clark, Sahu, Kalra, & Crowley, 1989). NPY is found in both central and peripheral neurons. The population of NPY neurons within the arcuate nucleus of the hypothalamus is clearly sensitive to alterations in energy balance and is involved in the metabolic and ingestive behavioral responses of this peptide (e.g., Cusin *et al.*, 1995; Wang *et al.*, 1997). Central administration of NPY has proven it to be one of the most potent stimulators of food intake (Clark, Kalra, Crowley, & Kalra, 1984; Stanley & Leibowitz, 1984), and it increases food intake in a wide variety of vertebrate species (DiBona, 2002). As with most studies of ingestive behavior, the majority of work on the orexigenic actions of NPY has focused on laboratory rats and mice; however, there is a rapidly increasing number of comparative studies suggesting that the physiological and behavioral actions of NPY are highly conserved across vertebrate taxa (Jensen, 2001).

NPY: Effects on Food Intake by Syrian and Siberian Hamsters

Although hamsters do not increase their food intake after a fast, but are physically capable of doing so (see above), they do increase their food intake after icv NPY injections. Thus, both Syrian (Kulkosky, Glazner, Moore, Low, & Woods, 1988) and Siberian (Boss-Williams & Bartness, 1996) hamsters given icv NPY profoundly increase their food intake comparably to laboratory rats. As for laboratory rats (Clark, Kalra, & Kalra, 1985), NPY inhibits female reproductive behavior in a dose-dependent fashion and increases food intake (Corp, Greco, Powers, Bivens, & Wade, 2001). Specifically, icv NPY decreases lordosis, but increases food intake in ovariectomized, steroid-primed female Syrian hamsters (Corp, McQuade, Krasnicki, & Conze, 2001). Furthermore, the effects of NPY on reproduction and food intake appear to be mediated by separate pathways as determined by using several NPY receptor subtype antagonists in conjunction with exogenous NPY (Corp, McQuade, Krasnicki, & Conze, 2001). Thus, the effects of NPY on feeding by Syrian hamsters appears to be mediated by the NPY Y5 receptor subtype, whereas the effects of this hormone on female reproductive behavior are mediated by the NPY Y2 receptor subtype (Corp, McQuade, Krasnicki, & Conze, 2001).

As is the case for leptin, considerably more research on the effects of NPY on food intake and energy balance has been accomplished using Siberian hamsters and a related species, Djungarian hamsters (*Phodopus cambelli*). As expected, food deprivation increases both NPY and prepro-NPY gene expression in the arcuate nucleus, although these changes were less robust than those reported in laboratory rats (Mercer *et al.*, 1995). Interestingly, despite marked decreases in body and lipid mass (~30–40%) by short-day exposed Djungarian hamsters, neither arcuate NPY content nor gene expression changes there in these animals (Mercer *et al.*, 1995). Generally, similar results have been shown for short-day-housed Siberian hamsters except that the predicted increase in NPY gene expression, based on their body and lipid mass decreases, was modest compared with long-day food-restricted hamsters (Reddy *et al.*, 1999). The naturally occurring elevation of food intake by Siberian hamsters in long versus short days (Wade & Bartness, 1984a) might suggest that food intake would be more readily stimulated by NPY in long days; however, the magnitude of the NPY-stimulated food intake at all doses is similar between long- and short-day-housed hamsters (Boss-Williams & Bartness, 1996) and both groups of animals show comparable levels of NPY gene expression (Reddy *et al.*, 1999).

Furthermore, short-day-housed Siberian hamsters display increases in NPY gene expression, but this increase is modest compared with long-day food-restricted hamsters, based on their respective body and lipid mass decreases (Reddy *et al.*, 1999). Although Siberian hamster arcuate NPY gene expression is not increased after short-day exposure, despite decreases in body and lipid masses as noted above, food deprivation increases hypothalamic NPY gene expression in these animals to the same extent in both photoperiods (Reddy *et al.*, 1999).

Although maintenance in short days does not appear to affect central NPY independently, short-day-housed hamsters are more sensitive to *food deprivation-induced* changes in NPY. Short-day-housed Djungarian hamsters significantly increase arcuate NPY gene expression (~200%) when food deprived for 24 hr (Mercer *et al.*, 1997) Moreover, these results do not appear to be due to changes in gonadal steroid hormones or total body fat *per se*, as neither castrated hamsters nor juvenile long-day hamsters matched for body masses of short-day hamsters displayed significant changes in NPY mRNA. Thus, the response of Siberian hamsters to acute energetic challenges, such as food deprivation, is similar to that of laboratory rats, but the response of Siberian hamsters to naturally occurring, seasonally appropriate fluctuations in body mass is not as tightly correlated with body fat levels as it is for laboratory rats (Mercer *et al.*, 1995).

It has been effectively argued (Mercer, 1998) that that the lack of a change in NPY gene expression by short-day-housed Siberian and Djungarian hamsters makes sense in that their short-day-induced decreases in body and lipid mass *are not* a deviation from their natural state of energy balance, as occurs with food deprivation. Therefore, these animals should not be expected to show similar compensatory changes in hypothalamic neuropeptide gene expression. That these animals are experiencing a "seasonal program" of body and lipid mass changes is borne out by the return to seasonally appropriate body masses after the lifting of food restriction at different points in their short-day decline in these measures (Steinlechner, Heldmaier, & Becker, 1983). How such a seasonal program controls the changes in neuropeptides, food intake, and body fat levels is unknown, but understanding these interactions within this naturally occurring context seems likely to provide insight into a more general understanding of energy balance.

NPY: Effects on Food Intake by Sheep

As with the long photoperiod-breeding hamster species above, short-day breeding sheep markedly increase food intake (~800%) shortly after (30 min) icv administration of NPY, whereas peripheral injections of the peptide have no effect suggesting a central site of action (Miner, Della-Fera, Paterson, & Baile, 1989). A central mode of action also is suggested by the presence of the NPY Y1 receptor subtype mRNA in the arcuate nucleus and PVN of the sheep hypothalamus (Dyer, Simmons, Matteri, & Keisler, 1997a, 1997b), a receptor subtype implicated in the stimulation of feeding by NPY in other species (e.g., Kanatani *et al.*, 1996, 1998; Lopez-Valpuesta, Nyce, Griffin-Biggs, Ice, & Myers, 1996). Furthermore, the seasonal peak in food intake (long "summer-like" days) and its nadir (short "winter-like" days; e.g., Clarke, Rao, Chilliard, Delavaud, & Lincoln, 2003; Gettys, Schanbacher, & Taylor, 1989) may be partially based on increases in the activation of the NPY system in the former given that arcuate NPY gene expression is significantly greater in long versus short days in sheep (Clarke *et al.*, 2003). Therefore, as for all other species tested (cf. Sipols *et al.*, 1996), sheep are responsive to the

orexigenic properties of NPY. Whether they are differentially susceptible to the appetite-promoting effects of NPY in long or short days, as the differential gene expression in the arcuate might suggest (Clarke *et al.*, 2003), remains to be tested.

NPY: Effects on Food Intake by Birds

The variety of birds species tested for feeding responses to neuropeptides is narrow, with work almost exclusively focused on domestic chickens. Icv injections of NPY, or the structurally related peptide PYY, markedly stimulates food intake by broiler chicks within 1 hr post-injection (Kuenzel, Douglass, & Davison, 1987). The dose of NPY that elicits maximal feeding is greater than that of PYY (i.e., 9 vs 5 μg, respectively), suggesting a higher potency for the latter peptide (Kuenzel *et al.*, 1987) Centrally administered NPY does not increase food intake by 2-day-old Leghorn chicks (Steinman, Fujikawa, Wasterlain, Cherkin, & Morley, 1987), an effect not due to the inability of these young animals to increase their food intake because both pancreatic polypeptide and naloxone increase food intake at this age (Steinman *et al.*, 1987). This lack of a NPY-induced stimulation of feeding is likely due to the peptide stimulating convulsions in that those chicks not convulsing increase their food intake (Steinman *et al.*, 1987). As with mammals, the site of action for NPY-induced feeding is probably central in chickens. Although the cell bodies of the NPY producing neurons are not in brain areas traditionally associated with feeding in mammals (i.e., lateral thalamus, hippocampus, caudal linear nucleus, and raphe nucleus of the brainstem; Boswell, Millam, Li, & Dunn, 1998), there is hyperstriatal, archistriatal, and neostriatal regions of the telencephalon that have NPY-immunoreactivity, but not gene expression, in Japanese quail (*Coturnix coturnix japonica*) and domestic chickens (Boswell *et al.*, 1998).

Regarding other avian species, short-day-housed white-crowned sparrows (*Zonotrichia leucophrys gambelii*) given NPY icv increase food intake at the higher doses tested compared with saline-treated birds, whereas long-day-housed (photostimulation for the reproductive system) birds have increases in food intake, but at doses 4–8 times lower than in short days (Richardson *et al.*, 1995) Therefore, increases in sensitivity to the appetite promoting effects of NPY seem to occur during the breeding season, a time when energy investments are high and seasonal body mass and fat peak (Wingfield, Hahn, Wada, & Schoech, 1997). Finally, icv NPY increases food intake by ring doves (*Streptopelia risoria*) at the same low doses as in long-day-housed white-crowned sparrows (Strader & Buntin, 2001).

NPY: Effects on Food Intake by Fish

Goldfish (*Carassiua auratus*), as with the other species discussed above, show increases in brain NPY gene expression after a fast (e.g., 72–75 hr), especially in the telencephalic-preoptic area, hypothalamus, and optic tectum-thalamus regions (Narnaware & Peter, 2001a). The fasting-induced increase in NPY mRNA in these brain areas reverts to non-fasting levels within 1–3 hr of refeeding, suggesting sensitivity to energetics in the central NPY system of this species (Narnaware & Peter, 2001a). Goldfish significantly increase their food intake after icv, but not intraperitoneal (ip) administration of exogenous NPY 2 hr post-injection, an effect completely blocked by a general NPY receptor antagonist (Bodkin *et al.*, 1996; Borer *et al.*, 1985, 1979; Borker & Gogate, 1981; Boss-Williams & Bartness, 1996; Boswell *et al.*, 1998; Boyer *et al.*, 1997; Broberger & Hokfelt, 2001; Browne & Borer,

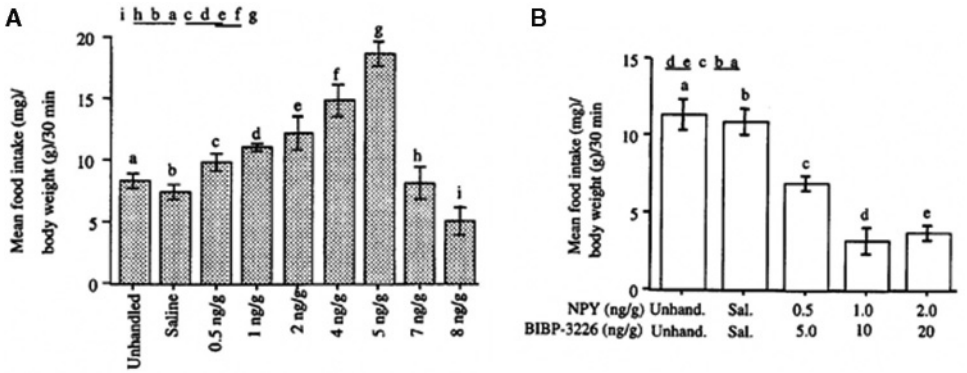

Figure 3. Effects of (A) NPY and (B) NPY + NPY Y1 receptor antagonist (BIBP-3226) on food intake and body weight in goldfish (from Narnaware *et al.*, 2000).

1978; Bungo *et al.*, 1999) suggesting a central site of action and a receptor-mediated effect (Lopez-Patino *et al.*, 1999). Furthermore, NPY-induced feeding is blocked by the NPY Y1-receptor antagonist BIBP-3226 suggesting involvement of this receptor subtype (Narnaware, Peyon, Lin, & Peter, 2000; Figure 3). In support of the role of the NPY Y1-receptor subtype in the NPY-induced increase in food intake by goldfish, icv NPY Y1, but not NPY Y2 receptor agonists, stimulate feeding in goldfish (de Pedro *et al.*, 2000), effects blocked by the general NPY receptor antagonist NPY (de Pedro *et al.*, 2000). Furthermore, as in laboratory rats and mice (e.g., Corp, McQuade, Krasnicki, & Conze, 2001; Iyengar & Simmons, 1999), the NPY Y5 receptor subtype may be involved in food intake by goldfish because a NPY Y5 receptor subtype agonist ([D-32Trp]NPY) increases food intake, but simultaneous stimulation of both receptor subtypes is not additive (Narnaware & Peter, 2001b). Because desensitization of either receptor subtype does not reduce the sensitivity of the other subtype, this suggests that NPY-induced increases in food intake by goldfish may be due to independent stimulation of both the Y1 and Y5 receptor subtypes (Narnaware & Peter, 2001b). The ability to determine the primary NPY receptor subtype responsible for the NPY-induced increases in food intake is not possible at this time for this, or any species, because highly specific receptor subtype agonists or antagonists are not available.

Teleost fish possess NPY in addition to a related neuropeptide, anglerfish peptide YG (aPY), as indicated by their isolation and characterization from brain and the peripheral nerves innervating pancreatic islets of the anglerfish (*Lophius americanus*) (Milgram, Balasubramaniam, Andrews, McDonald, & Noe, 1989; Noe *et al.*, 1989). No attempt has been made, however, to test the stimulatory effects on exogenous NPY on food intake by teleost fish.

NPY: Effects on Food Intake by Other Species

Nonhuman primates have been tested for the stimulatory effects of NPY on food intake yielding mixed results. NPY potently stimulates food intake in rhesus monkeys (Larsen *et al.*, 1999), but unlike other species, NPY does not increase food intake in baboons (Sipols *et al.*, 1996). Lastly, in the only study conducted on a reptilian species, NPY significantly reduced courtship behavior and stimulated food

intake of male red-sided garter snakes (*Thamnophis sirtalis parietallis*). The NPY-induced increase in food intake occurs with a substantial delay compared with other vertebrates (4–5 hr post-injection, Morris & Crews, 1990).

Collectively, the studies discussed above suggest that NPY acts as a potent orexigenic agent, stimulating food intake in virtually all species studied to date. In several cases, central injections of the hormone are required to elicit increased food intake, whereas peripheral injections are generally ineffective. As with leptin, these data suggest that NPY appears to act on central NPY receptors, likely Y1 receptors and possibly other receptor subtypes, to affect food intake. The precise mechanisms by which activation of NPY receptors triggers increased food intake and whether such mechanisms are conserved across taxonomically distinct species, however, require further research.

Cholecystokinin (CCK)

CCK is a polypeptide hormone secreted by the gastrointestinal tract that has been demonstrated to exert marked effects of gastric motility, bile secretions from the gall bladder, and exocrine pancreatic secretion, also is synthesized in the brain and appears to be a potent satiety factor. In terms of the latter, intraperitoneally administered CCK inhibits food intake in laboratory rats and, moreover, elicits a sequence of behaviors virtually indistinguishable from those exhibited after naturally occurring satiety or after infusions of food onto pre-gastric and gastric gut surfaces (Gibbs & Smith, 1982). This inhibitory effect of CCK on food intake also occurs after icv administration (Gibbs, Young, & Smith, 1973). The site of action for these effects of CCK is likely both peripheral and central (for review see Reidelberger, 1994).

CCK: Effects on Food Intake by Syrian and Siberian Hamsters

CCK decreases food intake in both Syrian and Siberian hamsters. For example, icv injections of CCK octapeptide (CCK-8) decrease food intake in a dose-dependent manner in Syrian hamsters (Miceli & Malsbury, 1983). Relatively large peripheral injections of CCK-8 (1.0–4.0 μg/kg) given to Syrian hamsters also decrease food intake, whereas smaller peripheral injections (e.g., 0.5 μg/kg) do not (Miceli & Malsbury, 1983). The site for the central effects of CCK in hamsters is unknown, but potentially could differ from that of laboratory rats in that the distribution of central- and peripheral-type receptor binding sites and peptide immunoreactivity is somewhat different from laboratory rats. Specifically, peripheral-, but not central-type CCK binding sites are found in the magnocellular PVN (Miceli & Steiner, 1989) and CCK-immunoreactivity occurs in the suprachiasmatic nucleus in Syrian and Siberian hamsters compared with laboratory rats that have both central- and peripheral-type binding sites (Miceli, van der, Post, Della-Fera, & Baile, 1987; Reuss, 1991). The receptor binding differences may help explain why CCK-8 injections into the PVN inhibit food intake by laboratory rats (e.g., Blevins *et al.*, 2000), but not by Syrian hamsters (Miceli & Steiner, 1989).

It is important to note that, unlike other peptide hormones (e.g., calcitonin gene-related peptide) that can act as "behavioral bombs" by triggering nonspecific incapacitating or debilitating effects on behavior (e.g., Bartness *et al.*, 1986), the effects of exogenous administration of CCK-8 on behavior appear specific to food intake (Miceli & Malsbury, 1983). In addition, peripheral injections of CCK-8 are as

effective in decreasing food intake during the day and night for female hamsters, but oddly only are effective in doing so during the night for males (Miceli & Malsbury, 1985). Injections of a CCK antagonist, proglumide, does not affect normal food intake or block the effects of CCK-8 administration on food intake by Syrian hamsters (Miceli & Malsbury, 1985). Long-day-housed Siberian hamsters decrease food intake after peripheral injections of CCK-8 administration, but similarly treated Chinese hamsters (*Cricetulus griseus*) do not (Billington *et al.*, 1984). After transfer of long-day-housed Siberian hamsters to short days, the same doses of CCK-8 inhibit food intake to a substantially greater degree than in long days; indeed, several low doses that were ineffective in suppressing food intake in long days readily inhibit food intake in short days suggesting photoperiodic changes in sensitivity to this satiety peptide (Bartness *et al.*, 1986). This enhanced sensitivity to the suppressive effects of CCK on food intake in short days suggests that the naturally occurring decreases in food intake of short-day-housed Siberian hamsters (e.g., Wade & Bartness, 1984a) may be due to an increased role of CCK and perhaps other satiety peptides (Bartness *et al.*, 1986).

CCK: Effects on Food Intake by Sheep

Consistent with the hypothesis that CCK is a natural satiety signal, its serum concentrations are significantly reduced after prolonged fasting of lambs and conversely increased after 20–50 min after suckling (Nowak *et al.*, 1997). It may not be surprising, therefore, that CCK potently inhibits food intake of adult sheep; thus, continuous application of CCK-8 into the lateral ventricles of sheep reduces feeding by ~40% during their feeding period, and food intake returns to normal levels within 24 hr after treatment stops (Della-Fera & Baile, 1980). A CCK receptor antagonist (L364–718) *increases* food intake of sheep (Dynes, Poppi, Barrell, & Sykes, 1998), as it does in laboratory rats (Reidelberger & Rourke, 1989; Reidelberger, Varga, & Solomon, 1991), but only after central, but not peripheral infusions (Dynes *et al.*, 1998).

CCK: Effects on Food Intake by Pigs

As with other species, CCK reduces food intake in domestic pigs (*Sus scrofa*). For example, infusions of CCK-8 into the jugular vein and carotid artery reduce food intake by ~65–70% of control values in pigs (Houpt, 1983). Furthermore, CCK appears to affect motivation to eat in addition to food intake *per se*; thus, pigs trained to perform an operant response to obtain food reduce their responding after iv administered CCK in a dose-dependent manner (Baldwin, Cooper, & Parrott, 1983). The CCK-A agonist A-71378, but not the CCK-B agonist pentagastrin, given iv increases food intake in the operant response model, but neither agonist reliably decreases food intake when administered centrally (Parrott, 1993). This latter finding suggests that stimulation of peripheral CCK A receptors underlie the inhibition of food intake by CCK in pigs and that central or peripheral CCK B receptors are not involved with feeding in this species (Parrott, 1993). Conversely, immunization against CCK via application of CCK antibodies (Pekas, 1991; Pekas & Trout, 1990), or administration of the CCK receptor antagonist MK-329 (Ebenezer, de la, & Baldwin, 1990) expectedly increases food intake in swine. Thus, the primary site of action for the inhibitory effects of CCK on food intake in pigs may be of peripheral origin.

CCK: Effects on Food Intake by Birds

CCK also may act as a satiety peptide in birds. Specifically, iv injections of CCK-8 suppress food intake in broiler and layer chickens (Savory & Gentle, 1983; Savory & Hadgkiss, 1984). This inhibitory effect of CCK on food intake is overcome as the length of the fast preceding CCK injection is increased (Savory & Gentle, 1983). The dual site of action for CCK seen in most other species (but apparently not pigs, see above) also occurs in chickens, although a stronger importance of central sites is suggested. That is, both icv and iv injections of CCK-8 reduce food intake in chickens, but the iv effects are not blocked by iv pretreatment with the peripheral CCK receptor antagonists L-364-718 or L-365-260. Pretreatment with L-364-718 icv, however, blocks the inhibition of food intake by icv CCK-8 (Rodriguez-Sinovas, Fernandez, Manteca, Fernandez, & Gonalons, 1997). In addition, central and peripherally administered CCK antagonists L-365-260 and L-364-718 increase food intake in chickens (Rodriguez-Sinovas *et al.*, 1997).

It is possible that the CCK-induced suppression of food intake is due to malaise, as is always the situation when a treatment decreases food intake. Indeed, conditioned avoidance tests in chickens show that CCK produces a mild aversion (Savory, 1987), perhaps suggesting that CCK is not a naturally occurring satiety signal in chickens. Because two potent stimulators of CCK, soybean trypsin inhibitor and phenylalanine, do not affect food intake by chickens, nor do the CCK-A receptor antagonists, devazepide (Choi, Furuse, Satoh, & Okumura, 1994) or MK-329 (Covasa & Forbes, 1994), the notion that CCK is an illness-inducing agent in this species and not a normal satiety signal seems supported. Alternatively, CCK may act as a natural agent in moderate doses, but cause nausea when administered at higher doses.

CCK dose-dependently decreases food intake when given peripherally to white-crowned sparrows (ip; Richardson, Boswell, Weatherford, Wingfield, & Woods, 1993). This effect was blocked by the CCK-A receptor antagonist L 365-260, suggesting that, as with mammalian species, the effects of CCK on food intake act via this receptor subtype in birds (Richardson *et al.*, 1993).

CCK: Effects on Food Intake by Fish

CCK appears to be an important satiety factor in goldfish. For example, goldfish brain contains CCK/gastrin-like immunoreactive neurons within the ventral telencephalon and diencephalons including the preoptic hypothalamus, as well as nerve fibers and endocrine cells in the gut (Himick & Peter, 1994). Moreover, centrally (3rd ventricle) or peripherally (ip) administered CCK-8 inhibits food intake by goldfish (Himick & Peter, 1994).

Food Intake Responses to High Fat Diets (HFDs)

An important experimental model used to discover the mechanisms underlying the role of palatability on food intake, as well as to induce obesity, is the feeding of HFDs (Mickelson, Takahashi, & Craig, 1955). This model has been used successfully for the past ~50 years in traditionally studied animal species such as laboratory rats and mice and has contributed enormously to our understanding of energy balance (for review see West & York, 1998). For example, laboratory rats

(Faust, Johnson, Stern, & Hirsch, 1978; Masek & Fabry, 1959; Mickelson *et al.*, 1955) and mice (Lemonnier, 1972; Lemonnier, Suquet, Aubert, de Gasquet, & Pequignot, 1975; West, Boozer, Moody, & Atkinson, 1995) maintained on a HFD undergo marked increases in total body fat. This diet-induced obesity is due, in part, to increased caloric intake (for review see Kanarek & Hirsch, 1977) and to decreased energy expenditure (e.g., Storlien, James, Burleigh, Chisholm, & Kraegn, 1986; cf., Schwartz, Young, & Landsberg, 1983). As with most other areas of research, however, much less is known about the effects of HFDs on food intake and energy balance in nontraditional animal species. The factors underlying resistance to diet-induced obesity may be revealed by studying inbred laboratory rats selected for susceptibility or resistance to diet-induced obesity (Levin, Dunn-Meynell, Balkan, & Keesey, 1997) or mouse strains that are differentially susceptible/resistant to HFD-induced obesity (West, Waguespack, & McCollister, 1995). As discussed below, however, several species show a natural resistance to HFD-induced obesity, whereas other species are naturally susceptible to HFD-induced obesity. These responses to HFDs are, by definition, shaped by evolutionarily forces rather than artificially selected. The examples of natural resistance to HFD-induced obesity in particular share several striking physiological characteristics that may offer opportunities to uncover evolutionarily based factors altering the consumption and/or obesity-producing effects of HFDs.

HFDs: Effect on Food Intake by Hamsters, Gerbils, and Jirds

Unlike laboratory rats and mice that maintain relatively constant levels of food intake and adiposity on an annual basis, many non-tropical rodent species undergo marked seasonal cycles of body and lipid mass that are regulated by environmental cues, predominantly the photoperiod (for review see Bartness & Wade, 1985). Interestingly, some of these species, such as Siberian hamsters (McElroy, Mason, Hamilton, & Wade, 1986; Wade & Bartness, 1983) and meadow voles (*Microtus pennsylvanicus*; J. Dark and I. Zucker, unpublished observations), do not increase body fat levels when fed HFDs. Specifically, long photoperiod-exposed Siberian hamsters fed a HFD (i.e., 2:1 ratio of chow:shortening) either do not increase caloric intake (Wade & Bartness, 1983) or slightly overeat this diet (~20%; McElroy *et al.*, 1986). In both cases, body and lipid mass do not increase, nor does HFD feeding block the naturally occurring short-day-induced decreases in body fat (Wade & Bartness, 1983). The resistance to HFD-induced obesity in the study where overeating occurred (McElroy *et al.*, 1986) was associated with an increase in sympathetic drive to brown adipose tissue (i.e., increased norepinephrine turnover) and guanosine diphosphate binding to mitochondria (i.e., a measure of thermogenic activity Rafael & Heldt, 1976) that would tend to mitigate the effects of increased caloric intake. Thus, these animals have a naturally occurring resistance to HFD-induced obesity either due to a lack of HFD-induced hyperphagia or, if the hyperphagia exists, a corresponding increase in energy expenditure.

By contrast to Siberian hamsters, Syrian hamsters also undergo seasonal cycles in body and lipid mass, but the response is opposite to that of Siberian hamsters (i.e., body fat increases in short compared with long days ([Bartness & Wade, 1984; Hoffman, Davidson, & Steinberg, 1982; Wade & Bartness, 1984b]). Syrian hamsters fed HFDs become impressively obese, but do so largely without overeating (Bartness & Wade, 1984; Wade, 1982, 1983) unlike many strains of laboratory rats and mice (e.g., Schemmel & Mickelsen, 1970; West *et al.*, 1995). The HFD-induced

obesity seen in long-day-housed Syrian hamsters is exaggerated by short-day exposure (Bartness & Wade, 1984; Wade, 1983; Wade & Bartness, 1984b) to the extent that these hamsters are unable to right themselves when placed on their backs. This obesity without overeating is accompanied by decreased sympathetic drive on brown fat (i.e., normal norepinephrine turnover), but surprisingly an increase in mitochondrial cytochrome C oxidase activity and guanosine diphosphate binding showing a dissociation of BAT thermogenesis from sympathetic activity (Hamilton, Mason, McElroy, & Wade, 1986).

Why would one hamster species, Siberian hamsters, be resistant to HFD-induced obesity and perhaps somewhat to HFD-induced overeating, whereas another hamster species, Syrian hamsters, be highly susceptible to HFD-induced obesity, but also, perhaps not HFD-induced overeating? In other words, do these two different, species-specific responses confer an adaptive advantage based on these hamsters' seasonal body fat responses? Siberian hamsters (e.g., Wade & Bartness, 1984a), similar to meadow voles (e.g., Dark, Zucker, & Wade, 1983), exhibit a fall (short-day) energetic strategy of decreasing their body mass. Although the more typical fall energetic strategy is to increase lipid deposition for later use when food is scarce in the winter (e.g., Syrian hamsters; Bartness & Wade, 1984; Hoffman *et al.*, 1982; Wade, 1983), it has been argued that the short-day-reduced body mass carries with it a reduction in maintenance energy requirements (e.g., Weiner, 1987). Thus, it is disadvantageous for species that exhibit this adaptive body fat nadir in fall/winter to fatten by eating a HFD in short "winter-like" days, or even in long days given that any lipid excesses here would need to be reduced in the fall (El-Bakry, Plunkett, & Bartness, 1999). Hence, it would be predicted that other species that exhibit short-day-induced body fat decreases also should be relatively resistant to HFD-induced obesity and perhaps HFD-induced increases in food intake. Consistent with this, Shaw's jird (*M. shawi*) is a desert rodent that decreases its body mass (fat) when exposed to short days (El-Bakry *et al.*, 1999) and, as predicted, HFD feeding has no effect on body mass, food intake, carcass lipid content, or WAT pad mass in long- or short-day-housed jirds compared with their standard lab chow-fed counterparts ([El-Bakry *et al.*, 1999]) Figure 4). Similar effects are seen in a closely related species, Mongolian gerbils (*M. unguiculatus*), that decrease their caloric intake in response to HFD-induced increase in caloric density (Kanarek, Ogilby, & Mayer, 1977) as well as in the bank vole (*Clethrionomys glareolus*) (Peacock & Speakman, 2001). Collectively, the mechanisms underlying resistance or susceptibility to HFD-induced obesity in species that exhibit seasonal body fat cycles are unknown. Nevertheless, it may be that the study of those species that are naturally resistant or naturally susceptible HFD-induced obesity and/or HFD-induced 1overeating, and that are studied in the context of their adaptations to their environments, may add new insights into the advantages and disadvantages of becoming obese when fed a HFD. For example, perhaps in these resistant species, HFD are not as reinforcing (i.e., palatable) as in species that do overeat; such differences may reflect differences in sensory processing of lipid-rich food. In terms of resistance to HFD-induced obesity, it may be that in these species, HFD-induced stimulation of thermogenesis in BAT mitigates the obesity-promoting effects of a HFD; indeed, as mentioned above, HFD-fed Siberian hamsters display exaggerated stimulation of BAT thermogenesis, as suggested by increased GDP binding, compared with other species (McElroy *et al.*, 1986). Although these possibilities are intriguing, they remain to be tested.

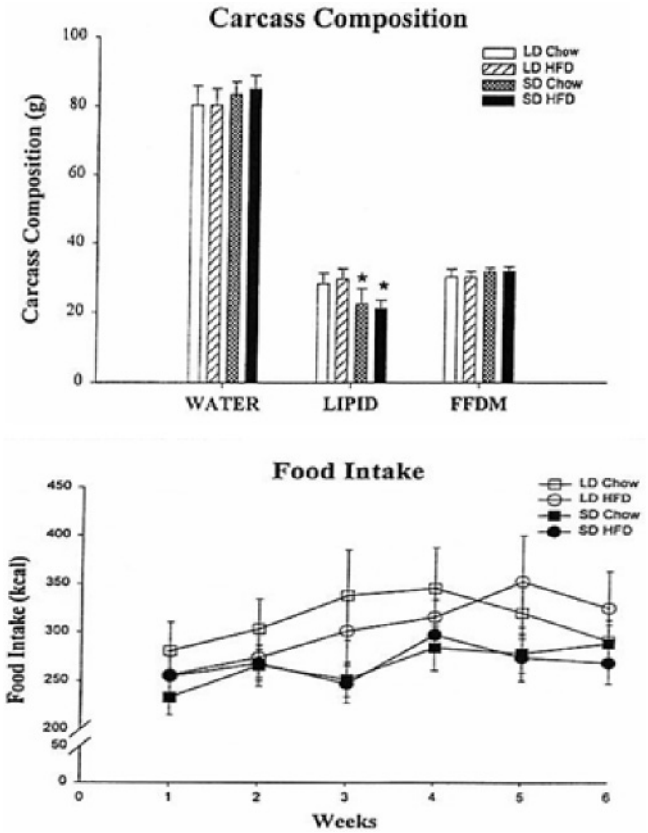

Figure 4. Lack of an effect of high-fat diet (HFD) on food intake and carcass lipid by Shaw's jird (from El-Bakry *et al.*, 1999). FFDM = fat-free dry mass; LD = long day; SD = short day (from El-Bakry *et al.*, 1999).

Appetitive Ingestive Behaviors

The role of foraging and food hoarding in the ingestive behavioral repertoire of several species, especially hamsters and laboratory rats, has been recently reviewed (Bartness & Day, 2003) and will only be summarized here. The animal behaviorist, Wallace Craig, coined the terms "appetitive" and "consummatory" for the two-part sequence of eating, drinking, and sexual behaviors in 1918 (Craig, 1918). He defined appetitive behaviors as those involved in seeking the goal object (food, water, a mate) and noted that these are flexible and non-stereotyped behaviors drawing animals into physical contact with these goals (Craig, 1918). In terms of food intake, these appetitive behaviors form the initial steps in the ingestive behavior sequence (Craig, 1918), emphasizing that animals must find food before they can eat it. He defined the second part of this sequence as consummatory behaviors (from *consummate* not *consume*), defining them as the final act once the goal object has been contacted and noting that they are reflexive and stereotyped behaviors (Craig, 1918); for food intake, this is the act of eating. The consummatory phase of ingestive behavior has received the most extensive study and several chapters in

this *Handbook* discuss various aspects of it. Foraging and food hoarding, two quintessential appetitive behaviors (for review see Bartness & Day, 2003; Vander Wall, 1990), however, are instructive for comparative analyses. Foraging has been studied extensively at the behavioral rather than the physiological level of analysis, especially in birds and is not reviewed here (for review see Stephens & Krebs, 1986).

Much of the research on the physiological mechanisms underlying food hoarding has been accomplished using laboratory rats that are not natural hoarders. Even their wild counterparts (*Rattus norvegicus*) are not thought to hoard food significantly because no food has been found in their burrows except for occasional observations of food buried near the burrow of lactating rats (Calhoun, 1962; Lore & Flannelly, 1978; Pisano & Storer, 1948; Takahhashi & Lore, 1980; Whishaw & Whishaw, 1996). With that caveat, several points stand out. First, there are species that use hoarding as an integral part of their behavioral energetic repertoire (usually animals possessing specialized anatomical structures to aid in transporting food, such as pouches [Vander Wall, 1990] [e.g., hamster and squirrel species]), where food hoarding serves essentially the same function as does the consequence of food intake—energy storage. Second, some of the peptidergic controls of ingestive behavior revealed through feeding tests with laboratory rats and mice in their home cages (unlimited food, no foraging) may be more directly involved in food acquisition (foraging) and storage (hoarding) than with feeding. Both of these have been assessed in studies of Siberian hamster foraging/hoarding. The ability to separate the appetitive from consummatory ingestive responses in these animals rests on the non-covariance of food intake and food hoarding in Siberian hamsters. With few exceptions, these animals neither overeat *and* "overhoard," nor undereat *and* "underhoard," thereby inferring some degree of separate underlying mechanisms controlling food intake and hoarding. In addition, changes in foraging (pellets earned in a running wheel-based delivery system) do not always covary with changes in food hoarding (Day & Bartness, 2001); thus, it can also be inferred that these two appetitive ingestive behaviors have at least partially separate underlying mechanisms.

As discussed above, hamsters do not overeat after a fast, but they do overhoard and, depending upon the foraging effort, foraging can increase. Specifically, after a 32-hr fast, food hoarding increases and then decreases as the foraging effort increases, whereas foraging (pellets earned) increases, but only at low foraging efforts. Two approaches have been used to get at the underlying mechanisms. Fasting produces numerous peripheral metabolic changes, and any of these or their combinations might underlie the increased hoarding and foraging. In one report, substances that alter metabolic fuel utilization (i.e., 2DG, MP, and their combination) or storage (i.e., insulin) did not affect hoarding (Bartness & Clein, 1994). These manipulations were acute, with injections occurring just before darkness leaving open the possibility that more chronic metabolic challenges are necessary to trigger increases in food hoarding or foraging such as those that are effective in blocking estrous cycles and reproductive behavior in Syrian hamsters (Schneider, Friedenson, Hall, & Wade, 1993; Schneider & Wade, 1989; Wade *et al.*, 1991).

Alternatively, decreases in lipid fuels *per se* could stimulate food hoarding/foraging. Several studies suggested that whenever body fat is decreased in hamsters, such as after fasts or during pregnancy/lactation, food hoarding increases (Bartness & Clein, 1994; Day *et al.*, 1999; Wood & Bartness, 1996, 1997). This apparent inverse relation between body fat and food hoarding, which has also been hypothesized for laboratory rats (Cabanac & Gosselin, 1996), was explicitly

examined utilizing the lipectomy model for testing body fat regulation (for review see Mauer, Harris, & Bartness, 2001). Removal of both inguinal and epidymal WAT pads triggers increases in food hoarding that are reversed as the remaining unexcised fat pads compensated for the surgical-induced lipid deficit by increasing their lipid stores until no body fat deficit is apparent (Wood & Bartness, 1997). These results contrast with a report of a lack of increased food hoarding in lipectomized rats (Michel & Cabanac, 1999), but interpretation of those findings is complicated because the method used to test food hoarding in the severely lipectomized rats required sequential food restriction to produce a within-animal determination of body mass versus food hoarding (Michel & Cabanac, 1999). Therefore, rats bearing this large lipid deficit were further energetically stressed by repeated restricted feedings. Subsequent studies using the foraging/hoarding system suggest that decreases in gonadal fat (e.g., parametrial fat pads) may be more important for the initial stimulation of food hoarding than overall decreases of body fat (Day & Bartness, 2001). That is, as the foraging effort is increased, parametrial fat mass, but not the mass of other fat pads or total carcass lipid, is decreased and food hoarding is increased (Day & Bartness, 2001). Although not completely established at this time, it seems that normal functioning of the gonads is somewhat dependent on ample lipid fuel stores in the gonadal fat pads (e.g., Srinivasan, Thombre, Lakshmanan, & Chakrabarty, 1986), and it is not surprising that behavioral responses to acquire and store more energy are triggered by decreases in the lipid content of these fat depots. How the brain senses these or other decreases in lipid energy stores is not known, but at least two possible mechanisms exist. First, as stated above, leptin can reflect total lipid stores and this might be one means of conveying that information to the brain; but how decreases in the lipid content of specific fat depots would be communicated by such a general circulating signal does not seem possible. The fasting-induced increases in food hoarding are attenuated by chronically administered leptin given peripherally to Syrian hamsters (Schneider & Buckley, 2003), but similarly administered leptin to Siberian hamsters is without such an effect (C. Rooks, D. Day, and T. Bartness, unpublished observations). Therefore, the role of leptin in appetitive ingestive behaviors is largely unexplored and unclear at present.

Alternatively, changes in lipid content of body fat in general, or of specific fat depots, may be signaled via sensory nerves innervating white fat and transmitting the information to the brain. This possibility is supported by the presence of 'sensory neurotransmitters' (Hill, Ralevic, Crowe, & Burnstock, 1996) in white fat, such as substance P (Fredholm, 1985), and by the labeling of dorsal root ganglia neurons after application of an anterograde tract tracer (true blue) to white fat (Fishman & Dark, 1987) in laboratory rats and FluoroGold in Siberian hamsters (Song, Warren, Youngstrom, & Bartness, 1996). It is not clear what is being sensed. Possibilities range from mechanoception of fat pad expansion and contraction (unlikely) to monitoring of lipolytic rate via chemoception (more likely). Regardless of the exact mechanism, it seems that decreases in body fat either generally or within critical lipid depots (gonadal fat; Day & Bartness, 2001) trigger food hoarding, as do lipid deficits generated surgically (Wood & Bartness, 1997), at least in Siberian hamsters. How and where such sensory information arising in peripheral lipid stores is reflected in neurochemical changes in the CNS is not known, but one possibility would be through changes in neuropeptide systems shown to reflect alterations in energy balance in laboratory rats and mice. For example, fasting induces increases in NPY and AgRP mRNA levels in the arcuate nucleus (i.e., Ebihara *et al.*, 1999; Sanacora, Kershaw, Finkelstein, & White, 1990; Schwartz, Sipols, Grubin, & Baskin,

1993) and also in the arcuate nucleus of Siberian hamsters, the latter showing no changes in orexin, or decreases in proopiomelanocortin gene expression (Mercer, Moar, Ross, Hoggard, & Morgan, 2000; Reddy *et al.*, 1999). Of course changes in gene expression do not necessarily reflect changes in neuropeptide release in the terminal fields, but they are often consistent with such changes (e.g., NPY release in the PVN with fasting; Beck *et al.*, 1990; Jain, Dube, Kalra, & Kalra, 1998; Sahu *et al.*, 1988). In addition, icv- or PVN-injections of NPY causes impressive increases in food intake (Clark *et al.*, 1984; Levine & Morley, 1984; Stanley & Leibowitz, 1984) in rats and other species (Larsen *et al.*, 1999; Moris & Crews, 1990) including Siberian hamsters (Boss-Williams & Bartness, 1996). NPY injected into the PVN of rats also stimulates behaviors suggestive of foraging (Harland, Bennett, & Gardiner, 1988; VanNess, DeMaria, & Overton, 1999). Finally, the failure of icv NPY to stimulate food intake in laboratory rats fed passively via intraoral catheters (Seeley, Payne, & Woods, 1995) has been effectively argued to indicate a role for this traditionally recognized "consummatory neuropeptide" as an "appetitive neuropeptide" (Woods *et al.*, 1998). In Siberian hamsters, 3rd-ventricular injections of NPY stimulate food intake (~100–500% increases), but food hoarding, is increased even more (up to ~1,100%; D. Day and T. Bartness, unpublished observations). The ingestive behavior responses of Siberian hamsters housed in the foraging/hoarding system are even more selective for AgRP. As noted above, AgRP is an intense stimulator of food intake in rats and mice, but in Siberian hamsters 3rd-ventricular AgRP either does not affect or decreases food intake, but it impressively stimulates food hoarding (Day & Bartness, 2004). Thus, many neuropeptides, rather than simply triggering or curtailing food intake *per se*, are likely involved in more subtle, complex regulation of food-related behaviors. Clearly, further studies with these neuropeptide stimulators of ingestive behavior are required for a deeper understanding of their role in responding to changes in peripheral lipid energy stores and in triggering consequent appetitive ingestive behaviors.

Conclusions

The examples given above across a wide range of species indicate that "experiments of nature" afford scientists of ingestive behavior opportunities to obtain a different, and frequently telling insight into the diversity of this behavior. We selected examples of species that naturally exhibit features of ingestive behavior that are often studied in the laboratory through significant and often highly invasive physiological manipulations performed on more traditional species (rats and mice) or via genetic engineering or selective breeding of these species. We hope that the reader of this trek through the control of food intake across diverse regions of the animal kingdom might more fully appreciate the wide range of feeding strategies and physiological responses of these nontraditionally studied species. Although it is often the view that the ingestive behavior responses of laboratory rats and mice are the "gold standard" by which all others should be compared, it could be just as easily argued that these domesticated and inbred animals are the oddities because of the lack of evolutionary pressures shaping their ingestive and other behaviors/physiology during the last ~100 years of captive breeding. As always, perspective is in the eye of the beholder.

In summary, several points can be drawn from the food intake responses of the species reviewed here in terms of the use of these various species for research on ingestive behaviors: (1) there are naturally occurring fasts of prolonged duration or

more modest reductions in food intake that could offer insight into satiety mechanisms, (2) glucoprivation and lipoprivation are not uniformly stimulators of food intake, (3) there are naturally occurring instances of resistance and susceptibility to HFD-induced obesity that seem to make sense given the animals' behavioral ecology, and (4) perhaps a reason for the ever-expanding list of peptides that stimulate or inhibit food intake is that some may function to draw animals to or away from food (appetitive) (i.e., by affecting foraging and other food-seeking behaviors), rather than affecting ingestive (consummatory) behavior directly. Thus, by studying the differences in physiological responses to the same experimental treatment across a wide range of species, we are able to gain valuable perspective on the organization of energy balance systems, the relative importance of specific sensory input signals, as well as other environmental factors that may modify food ingestion in real-world situations.

Acknowledgment

This work was supported, in part, by NIH Research Grant R0-1 DK-35254 to TJB.

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The Control of Eating Behavior in Free-Living Humans

John M. de Castro

Even though eating behavior has been intensively studied over the last century, an understanding of how it is regulated has been elusive. The research has not produced an effective therapeutic intervention for being over- and underweight. In fact, the problem of overweight and obesity, rather than being resolved, has increased substantially (Flegal, 1999; Flegal *et al.*, 2002; Mokdad *et al.*, 1999, 2003; Ogden, Flegal, Carroll, & Johnson, 2002). The intent of the present review is to present what has been learned about the factors that affect intake in humans. An attempt is made to understand whether intake is regulated, and if it is, to what extent, and what controls function to produce this regulation. Finally, a new general model of intake regulation will be presented that attempts to integrate the influences of multiple physiological systems with the influences of environmental, psychological, and socio/cultural variables. The ability of the model to account for the phenomena of intake regulation is assessed.

The review focuses on the intake of normal free-living humans with no constraints on their food consumption. This is an important condition. It is a thesis of this review that the difficulty in developing an understanding of the nature of intake controls in humans is, at least in part, due to the fact that there has been a relative paucity of research performed in the natural environment. In general, it appears that laboratory research produces results that are valid for the lab, but miss essential variables that are critical for the control of intake in the real world. This approach results in an overestimation of the importance of some variables, an underestimation of the importance of others, and a failure to recognize other salient influences. Most chapters in this volume will focus on the extraordinary

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Neurobiology of Food and Fluid Intake, 2nd Ed., Volume 14 of *Handbook of Behavioral Neurobiology*, edited by Edward Stricker and Stephen Woods, Kluwer Academic / Plenum Publishers, New York, 2004.

advances in the understanding of intake regulation that has been obtained from laboratory studies. Consequently the current review emphasizes the understandings that have been obtained in the real world of humans.

The reliable and valid measurement of intake and other variables of interest in real-world settings present a new set of difficulties and challenges. In fact, this difficulty has likely been the primary deterrent to research in natural environments. However, considerable evidence suggests that these measurement difficulties are overestimated. In fact, it appears to be fairly easy to measure eating and other variables of interest in humans in a relatively reliable and valid way during the everyday course of life. While the methods have imperfections, the errors that are produced can be understood and factored into the reasoning process, and meaningful conclusions can be made from the data.

The Diet Diary Method For Measuring Real-World Intake

Although a number of methods can be used to estimate the nutrient intakes of humans (see Thompson & Byers, 1994, for a review), they are primarily designed to measure the overall levels of intake. Unfortunately, these measures are of limited utility for identification of the factors that influence or control intake since they do not measure the detailed pattern of eating behavior. On the other hand, the detailed eating behavior by humans in their natural environment along with contextual and psychological variables can be measured with a 7-day diet-diary technique (de Castro, 1994a; 1999a). People are simply required to make detailed records of their intake in a pocket-sized diary for 7 consecutive days. In addition, they are asked to record their feelings and the nature of the environmental context. This is a tedious and somewhat onerous process. We have found that the 7-day period is about the maximum acceptable duration for maintaining the subjects' motivation and tolerance of diary recording and still produce regular, stable, and interpretable data.

To help ensure that reliable and valid records will be kept, the participants are offered a contingent reinforcement, a detailed nutritional composition analysis of the reported diets. The subjects usually express great interest in this report and have participated willingly, interest being the only incentive for participation (de Castro & Kreitzman, 1985; de Castro, McCormick, Pedersen, & Kreitzman, 1986). The participants are informed that record keeping often produces a change in nutrient intake and they should eat normally as their dietary nutrient composition report will be of use to them only if it accurately reflects their normal intake. As another motivator and validity check, two individuals who ate with the subject during the recording period are contacted and asked to verify the subject's diary entries. The subjects are aware that this check will be performed. No disconfirmations have been found in over a thousand such validity checks. About 45% of the meals are fully validated without prompting, 50% are verified with prompting, while 5% cannot be recalled.

Recently, a new element has been added (de Castro, 1999a). Subjects are given cameras and asked to take a picture of the foods they are eating prior to ingesting them. The diaries are then coded, as usual, without the benefit of the pictures and then independently coded using the photographs to aid in the coding. Inclusion of the pictures increases the estimates of the amounts of the macronutrients

ingested in the meals by 6% to 9%. Hence, this procedure helps overcome a potential problem of underreporting. In addition, it is possible that the fact of taking the pictures increases the accuracy of the written records since the subjects know that the pictures will be seen along with their entries. In addition, when the pictures were included in the coding, the magnitudes of the relationships between a number of variables that are associated with meal size and meal frequency did not differ. Since most of the diet-diary reports summarized in this chapter were recorded without cameras, the equivalence of these meal pattern relationships is particularly important and indicates that these relationships are unaffected by underreporting.

There is reasonable agreement between diet-diary records repeated after as long as 2 years (Adleson, 1960; Block, 1982; Heady, 1961; Livingstone *et al.*, 1990; St. Jeor, Guthrie, & Jones, 1983). Hence, the 7-day diet-diary procedure has good reliability. A more difficult issue is the determination of the veracity of self-reported intake records. To assess this issue, participants were asked to keep a 7-day diary in two empirical tests (Gersovitz, Madden, & Smicikalas-Wright, 1978; Krantzler *et al.*, 1982). Unknown to the participants, the experimenters surreptitiously measured the actual amount of food consumed at lunch. In both studies, 80% to 90% agreement was found between the intake that was recorded in the diaries and the actual amounts eaten. Hence, the diet-diary reports of intake appear to have an acceptable level of reliability and validity.

That said, there is considerable evidence that self-reported intakes are about 20% below the energy requirements of weight-stable, normally active individuals (Bandini, Schoeller, Cyr, & Dietz, 1990; Goran & Poehlman, 1992; Lissner *et al.*, 1989; Livingstone *et a1.*, 1990, 1992; Mertz *et al.*, 1991; Prentice *et al.*, 1986; Seale & Rumpler, 1997). This difference appears to be due to a combination of underreporting and reactivity to the measurement procedure producing a decrease in intake during the recording period (Goris & Westerterp, 1999; Goris, Westerterp-Plantenga, & Westerterp, 2000). To assess the degree to which the reported intakes are representative of the participants' typical daily intakes, reported intakes were compared to an approximated basal metabolic rate (BMR_{est}) that was estimated from the participant's weight considering age and gender (Schofield, Schofield, & James, 1985). The ratio ($EI : BMR_{est}$) of the reported daily food intake (EI) to the BMR_{est} was calculated for each participant. A reasonable cutoff for identifying unrepresentative intake is $EI : BMR_{est} < 1.1$ (Black *et al.*, 1991; Goldberg *et al.*, 1991). This cutoff includes participants whose intake is at least 10% above their estimated BMR. For the entire sample, the mean $EI : BMR_{est}$ was 1.28 (0.013). This value is about 18% below the expected level of 1.55 for the average intake of similar subjects (Black *et al.*, 1991) and is similar to the levels of underestimation produced with the doubly labeled water technique (Bandini *et al.*, 1990; Goran & Poehlman, 1992; Livingstone *et al.*, 1990; 1992; Prentice *et al.*, 1986).

To investigate the impact of underestimation on the outcomes of diet-diary studies, an analysis was performed comparing the intakes from participants who were above and below the $EI : BMR_{est} = 1.1$ cutoff (Black *et al.*, 1991; Goldberg *et al.*, 1991). The participants who reported intakes that were above the cutoff had average meal sizes that were 6.6% larger than the participants who were below the cutoff. However, the relationships between meal size and other significant variables did not differ between groups. The correlations between each of six different variables that are known to affect meal size and the size of the meals ingested were calculated for both the low and acceptable reporting groups (Figure 1). The correlations between meal size and the number of people present, the time of day of the

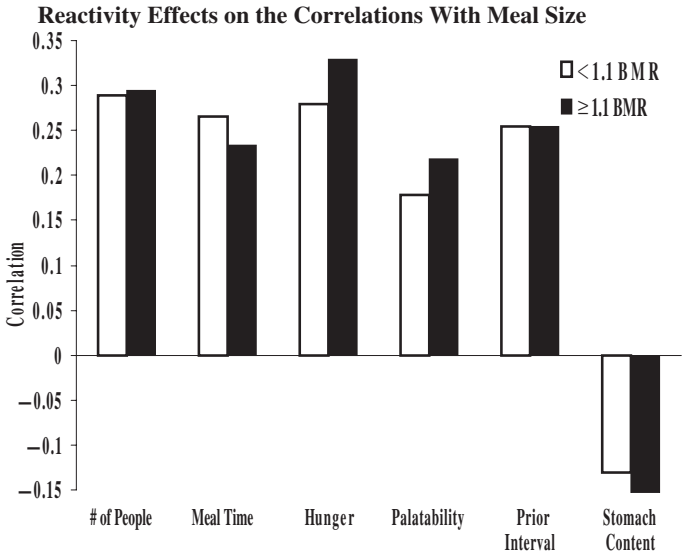

Figure 1. The mean correlations between the amounts ingested in the meals (Meal Size) and the number of other people present at the meal, the minute of the day of meal initiation, the self-ratings of before-meal hunger, the self-ratings of meal palatability, the amount of time since the last meal, and the estimated before-meal stomach content of food energy, for subjects whose intake was less (white bar) and greater (black bar) than 10% above their estimated basal metabolic rate.

meal, self-reported hunger, self-rated palatability, the duration of the interval prior to the meal, and the estimated content of the stomach at the beginning of the meal did not differ between groups. Hence, the relationships between meal size and the factors that affect eating are not changed by low reporting. This indicates that the diet-diary procedure is reliable and valid, and that measurement problems do not affect the validity of the conclusions that can be reached about the factors that control intake.

Intake In The Real World versus The Laboratory

Comparisons of the intakes of free-living normal humans with those obtained in laboratory studies have revealed significant shortcomings with laboratory research. In particular, constraints on eating often are removed or missing in the lab, facilitatory influences often are controlled or eliminated, the importance of variables can be overestimated, and important influences can be missed because of the short duration of laboratory studies. Each of these issues is considered in turn.

Real-World Constraints on Eating Are Often Removed or Missing in the Lab

There are circumstances in the real world that constrain the amount and timing of intake that often are absent in the laboratory setting. A major factor that profoundly influences real-world intake is the cost of food. The fact that the food is free in laboratory studies can lead subjects, particularly college students, to marked over-ingestion or gorging. As an example, a typical naturalistic lunch averages

2.4 MJ (de Castro, Brewer, Elmore, & Orozco, 1990). Yet in laboratory studies intakes often are seen at more than double these levels, from 4.6 to 7.3 MJ (de Graaf & Hulshof, 1996; Rolls *et al.*, 1991, 1998; Shide Caballero, Reidelberger, & Rolls, 1995). In fact, the cost of food, or the lack thereof, may be the most potent variable present in these studies.

In the real world, constraints on the timing of meals subtly alter the pattern of intake. In the lab, rats and humans delay their next meal by an amount proportional to the amount eaten in the preceding meal (Bernstein, Zimmerman, Czeisler, & Weitzmen, 1981; de Castro, 1975; LeMagnen & Tallon, 1966, 1968). In contrast, humans in the real world adjust meal size based upon the period of time since they last ate (Figure 1, prior interval; de Castro & Kreitzman, 1985; de Castro *et al.*, 1986). The key difference is that in the natural environment the timing of meals is constrained by external schedules dictated by work and social commitments. The individual often is not free to adjust when to eat. So, regulation occurs by adjusting how much is eaten. Indeed, when meal-timing constraints were imposed on rats in the laboratory, the real-world humanlike pattern occurred, such that the interval prior to the meal predicted the meal size (de Castro, 1986, 1988b).

In French culture, dining is a relatively important event, and work and socializing often are scheduled around eating. In a sense, eating is less constrained by outside schedules in France. This cultural difference is reflected in a difference in meal patterns, with meal size in France significantly related to the duration of the following interval (de Castro, Bellisle, Feunekes, Dalix, & De Graaf, 1997). Collectively, these results suggest that the control system is quite flexible and can adjust intake depending upon the environmental and social context, a result not seen in the laboratory.

Real-World Facilitatory Influences on Eating Often Are Controlled or Eliminated in the Lab

Many important variables that affect intake are controlled or eliminated in laboratory studies. For example, studies usually are scheduled around the convenience of the experimenter and the participants. As such, intake usually is studied during the daytime on weekdays and around lunch time. Unfortunately, this strategy misses important diurnal or circadian influences on intake since humans eat a substantial fraction of their total intake late in the day (de Castro, 1987a). In addition, humans eat differently on the weekends than they do during the week, eating 8% more food and being more responsive to social influences (de Castro, 1991b). A highly significant facilitatory influence on intake that usually is controlled or eliminated in the laboratory is the effect of the presence of other people. Normally, in laboratory research, eating is studied with the individual isolated. In the real world, however, meals eaten with other people are 44% larger than meals eaten alone (Figure 1, # of people; de Castro & de Castro, 1989). Controlling social effects in the lab eliminates this salient influence on intake from being observed or documented.

The Importance of Certain Variables Can Be Overestimated in the Laboratory. Frequently, real-world research projects are criticized because the variables studied account for only a small proportion of the variance. However, this feature really is a strength of the method because it reveals the actual importance of the variable. In contrast, laboratory research designs control or eliminate most

of the sources of variation in the dependent variable, reducing the total variance. As a result the independent variables can appear to account for large proportions of the variance. This difference leads to an overestimation of the importance of variables studied in the laboratory and an underappreciation of the salience of variables observed in real-world contexts.

Laboratory research has resulted in the conclusion that fluid intake is controlled by various systemic variables including plasma osmolality (Fitzsimons, 1961a; Gilman, 1937) and blood volume (Fitzsimons, 1961b; Stricker, 1968). The fluid intake of people in the real world, however, occurs primarily in association with eating, and the amount and timing of fluid ingestion are primarily determined by eating (de Castro, 1988a). In addition, fluid intake and thirst are unrelated to the osmotic characteristics of ingested nutrients (de Castro, 1991a). Phillips, Rolls, Ledingham, & Morton (1984) observed water intake by healtly men during their working hours along with the contents of the blood and urine. No changes were observed in body fluid variables associated with spontaneous fluid intake. Hence, there is evidence that in contrast to laboratory studies, osmotic and volumetric stimuli are relatively unimportant for everyday real-world intake.

Another variable whose importance may be overestimated in laboratory studies is the hedonic pleasure derived from food. In the laboratory, palatability has been shown to have a major impact on the amounts and types of foods ingested (Bobroff & Kissileff, 1986; Guy-Grand, Lehnert, Doassans, & Bellisle, 1994; Spiegel, Shrager, & Stellar, 1988; Yeomans, 1996; Yeomans, Gray, Mitchell, & True, 1997). Likewise, real-world meals that were rated high in palatability were 44% larger than meals that were lowly rated (de Castro, Bellisle, & Dalix, 2000; de Castro, Bellisle, Dalix, & Pearcey, 2000). Only 9% of self-selected meals, however, are rated as unpalatable. As a result, palatability accounts for only 4% of the variance in meal size (Figure 1, palatability). In the natural environment people maximize palatability. Thus, palatability has only a small influence on intake in the real world in contrast to its huge impact in the laboratory.

Important Influences Can Be Missed Because of the Short Durations of Laboratory Studies. One of the drawbacks of laboratory research is the great difficulty in studying the behavior of the participant for an extended period of time. It is unusual for a laboratory research project to investigate intake for longer than a few hours. As a result, laboratory studies can miss longer-term and rhythmic effects on intake. These include adjustments that occur days later (de Castro, 1998a), as well as daily, weekly, and seasonal changes in eating behavior (de Castro, 1987a, 1991b, 1991c). For example, during winter, spring, and summer daily intakes are equal. In the fall, however, intake increases by 11% to 14%. This finding further illustrates the superiority of real-world studies for the exploration of longer-term processes.

To some extent, the above discussion has been an overstatement of the case for real-world studies. In point of fact both laboratory and real-world studies are important in the unraveling of the complexities of food intake regulation. Each has its strengths and weaknesses. Fortunately, the weaknesses of one often are the strengths of the other. In the past, however, the scientific analysis of food intake has overemphasized laboratory research and downplayed the importance or meaningfulness of real-world research. Basic scientists often do not adequately value the results of real-world research due to the lack of tight controls that scientists have been taught to treasure. Balance is needed, and this means that far more real-world

research is needed. It can be reliably and validly performed and can teach valuable lessons that are not always evident in the laboratory.

Is Intake Controlled or Regulated in Humans?

Throughout most of the modern history of the study of intake it has been assumed that a single physiological regulatory process controlled intake, and research was aimed at discovering its nature. A number of single-factor models were proposed including the peripheral model of Walter Cannon (1929), the glucostatic model of Mayer (1996; Campfield, Smith, Rosenbaum, & Hirsch, 1996), the lipostatic model of Kennedy (1953; Mercer & Speakman, 2001), the thermostatic model of Brobeck (1948), and several additional modern models that involve factors such as leptin (Friedman & Halaas, 1998), body fat mass (LeMagnen, 1984), CNS insulin (Woods, Schwartz, Baskin, & Seeley, 2000; Woods & Seeley, 2000), or hypothalamic neuropeptide-Y (Tomaszuk, Simpson, & Williams, 1996). These models typically include a negative feedback loop between intake and some physiological factor such that it affects intake and in turn is affected by intake, either directly or indirectly. These models are homeostatic and postulate that particular levels of one or more parameters are defended.

Inheritance of Body Size and Metabolic Processes

These homeostatic systems are based on a physiology that is primarily determined by the genes. It stands to reason then that the defended levels and the negative feedback mechanisms that are an integral part of these homeostatic systems also would be determined by the genes. Consistent with this hypothesis, there is considerable evidence supporting genetic influence on body size and intake. Adoption and twin studies demonstrate that height and weight are strongly influenced by heredity (Allison *el al.*, 1996; Bouchard, 1991; Bouchard *et al.*, 1985, 1986; de Castro, 1993a, 1999c, 2000b; Hewitt, Stunkard, Carroll, Sims, & Turner, 1991; Price, Cadoret, Stunkard, & Troughton, 1987; Sorenson, Price, Stunkard, & Schulsinger, 1989; Stunkard, Foch, & Hrubec, 1986; Stunkard, Sorenson *et al.*, 1986; Stunkard, Harris, Pedersen, & McClearn, 1990), and that the effect of heredity on body weight remains evident even after accounting for the effect of height (de Castro, 1993a). There also are strong genetic influences on the composition of the body (Bouchard, Savard, Depres, Tremblay, & Leblanc, 1985; Bouchard, Perusse, Tremblay, & Leblanc, 1986; Brook, Huntley, & Slack, 1975) and even on the metabolic response to feeding (Poehlman *et al.*, 1986a, 1986b, 1986c) including the tendency to store energy as either lean tissue or fat (Bouchard, 1991; Bouchard *et al.*, 1990).

Even though there appear to be clear genetic influences on body size, the control seems to be weak. Homeostatic models predict that the overall constituents of the body should remain relatively stable, and that body weight should remain within a limited range over prolonged periods of time. Body weight changes, however, can occur at any time over the life span of the individual and be maintained at the new levels (Pearcey, 2000). A more telling observation is that over the last few decades there has been a marked increase in body weight in the population in many countries, especially in the United States (Flegal, 1999, Flegal *et al.*, 2002; Mokdad *et al.*, 1999, 2003; Ogden, Flegal, Carroll, & Johnson, 2002). These findings suggest that body size is not as tightly regulated as would be predicted based upon homeostatic control.

Inheritance of Intake Levels and Pattern

It would be reasonable to assume that if body size is influenced by inheritance, then the nutrient intakes that are necessary for the growth and maintenance of body mass should also be influenced by inheritance. In fact, twin studies indicate that ~42% of the variance in daily energy intake is determined by heredity (de Castro, 1993a, 1998b, 1999b, 1999c, 2000b; Faith, Rha, Neale, & Allison, 1999). Surprisingly, this influence of heredity appears to be independent of body size as the effect still is highly significant even after accounting for the effects of height, weight, gender, and age (Figure 2, left; de Castro, 1993a). This independence of intake from body size could be due to the fact that the individuals had a stable body weight at the time of measurement. Heightened intake is characteristic of individuals who are accumulating body mass (Pearcey & de Castro, 2002). Had the twins been measured while their weight was increasing, the relationship with intake would, in all likelihood, have been in evidence. In addition to overall intake, carbohydrate, fat, protein, alcohol, and water intakes all are significantly affected by inheritance (de Castro, 1993a) and to some extent they all are independent of overall intake (de Castro, 1993b). Hence, genes have separate and independent effects on height, weight, overall daily intake, and individual macronutrient intakes.

It would be reasonable to assume that if overall daily intake is influenced by inheritance then the processes that underlie total intake, meal size and frequency, also should be influenced by inheritance. In fact, meal patterns are affected by a number of independent genes (de Castro, 1993a). Meal sizes clearly are influenced by the overall level of intake. Heredity, however, still accounts for 28% of the variance in the average meal size even after accounting for daily intake (Figure 2, right). In addition, the number of meals ingested each day also is strongly affected by the level of daily intake, but heredity still accounts for 34% of the variance in the meal frequency after accounting for daily intake (de Castro, 1993a).

These combined results are in agreement with the predictions of homeostatic models that suggest that inheritance has a major role in the determination of body size and nutrient intake. Indeed the results indicate that genes have multiple influences throughout the array of processes governing body size and intake. Genes have separate and independent effects on overall intake, the macronutrient composition of intake, meal size, and meal frequency. It should be noted however, that considerable variance in intake is not accounted for by heredity and likely is due to the environment. For example, whereas 42% of the variance in overall intake can be ascribed to inheritance, 58% is ascribed to environment, and 28% and 34% of the variance in meal size and frequency are ascribed to genes, but 72% and 66% respectively are ascribed to environment. The relatively large magnitude of environmental effects suggests that processes that are not homeostatic are involved in the control of intake.

Meal-to-Meal Compensatory Responses

Another method to ascertain the degree of control of intake is to investigate the degree of compensation for deviations from constancy. The homeostatic models predict that regulation will be tightly controlled and that no stable long-term deviation will be tolerated. But compensation and stability have been difficult to document. In uncontrolled settings prolonged gains in body weight occur (Flegal, 1999; Mokdad *et al.*, 1999; Pearcey, 2000) and there is a lack of complete compensatory responses (De Graaf & Hulshof, 1995; DeGraaf *et al.*, 1997; Prentice, 1998; Yao & Roberts, 2001). Even in the laboratory with animal models, high-fat and

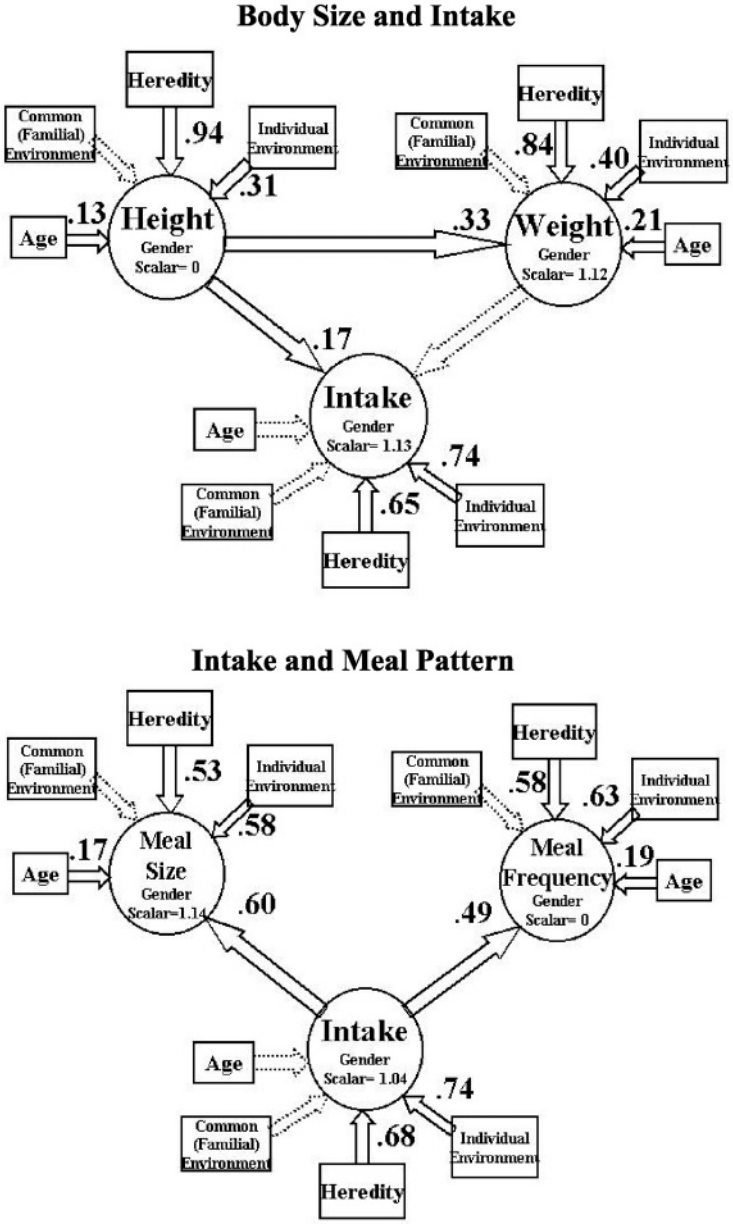

Figure 2. Linear structural model and the most parsimonious model fitting the twin data for height, weight, and total daily intake (top) and for total daily intake and meal size and frequency (down). Statistically significant paths are represented by the solid path arrows. The path coefficients for these estimated relationships are provided beside the arrows. Dashed arrows represent nonsignificant paths. For all remaining parameters, removing any one leads to a statistically significant reduction in the model's account of the observations.

highly palatable diets produce overeating and obesity (Cleary, Phillips, & Morton, 1999; Sclafani & Springer, 1976; Wade & Bartness, 1984). Hence, although the negative feedback system influences intake, compensatory responses are weak and transitory, and there are powerful influences on intake that are not compensated.

Homeostatic models predict that when intake is high on one occasion, a compensatory response should occur to produce a decrease in intake on a subsequent occasion. There is, however, no evidence of such compensation from one meal to the next as there are no significant correlations between amount eaten in a meal and the amount eaten in the subsequent meal by animals (de Castro, 1975; LeMagnen & Tallon, 1966, 1968) or humans (Bernstein *et al.*, 1981; de Castro *et al.*, 1986). Nonetheless, meal-to-meal compensation could occur by adjustment of the length of time between meals. Indeed, there is a significant correlation between the duration of the interval prior to the meal and the size of the meal (Figure 1, prior interval; de Castro & Kreitzman, 1985; de Castro *et al.*, 1986). This finding suggests that intake control may occur by adjustments to the amount eaten in the meal based upon the time since the last meal.

One possible intermediary between the passage of time and intake is the content of the stomach. Over the interval between meals the stomach empties at a slow rate. It makes intuitive sense that any food remaining in the stomach at the time of the meal would have a negative effect on the amount ingested (de Castro & Kreitzman, 1985). Since the stomach empties in a predictable way (Hopkins, 1966; Hunt & Knox, 1968; Hunt & Stubbs, 1975), estimates can be made of the amount emptied over a given interval and thereby the amount remaining at the time of a second meal. As expected, there was a significant negative correlation between the amount estimated to be in the stomach at meal initiation and meal size (Figure 1, stomach content; de Castro, 1987b, 1988b; de Castro & Kreitzman, 1985; de Castro *et al.*, 1986). This negative correlation was found regardless of whether meal size and/or stomach content were expressed in terms of total food energy, carbohydrate, fat, or protein (Figure 3, left). Hence, the more food energy or macronutrients present in the stomach at the beginning of a meal, the less will be eaten.

The magnitudes of these relationships, however, are small. The relationship between the prior interval and meal size accounts for only 4% to 9% of the variance. In addition, the relationship between estimated stomach contents and intake accounts for only 6% of the variance in meal size. In addition, if meal-to-meal compensation was a major factor in the control of intake, then daily intake should be

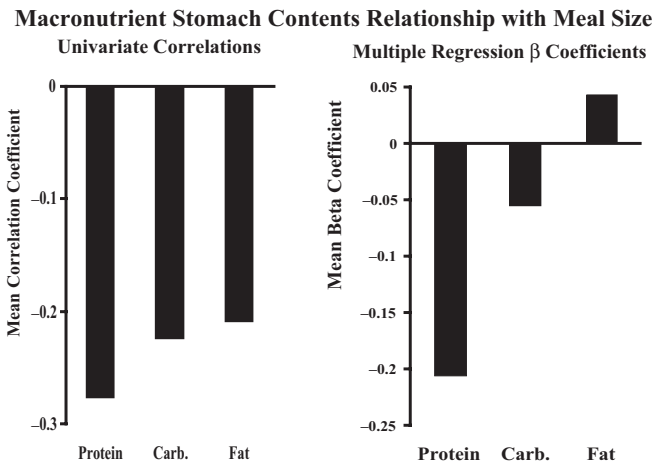

Figure 3. The relationship between the estimated before-meal stomach contents of the carbohydrate, fat, and protein and the amount of total food energy ingested in the meal expressed as univariate correlations (left panel) or as mean β coefficients from the multiple linear regressions (right panel).

fairly constant. In fact, there is considerable day-to-day variation in food intake (Balogh, Kahn, & Medalie, 1971; de Castro, 1998a; Edholm, Fletcher, Widdowson, & McCance, 1955, Edholm *et al.*, 1970; Hankin, Reynolds, & Margen, 1967; Hartman *et al.*, 1990; Morgan, Johnson, & Goungetas, 1987; Tarasuk & Beaton, 1991). Hence, it appears that meal-to-meal compensation is a weak form of control of intake.

Within-Day Compensatory Responses

If food intake was regulated over the course of each day, then compensation should be apparent following the ingestion of a particular food or beverage by a reduction in the ingestion of other foods or beverages. This notion implies that food intake in one form will result in less ingestion of other forms. Contrary to this prediction, within individual meals or over the entire day, energy derived from alcohol is added to and does not displace the energy derived from other sources (de Castro & Orozco, 1990; Orozco & de Castro, 1991). In general, on days when people voluntarily ingest a particular food, they ingest more total energy than on days they don't (de Castro, 1993c). Furthermore, the amounts of particular foods ingested correlate positively with total amount ingested overall or in meals but do not correlate when the calories attributable to the food are removed. In other words, consuming an individual item such as ice cream did not affect the amounts of other items ingested. Hence, intake is quite elastic and can be significantly influenced by the presence or absence of particular constituents. This finding indicates that compensatory regulation does not occur within the day.

Day-to-Day Compensatory Responses

If short-term mechanisms were completely responsible for regulation, then the total amount of food energy ingested in a day would be fairly constant, provided that activity levels also were relatively constant. However, food energy intake actually varies considerably from day to day (Balogh *et al.*, 1971; de Castro, 1998a; Edholm *et al.*, 1955, 1970; Hankin *et al.*, 1967; Hartman *et al.*, 1990; Morgan *et al.*, 1987; Tarasuk & Beaton, 1991). Hence, in order for regulation to occur there must be a compensatory mechanism available to increase intake in response to a prior day's deficit and/or decrease intake in response to a prior day's surfeit. This should produce a negative correlation between food energy intake on 1 day and the amount ingested on the next day. Such a negative autocorrelation, however, has not been observed. Those reported have been small and predominantly positive (Morgan *et al.*, 1987; Tarasuk & Beaton, 1991).

Using the daily intakes reported in 7-day diet-diaries autocorrelations were calculated between the amounts ingested in 1 day and in each of the 4 subsequent days (de Castro, 1998a). As in prior studies, the correlations between food intake in 1 day and that occurring in the following day were not significant. However, the autocorrelations with 2- and 3-day delays were statistically significant (Figure 4). The 2-day lag autocorrelations were significantly stronger than with other delays. Interestingly, there are macronutrient specific effects. Carbohydrate intake has a larger negative autocorrelation than either fat or protein with the amount of carbohydrate ingested. Fat intake has a larger negative autocorrelation than either carbohydrate or protein with the amount of fat ingested. Similarly, protein intake has a larger negative autocorrelation than either carbohydrate or fat with the

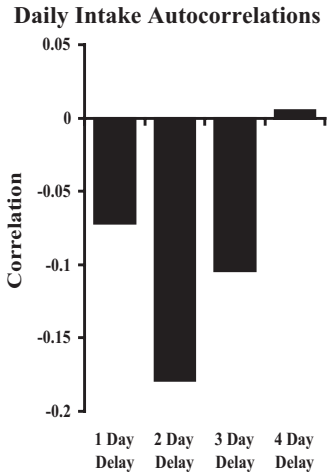

Figure 4. Autocorrelations calculated between the amounts ingested on a day and the amounts ingested 1, 2, 3, and 4 days later.

amount of protein ingested. In other words, carbohydrate appears to maximally effect carbohydrate, fat to maximally effect fat, and protein to maximally effect protein, and the effect is greatest 2 days later (de Castro, 1998a, 2000a).

It must be noted, however, that while significant, this delayed negative feedback is very weak. The autocorrelations account for less than 3% of the variance in daily intake. In addition, compensation does not appear to occur via adjustments in activity levels. The correlations between recorded activity levels and intake on the same day, the next day, or 2 days later are not statistically significant (de Castro & Pearcey unpublished; Edholm *et al.*, 1955, 1970) and the correlations between intake and the recorded activity levels on the same day, the next day, or 2 days later also are not significant (de Castro & Pearcey, unpublished). Hence, on a meal-to-meal or day-to-day basis, there is minimal compensation, indicating that homeostatic mechanisms have only weak effects on short-term intake.

Uncompensated Factors

A broad spectrum of factors influence intake but are not affected by intake. These are called uncompensated factors. These include social facilitation, dietary restraint, daily, weekly, and seasonal rhythms, cost and availability of food, palatability, and energy density. These uncompensated factors can have considerable effects on intake that may be amplified by the absence of a negative feedback loop.

Social Factors

Social Facilitation of Intake. Human beings are social animals whose behavior is profoundly affected by social influences. "Of all the stimulation that impinges on the organism in its lifetime, stimulation from social sources is most important" (Zajonc, 1980, p. 50). This stimulation markedly affects food intake. Meals eaten with others are 44% larger than meals eaten alone (de Castro & de Castro, 1989), including larger amounts of carbohydrate, fat, protein, and

alcohol. Similarly, people eating in groups in restaurants or coffee shops eat more than patrons eating by themselves (Klesges, Bartsch, Norwood, Kautzman, & Haugrud, 1984; Sommer & Sommer, 1989; Sommer & Steele, 1997), and soldiers eating in small groups eat more than when eating alone (Hirsh & Kramer, 1993). The number of people present is positively correlated with the amount eaten even when meals eaten alone are excluded (de Castro & de Castro, 1989; Figure 1, # of people; also Figure 5). One other person present at the meal is associated with a 33% increase in meal size while 47%, 58%, 69%, 70%, 72%, and 96% increases are associated with 2, 3, 4, 5, 6, and 7 or more people respectively. This orderliness can be adequately described by a power function (de Castro & Brewer, 1992), as is the case in general for social facilitation phenomena (Latane, 1981).

It is possible that the presence of other people might act by extending the amount of time spent at a meal and thus increasing the amount eaten (de Castro, 1990). The verbal interactions occurring during social meals may cause a person to linger over the meal and eat more. In fact, the rate of intake is the same regardless of the social conditions, and the duration of the meal is extended when other people are present (de Castro, 1990; de Castro & Brewer, 1992; Feunekes, De Graaf, & Staveren, 1995). Further, this hypothesis is supported by diet-diary findings that social facilitation occurs maximally when eating with friends, family, or a spouse (de Castro, 1994b). In addition, in the laboratory, social facilitation occurs only when the participants are acquainted and does not occur when strangers are paired together (Clendenen, Herman, & Polivy, 1994). Hence, the time extension hypothesis is a viable explanation of social facilitation of food intake.

It is possible that this relationship between the presence of other people and meal size is secondary to covariation produced by a third factor. However, strong, positive correlations between meal size and the number of other people present are found separately for breakfast, lunch, or dinner, for meals eaten in restaurants, at home, or elsewhere, for meals with or without alcohol, for snacks only or meals only (de Castro *et al.*, 1990), and for meals eaten during weekdays or during weekends (de Castro, 1991b). Hence, social facilitation is an important determinant of eating under a great many conditions.

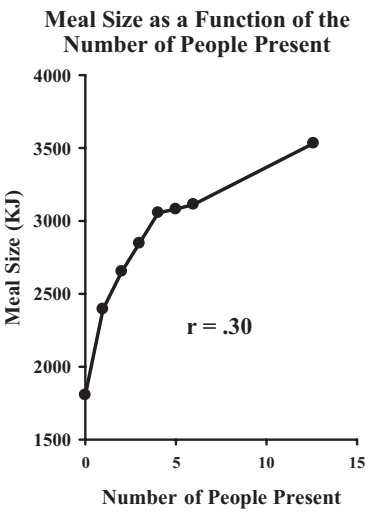

Figure 5. Mean meal size as a function of the number of other people present at the meal.

Even with all this evidence, because of the observational nature of the research, it cannot be concluded without an active manipulation that the presence of other people causes increased intake. So, the number of other people present was manipulated experimentally by instructing people to eat only by themselves, eat normally, or eat only with other people. In comparison to the normal condition, 11% less was eaten per day when people were instructed to eat alone (Redd & de Castro, 1992). In addition, in the laboratory, more food is ingested when individuals are placed in groups than when they eat alone (Berry, Beatty, & Klesges, 1985; Edelman, Engell, Bronstein, & Hirsch, 1986). Also, when subjects are required to eat a test meal with one or three other people, they eat significantly more than when alone (Clendenen *et al.*, 1994). Hence, the presence of other people causes an increase in intake at meals.

Modeling. In general, when people are paired with someone who eats a large amount of food, they increase their intake (Conger, Conger, Costanzo, Wright, & Matter, 1980; Goldman, Herman, & Polivy, 1991; Polivy, Herman, Younger, & Jaeger, 1979). Thus modeling is another way that social influence can affect ingestive behavior. This has long been known to occur with nonhuman animals (Bayer, 1929; Hsia & Wood-Gush, 1984), but it is true of humans as well. For example, Nisbett and Storms (1972) invited subjects to taste test crackers alone or paired with a confederate who ate either one or twenty crackers. Normal weight subjects ate 29% less with the low intake "model" and 25% more with the high intake "model" than when alone. Roth, Herman, Polivy, & Pliner (2001) left notes indicating prior subjects, behavior. Their participants ate more or less depending upon the declared behavior of the "model." Hence, the modeling effect occurs even when the model is not physically present. In the natural environment females eat more when they eat with males than they do when they eat with other females (de Castro, 1994b). Hence, modeling can affect intake in the real world as well as the lab.

Environmental Factors

Time of Day. There are clear diurnal or circadian influences on intake (de Castro, 1987a). In the real world, average meal size increases as the day progresses up to the time of sleep (Figure 6, left) and the following interval until the next meal gets shorter and shorter (Figure 6, center). This pattern is true for both North Americans and Europeans (de Castro *et al.*, 2000). The satiety ratio is defined as the duration of the interval after the meal divided by the meal size, and gauges the duration of satiety produced per unit of food energy ingested. This satiety ratio markedly declines over the course of the day and becomes quite low during the late evening (Figure 6, right; de Castro, 1987). Such a pattern also can be discerned in rats in the lab (LeMagnen & Devos, 1984; Rosenwasser, Boulos, & Terman, 1981). This finding suggests that the satiating effect of food decreases over the course of the day. Indeed, in humans, eating a large proportion of intake in the morning is associated with lower overall intake while eating a high proportion of intake in the evening is associated with higher overall intake (de Castro, 2003).

Day of the Week. Humans eat significantly more on weekends than on weekdays (de Castro, 1991b, 1991d; Jula, Seppanen, & Alanen, 1999; Lyons *et al.*, 1989). On average there is an 8% increase (145 kcal/day) for the Friday, Saturday,

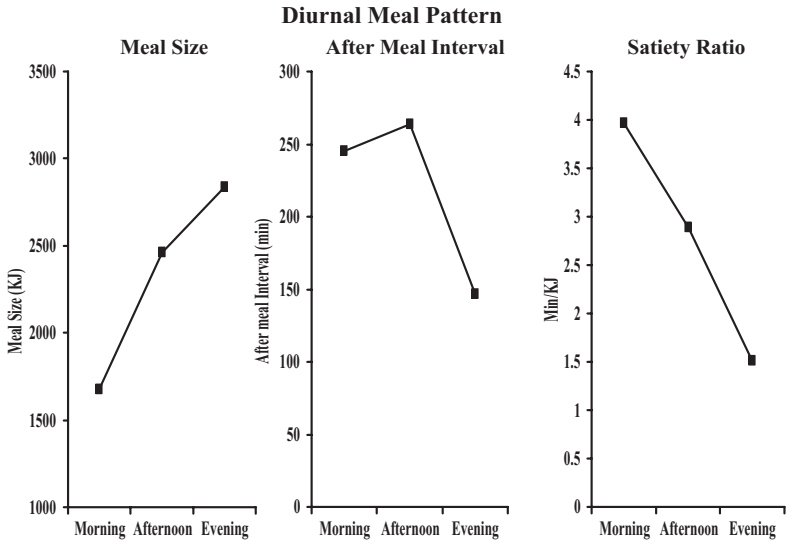

Figure 6. Mean meal sizes (left panel), interval until the following meal (middle panel), and the satiety ratio (right panel) observed during the morning, afternoon, and evening periods.

and Sunday intakes, and this effect results from higher intakes of all macronutrients and alcohol. The higher weekend intakes result from an 11% increase in meal size with no change in meal frequency. The increased meal size, in turn, results from a 20% increase in the duration of the meals with no change in the rate of intake. Thus people eat for longer periods of time on weekends, which results in larger meals and greater overall intakes.

The single factor with the largest influence on weekend intake is social facilitation (de Castro, 1991b, 1991d). The correlations and slopes of the regression lines between meal size and the number of other people present at the meal are larger on weekends than on weekdays. Hence, the presence of other people has a greater impact per person on weekends. In addition, there were 43% more people present at the meals on weekends than weekdays. Thus, not only is the impact per person higher, but the number of persons present also is greater on weekends. Moreover, weekend meals generally are less constrained by external, work, or class schedules. The greater flexibility to extend the duration of the meals on weekends allows social effects to be fully expressed. Thus, the heightened intake on weekends results from increased social facilitation.

Phase of the Moon. Surprisingly, there are small variations in nutrient and meal intakes of humans over the lunar cycle of humans (de Castro & Pearcey, 1995). For both men and women, there is a reliable, 8% increase in meal size at the time of the full moon relative to the new moon, and a larger, 26%, decrease in alcohol intake with the full moon. It is not likely that these lunar effects on intake result from direct illumination effects (Templer, Veleber, & Brooner, 1982). If illumination level was critical then the effect should be present only for nighttime. Yet, the meal size differences during the full moon phase are present for both day and nighttime.

Season. There are seasonal variations in nutrient and meal intakes and in hunger (de Castro 1991c). Daily intake increases by 14% in the fall, primarily by

increased carbohydrate intake. This increase results from larger meals and a faster rate of intake. Interestingly, newborn infants also increase weight and BMI maximally in the fall (Xu, Wang, Guo, Cheung, & Karlberg, 2001). The magnitude of the effect in adults is striking, with a 222 kcal/day increase in the fall relative to the other seasons (de Castro, 1991c). Extrapolating this intake over the entire fall season predicts an increase of over 20,000 kcal. If it were all converted to fat, it would increase body weight by more than 2.5 kg. The increased intake during the fall cannot be accounted for by increased thermoregulatory requirements since intake does not increase during the winter, when thermoregulatory needs are greatest. Rather, it appears to result from a seasonal change in hunger and satiety processes. The level of hunger reported at the end of the meal increases in the fall (de Castro, 1991c). In addition, food has a lessened effect on hunger as the negative correlation between the amount eaten and after-meal hunger is smaller in the fall. Hence, the larger meals appear to be tolerated as a result of a lessened effectiveness of the nutrients to induce a state of satiety during the fall. Regardless, it is striking that such a salient seasonal rhythm occurs in humans living in modern environments with artificial lighting and temperature control.

It makes ecological and evolutionary sense that a heightening of food intake would occur in the fall when supplies are usually abundant, as if in preparation for the winter when supplies are scarce. This same phenomenon occurs in many animal species (Bartness, Demas, & Song, 2002; Hunter & Nagy, 2002; Thiery *et al.*, 2002).

Location. Where food is eaten can make a difference. In either full service or fast food restaurants, meals are significantly larger and contain more of each macronutrient and alcohol than at home or in other locations (de Castro *et al.*, 1990). This effect is due in part to social facilitation as there are significantly more people eating with the individual in restaurants than at home (de Castro *et al.*, 1990) and in part to large portion sizes served, particularly in fast food restaurants (Nielsen & Popkin, 2003). In addition, there has been an increase in the frequency of restaurant meals and a decrease in home meals in modern society (Nielsen, Siega-Riz, & Popkin, 2002). This societal shift toward larger portions eaten in restaurants could, at least in part, be responsible for the societal increase in body weight (Binkley, Eales, & Jekanowski, 2000; McCrory, Suen, & Roberts, 2002).

Psychological Factors

Dietary Restraint. Humans differ in the degree to which they attempt to assert control over their food intake. Many eat whatever and whenever they want, while others attempt to actively restrict their intake, with varying degrees of success. The most commonly used instruments to measure the degree of eating restraint are the Three-Factor Eating Questionnaire (Stunkard & Messick, 1985) and the Restraint Scale (Herman & Polivy, 1980). Employing these instruments, restrained eating has been studied extensively in laboratory settings (see Lowe, 1993, for a review). In general, lower overall intake, especially of fat and carbohydrate, is associated with higher restraint scores (Kirkley, Burge, & Ammerman, 1988; Laessle, Tuschl, Kotthaus, & Prike, 1989; Mulvihill, Davies, & Rogers, 2002; Tuschl, Platte, Laessle, Stichler, & Prike, 1990; van Strien, Frijters, van Staveren, Defares, & Deurenberg, 1986; Wardle & Beales, 1987; Wardle *et al.*, 1992). In addition, in natural settings, restraint is associated with lower and less variable intakes, especially of fat and carbohydrate, and the ingestion of smaller and less variable meals (de Castro, 1995).

PALATABILITY. Palatability is a hypothetical construct that stands for the stimulus qualities of an ingestible substance that affects its acceptability (Kissileff, 1976; Rogers, 1990). Palatability is influenced by both learned and innate factors (Rogers, 1990), and has a potent effect on the desire to eat and the amount ingested (Bobroff & Kissileff, 1986; DeGraaf, De Jong, & Lambers, 1999; Guy-Grand *et al.*, 1994; Hill, Magson, & Blundell, 1984; Rogers & Schutz, 1992; Spiegel *et al.*, 1988; Yeomans, 1996; Yeomans *et al.*, 1997). In addition, the intake of food of a specific flavor can have a short-term suppressive effect on the palatability of that food, in a process termed sensory-specific satiety (Rolls, Rolls, Rowe, & Sweeney, 1981; Rolls, Rowe, & Rolls, 1982). This effect, however, dissipates rapidly. Also, there is little change in palatability ratings as a result of ingesting the meal (de Castro, Bellisle, & Dalix, 1999; de Castro *et al.*, 2000). Hence, for the most part palatability may be considered an uncompensated factor.

Self-reported palatability has a positive relationship with the amount ingested in meals (Figure 7, right), with people ingesting 44% larger meals when the food was highly palatable (de Castro, Bellisle, & Dalix, 2000; de Castro *et al.*, 2000). The enlarged meals are related to an increase in meal duration and not the rate of intake. Correlations between the palatability ratings and meal size are positive and significant (Figure 1, palatability), but account for only 4% of the variance in meal size for North Americans (de Castro *et al.*, 2000) and 2% for the French (de Castro, Bellisle, & Dalix, 2000). A range restriction may account in part for the small magnitude of the correlations, as only 9% of meals are rated as unappealing (Figure 7, left). In addition, after separating meals ingested at home, restaurants, work, and

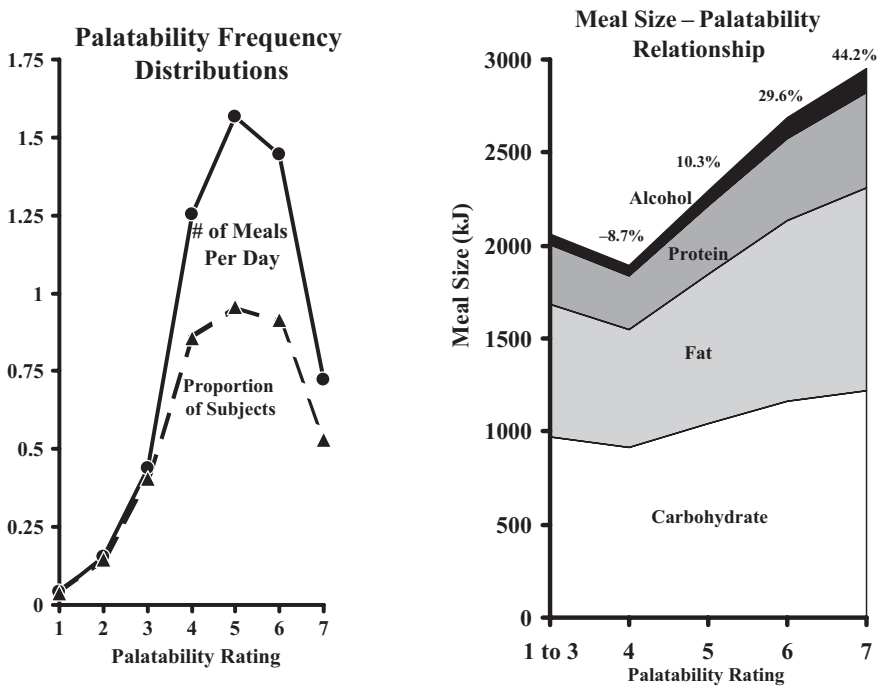

Figure 7. The number of meals per day and the proportion of subjects who used each of the seven levels of palatability ratings (left panel) and the relationship between the palatability ratings and the mean meal intakes of the macronutrients (right panel).

other locations, the correlations between palatability and meal size are significant for each individual location. Hence, even though meal sizes vary in different venues and the rated palatability of these meals varies with location, meal size is related to palatability regardless of location.

Dietary Composition

Alcohol. Alcohol is a major component of the typical American diet. It has been estimated that 2.7 gallons of alcohol are consumed annually per person 14 years and older, a per capita intake of about 160 kcal/day (Windham, Wyse, & Hansen, 1983). Interestingly, the intakes of carbohydrate, fat, or protein by drinkers are the same as that of nondrinkers, but overall daily intakes are larger due to calories derived from alcohol (de Castro & Orozco, 1990). In addition, drinkers ingest comparable amounts of macronutrients on days they drink as on days they do not drink, so more total energy was ingested on drinking days simply because of the additional calories derived from alcohol. In one study, alcohol intake was manipulated by having moderate drinkers either drink normally or refrain from alcohol for a 5-day period (Orozco & de Castro, 1991). During the normal week, 218 kcal/day more was consumed than during the abstinence week while the amounts of carbohydrates, proteins, or fats did not differ. Hence, alcohol supplements rather than displaces energy derived from macronutrients, and the derived calories appear to be unregulated (Hetherington, Cameron, Wallis, & Pirie, 2001). As a result, alcohol ingestion may lead to an increase in total energy intake (Yeomans, 1999).

Density. Food contains not only energy and macro- and micronutrients, but also nonabsorbable fiber and water. As a result meals can vary greatly in energy content, as well as in weight and energy density. It has been postulated that intake may be regulated not on the basis of its energy content but on its weight (Rolls *et al.*, 1998). If so, then ingestion of foods that contain more energy relative to their weight (i.e., high-density food) would result in higher energy intake while ingestion of foods that contain a large amount of water or some other nonnutritive filler (i.e., low-density foods) should result in lower overall energy intake. In fact, diet density has been found to be significantly associated with intake (Bell & Rolls, 2001; Prentice, 1998; Yao & Roberts, 2001).

Dietary energy density can be measured as the total energy ingested over the day divided by the total amount in grams ingested including all liquids, or it can be measured by the same calculation excluding liquids from drinks. Regardless, the density of meals is strongly correlated with their total energy intake (de Castro, 2004a). Also, the average density of intake over the entire day is positively correlated with the total energy intake. Consistent with this relation, the magnitude of the differences between the members of identical twins in the density of their diet is positively related to the magnitude of the differences in their daily energy intake (de Castro, 2003). Hence, the density of the ingestate is a major determinant of overall energy intake.

Since dietary density clearly is related to daily energy intake, and energy intake in turn is related to body weight and adiposity, it is possible that the incidence of overweight and obesity is related to the density of the diet. But, identical twin pairs whose members differ in average dietary density do not differ in body weight or BMI. In addition, dietary density was not correlated with body weight or BMI in

a sample of over 800 individuals (de Castro, in preparation). Hence, dietary density affects short-term, meal-to-meal, and day-to-day intake, but does not appear to have long-term effects on body weight and fatness.

Compensated Factors

There are a large variety of factors that both affect and are affected by intake called compensated factors. Most are of the physiological, homeostatic type. Unfortunately, in humans in real-world environments, the ability to monitor the levels of these factors is limited. As a result, we can attain only a glimpse of their influences by investigating those that can be measured or estimated and by looking at naturally occurring disruptions of the physiological systems. To date, evaluations have been made of hunger, the estimated stomach content, and the disruption of the glucoregulatory system as occurs in Type I diabetes.

Hunger

Hunger is compensated in that it affects intake and intake affects it. Hunger is subjective, and as such it is assessed with a self-report. Hunger ratings prior to a meal are strongly correlated with the meal size, (Figure 1, hunger). Its nature as a compensated factor is reflected in the fact that the more the food that is eaten in a meal, the lower the level of hunger at its completion (de Castro, 1993d, 1999b; de Castro & Elmore, 1988). It is possible that hunger's influence is due to the fact that it mirrors the levels of other factors such as the stomach content. The relationship of hunger with meal size, however, remained significant in a multiple regression analysis with hunger, estimated stomach contents, the number of people present, and the time of day as independent variables and meal size as the dependent variable (de Castro, 2002). Hence hunger's relationship with meal size is not a secondary consequence of its covariation with these other factors. Rather, it is an independent compensated influence on intake.

Stomach Content

The energy content of the stomach is compensated in that it influences and is influenced by intake. The amounts of nutrients that are present in the stomach at any time can be estimated with a simple equation that uses the amount eaten in meals as an indicator of stomach filling and the passage of time as an indicator of stomach emptying. This estimate of stomach contents at the initiation of the meal is negatively correlated with the size of the subsequent meal (Figure 1, stomach content; de Castro 1987b, 1988b; de Castro & Kreitzman, 1985; de Castro *et al.*, 1986). This negative correlation is seen regardless of whether meal size and/or stomach content are expressed in terms of total food energy, carbohydrate, fat, or protein intake (Figure 3, left).

The larger the meal the more of each macronutrient is eaten. As a result the intake of all macronutrients is positively correlated making it difficult to discern the independent impact of each macronutrient. However, multiple regression predictions of the meal size on the basis of the before-meal stomach contents of the three macronutrients reveal that protein in the stomach has a large and significant restraining effect on intake, while fat and carbohydrate have no such effects

(Figure 3, right) (de Castro, 1987b, 2000a). In addition, the effects of stomach protein are more pronounced on the intake of protein than on the intake of either carbohydrate or fat.

Blood Glucose: Type I Diabetes

The levels and availability of circulating glucose have long been thought to be an important compensated factor in the control of food intake (Mayer, 1996). Low blood glucose or low glucose availability was thought to create hunger and initiate eating (Campfield *et al.*, 1996), while high levels of glucose in the blood or high glucose availability were thought to create satiety and the cessation of intake. The primary anabolic hormone, insulin, also has been hypothesized to be involved either directly in the control of food intake and body weight, through direct effects on the brain, or indirectly via its influence on circulating glucose (Mayer, 1953; Woods *et al.*, 2000). In the lab, under tightly controlled conditions, continuous measurements of blood glucose levels have revealed a clear relationship between changes in glucose levels and meal intake (Campfield *et al.*, 1996).

In free-living humans it is not currently feasible to continuously measure circulating glucose levels. The role of glucose in regulating intake, however, can be glimpsed by studying a naturally occurring disruption of this system, diabetes. In Type I diabetes the pancreas loses its ability to produce and release insulin into the blood in response to food intake (Gutniak, Grill, & Efendic, 1986) or the presence

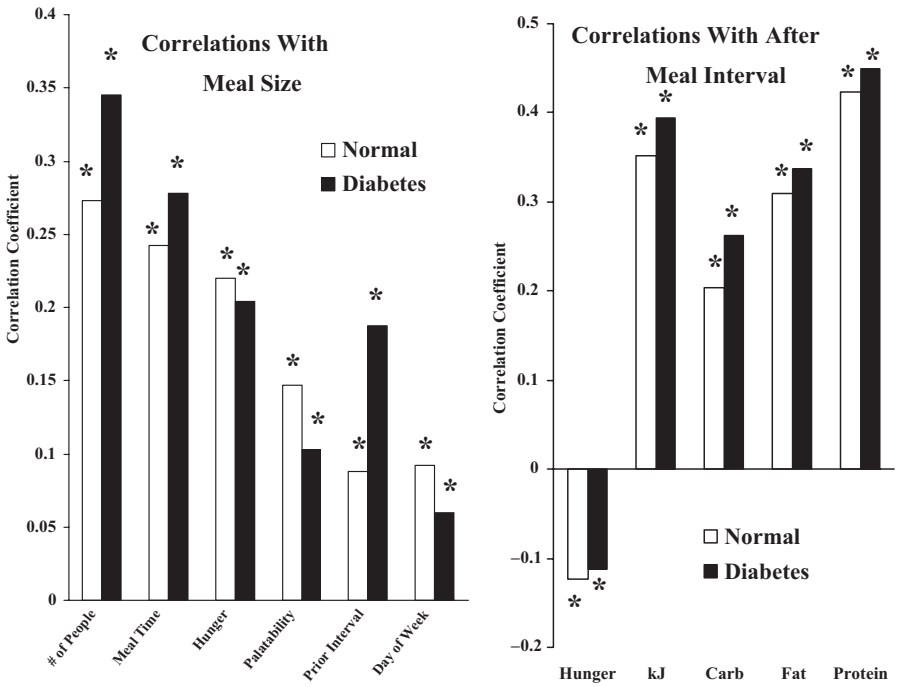

Figure 8. Mean correlations between meal size and the number of other people present at the meals, the average time of the meals during the day, the self-rated hunger, palatability, duration of the prior interval, and the day of the week (left panel) and the mean correlations between after-meal interval and the self-rated hunger, and the meal size for the participants who were without or with diabetes. * indicate that the correlation was significantly different from 0 ($p < 0.05$) as assessed with a z test.

of palatable foods (Teff, 2000). Treatment with insulin can normalize mean plasma glucose levels, but cannot normalize the short-term phasic relationships of insulin secretion with internal and external stimuli. Hence, the study of the intake of humans with Type I diabetes could shed some light on the degree of involvement of the insulin–glucose system in the control of intake.

In animal models, Type I diabetes produces large changes in intake and the meal pattern (de Castro & Balagura, 1975). Thus, it is surprising to find very few differences in food intake and meal patterns between diabetic humans and healthy controls except that the diabetics ingest more protein than controls, and eat more frequent and slightly smaller meals (de Castro *et al.*, 2002). Patients with diabetes, however, are instructed to incorporate more protein in their diet and to eat smaller meals more frequently. Hence these differences may simply reflect compliance with their dietary instructions. Correlations between meal size and the after-meal interval also were normal in diabetics (Figure 8), as were the correlations between meal size and the number of people present, the time of day, hunger, palatability, and the day of the week (Figure 8). Hence, the responses to sociocultural and subjective variables are not mediated by insulin-induced adjustments of blood glucose such as occur with "cephalic phase" responses (Teff, 2000). The transient fluctuations in blood glucose levels do not appear to be major factors in the control of intake in the real world. Hence, as with the other compensated factors, the glucose regulatory system influences intake but is not a major factor.

The Behavioral Genetics of Factors Affecting Intake

It is clear from the above discussion that intake is affected by a wide range of both compensated and uncompensated factors, with each factor responsible for only a small portion of the variance. It also is clear that compensated factors are relatively weak and probably not capable of adjusting intake sufficiently to compensate for the influences of the uncompensated factors. Hence, these data imply that intakes, and as a consequence body weight, should be highly variable and dependent upon uncompensated environmental influences. However, data on inheritance of intake and body weight suggests the opposite. These two possibilities, however, are not necessarily mutually exclusive. Inheritance might affect both the compensated and uncompensated factors. Indeed, evidence suggests that the genes have ubiquitous effects on all aspects of the system. The integral of these influences is to produce stability of intake and body weight, that is, a "settling point." This stability will remain as long as the environment remains stable. A change in the environment can, however, produce a new "settling point." This hypothesis assumes that inheritance affects all aspects of the system, including the levels and responsiveness to both compensated and uncompensated factors.

Effect on Compensated Factors

Stomach Content. The contents of the stomach at the time of a meal exert a restraining influence on intake (de Castro *et al.*, 1986). There are significant genetic influences on the amount in the stomach before and after a meal (Figure 9, left panel, stomach content; de Castro, 1999c). Some individuals eat their meals with the stomach relatively empty while others eat with it relatively full, and this individual difference is affected by inheritance. The responsiveness of the individual to

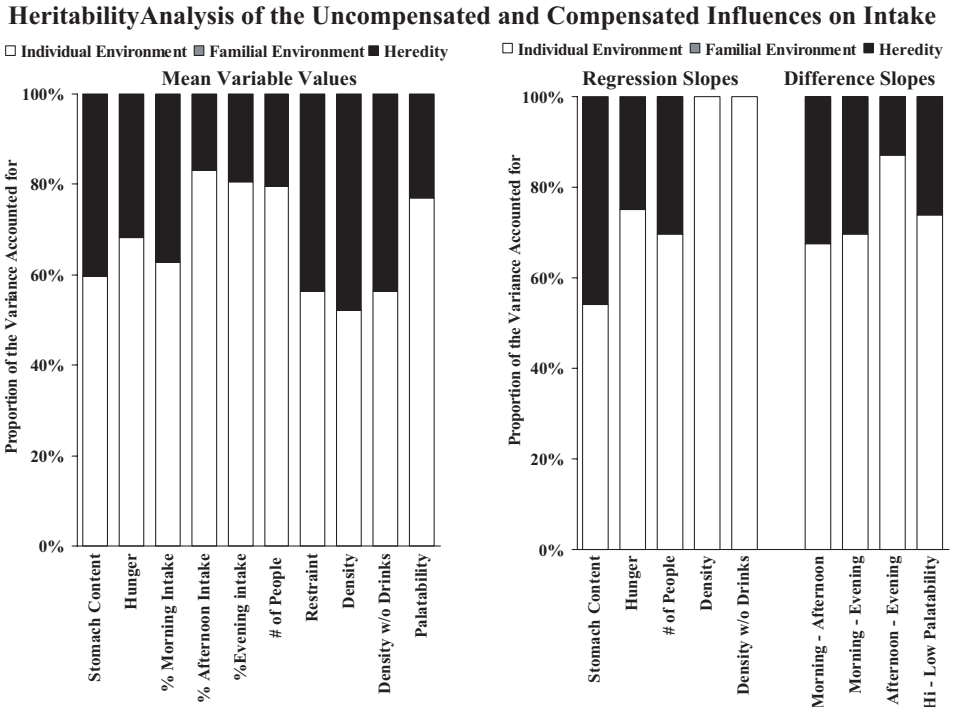

Figure 9. The proportion of the variance in the factor means (left panel) and the slopes of the relationships between the factors and the meal size (right panel) that could be accounted for by the individual environment (white), family environment (striped), and heredity (black) in the linear structural modeling heritability analysis of the twin data.

the content of the stomach can be estimated by calculating the slope of the relationship between the amount in the stomach and the amount eaten. There are significant heritabilities for both the correlation and the slope of the regression line between the before-meal stomach content and the meal size (Figure 9, right panel, stomach content), indicating that individual differences in the impact of the stomach content on meal size are inherited. Hence, heredity influences how full the stomach is when individuals begin to eat and the suppressive effect that fullness has on intake.

Hunger. Subjective hunger has a positive influence on the amount eaten in meals (de Castro & Elmore, 1988). Twin studies revealed significant genetic influences on the levels of self-rated hunger before the meal (Figure 9, left panel, hunger; de Castro, 1999b). In addition, the responsiveness of the individual to hunger is influenced by heredity. Significant heritability factors were observed for both the correlation and the slope of the regression between hunger before the meal and meal size (Figure 9, right panel, hunger). Genes also affect the influence of intake on hunger. There are significant heritabilities for the after-meal hunger ratings and the change in ratings over the meal (de Castro, 1999b). Thus, heredity influences reported hunger, the facilitative effect of hunger on subsequent intake as well as how hungry the individuals are when they have finished eating, and the impact of the meal on hunger.

In sum, the levels of compensated factors and the individual's responsiveness to them are influenced by heredity, as is the reciprocal effect of intake on compensated factors. An important conclusion from this analysis is that the

compensated factors individually produce only small effects on intake. In addition, the degree of response to both compensated and uncompensated factors differs between individuals and this is to some extent due to heredity. This conclusion supports the notion that inheritance affects all aspects of the system, including the levels and responsiveness to compensated factors.

Effect on Uncompensated Factors

Diurnal Rhythms. Inheritance also influences uncompensated factors. There is a diurnal rhythm of intake, with the amounts ingested increasing over the day (de Castro, 1987; de Castro *et al.*, 1997; Westerterp-Plantenga, 1999). The twin data, demonstrated that the time when people eat is affected by heredity (de Castro, 2001a), with the proportion of daily intake occurring in the morning, afternoon, and evening influenced by heredity (Figure 9, left panel). In addition, the differences in intake between the morning and afternoon, the morning and evening, and the afternoon and evening, are significantly heritable (Figure 9, right panel). These findings indicate that genetic factors affect not only when an individual will tend to eat, but also how big an effect the selection of that time will have on their intake.

Social Facilitation. There are heritable individual differences in the number of eating companions with whom twins eat (de Castro, 1997; Figure 9, left panel). Heredity also influences the nature of the companions with whom the individual eats, such as family, friends, and spouse. Heredity also affects how much intake is increased by the presence of other people. The slope of the regression between the number of people present and the meal size is quite steep indicating that the amount eaten in a meal is on average increased by over 20% (70 kcal) for each other person present at the meal. Both the correlation and the slope of the regression are heritable (Figure 9, right panel). Hence, genetic factors affect the number and types of eating companions and the magnitude of the effect that these companions have upon intake.

Restraint. Genes influence the degree to which the individual restrains intake (Figure 9, left panel; de Castro & Lilenfeld, 2003). There also are considerable influences of the environment on restraint. Indeed, identical twins often have restraint differences that likely are due to environmental differences. These differences can have just as potent an influence on behavior as inherited characteristics. An analysis was performed of the differences in body size and dietary restraint between individuals and their identical twins. Intrapair differences in cognitive restraint correlated significantly with the intrapair differences in body weight and body mass index (de Castro, 2003; de Castro & Lilenfeld, 2003) implying that the environment as well as heredity can influence dietary restraint and that both influence body size.

Dietary Density. The density of the diets reported by twins is affected by inheritance, accounting for over 40% of the variance (de Castro, unpublished). This effect was independent of the inclusion of drinks in the density calculations (Figure 9, left panel). Additionally, dietary density differences between members of identical twin pairs were positively related to differences in daily intake but were not related to differences in body size (de Castro, 2003). Also, heredity did not

influence the relationship between density and intake (Figure 9, right panel). Hence, individual differences in responsiveness to density are not due to heredity, and dietary density does not influence body size. Thus, the genes influence intake via effects on preferred dietary density but not its impact.

Palatability. Twin studies of the relationship between palatability and food intake demonstrated that inheritance accounts for 23% of the variance in the ratings of palatability (Figure 9, left panel; de Castro, 2001b). Similarly, genes influenced the amounts ingested in meals that were rated low in palatability and also those rated high in palatability. In addition, the difference in the amount ingested between low and high palatable meals is a metric of the responsiveness of the individual to palatability and also is heritable (Figure 9, right panel), accounting for 17% to 46% of the variance. Hence, genes influence the preferred level of food palatability and the reactivity of the individual to that palatability.

Multiple Independent Genetic Influences. Because many of these variables are intercorrelated, the apparent heritability of one or more variables may be an indirect effect resulting from covariation with another factor. To investigate this possibility, a multiple regression analysis was performed using the stomach contents, the number of other people present, the hunger ratings, and the time of day as predictors of meal size. Significant genetic effects were present for the standardized regression coefficients of all four variables (de Castro, 2002a). Hence the genes have primary influences on these variables' ability to affect food intake.

In summary, inheritance has independent influences on compensated and uncompensated factors. Heritable individual differences are present in the preferred levels of, and responsiveness to, these factors. That there are heritable preferred levels of these factors leads to the interesting suggestion that even external stimuli may have inherited "settling points." These "settling points." probably do not have specific physiological bases, but instead may result from inherited psychological characteristics. For example, the heritable preferred level of the number of other people present at meals may result from an inherited extraversion or sociability factor. Regardless, the operation of this factor causes the individual to seek out preferred levels of companionship. Functionally, this is not different from an individual seeking a preferred level of blood glucose or fat pad mass. What differs is the nature of the underlying mechanisms.

A General Model of Intake Regulation

Human food intake is affected by a wide range of compensated and uncompensated factors, each of which accounts for a small portion of the variance. Hence, any model that attempts to explain the control of intake must account for how intake can be controlled in the face of a complex array of influential variables with large individual differences. These elements recently have been incorporated into a general model of intake regulation (de Castro & Plunkett, 2002) that is presented schematically in Figure 10. The model separates uncompensated and compensated factors, and each factor has a preferred level that is influenced by heredity. The model also specifies that each factor has an individual impact factor or weight, that specifies the magnitude of its effect on intake. In the model, these weights are numbers between -1 and $+1$, are assumed to differ among individuals, and are influenced by heredity.

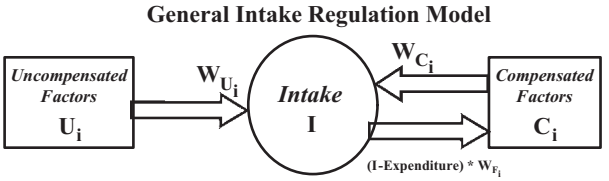

Figure 10. The general intake regulation model wherein intake (I) is controlled by two sets of factors; compensated factors (C_i) that both affect and are affected by intake via negative feedback loops and uncompensated factors (U_i) that affect but are not affected by intake. Inheritance affects the system by determining the preferred level for intake, and compensated and uncompensated factors and also by determining the level of impact of the factors on intake (W). Factors that are affected by heredity are indicated in bold.

Simulation of the Model

A computer simulation of the model was implemented to test its model's response to hypothetical changes like those that occur in the natural environment. The model's behavior could be well represented by a simple instantiation that included only four hypothetical uncompensated factors and four hypothetical compensated factors in addition to body weight. The values chosen for the model's parameters are presented in the top left corner of Figure 11. The values were arbitrarily chosen except that the sum of all positive and negative weights was set at 0 and the model was set to maintain a 60 kg body weight.

To simulate a change in the environment, the level of one of the uncompensated factors was doubled (Figure 11, top right, $x = 0.4$). The model responded with a rapid rise in the predicted body weight. At first the body weight output became unstable and oscillated, but the output then settled at an elevated body weight that was maintained as long as no further changes were made. To simulate individual differences in responsiveness, the weight for the factor was additionally manipulated. The body weight outputs of the model using seven different weights also are presented in Figure 11. Doubling the factor with a low weight produced a smaller predicted body weight increase than when a larger weight was used. Hence the model predicted that the same environmental change can result in very different effects on body weight depending upon an inherited responsiveness to the stimulus. The new level of body weight was maintained despite compensation built into the model. In other words, the model predicted that an increase in an environmental factor that promotes intake would result in a new, higher body weight, in spite of internal physiological, compensated systems that work to oppose it.

Homeostatic models of human food intake, such as the glucostatic model of Mayer (1996; Campfield *et al.*, 1996), predicted that compensatory adjustments occur on a meal-to-meal or day-to-day basis. The data, however, suggest that such adjustments, although present, are weak and do not produce a stable intake. Prior models also cannot account for the striking increase in obesity that has occurred over the last several decades. In addition, prior models postulated that a single factor is responsible for the control of ingestive behavior, yet it is clear that many separate systems are involved. Finally, these older models ignore the existence of highly influential environmental stimuli that have profound effects on intake.

The model in Figure 10 overcomes these problems, postulating that two extensive sets of factors are simultaneously active. Compensated factors both affect and are affected by intake while uncompensated factors affect but are not affected by

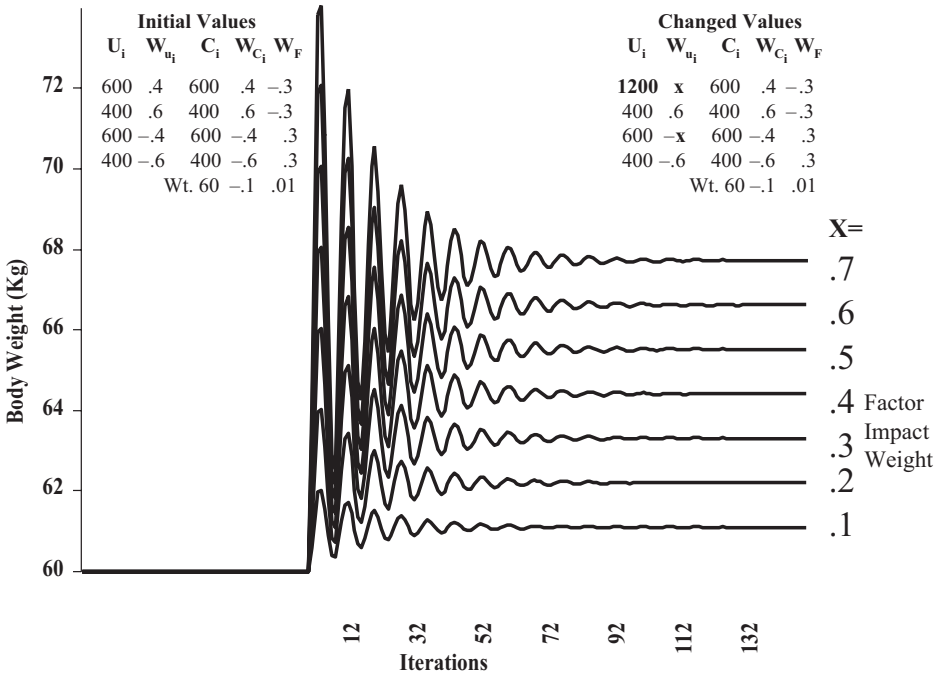

Figure 11. Results of iterations of a computer simulation of the general intake regulation model in response to a doubling of one uncompensated factor with seven different levels of impact, weights. Four hypothetical compensated factors and four hypothetical uncompensated factors with varying weights were set (parameterization is presented in the upper left) to produce a stable output from the model of 60-kg body weight. One uncompensated factor's level was doubled (parameterization is presented in the upper right) at Iteration 0. Seven iterations were performed with differing weights for the doubled uncompensated factor ("x" in upper right parameterization).

intake. Each factor also has a preferred level that is influenced by heredity. These preferred levels for the compensated factors are analogous to the concept of "set points" in prior models, but extend to uncompensated, environmental, factors. Finally, the new model postulates that the responsiveness of the individual to each factor is considerably less than perfect and that aspects of the environment are influenced by heredity. Since the genes directly affect only the individual's physiology, there are obviously intermediaries underlying the effects on the environment. For example inborn differences in personality characteristics such as extraversion (Jang, Livesley, & Vernon, 1996; Saudino, Pedersen, Lichtenstein, McClearn, & Plomin, 1997), circadian oscillators (Kolker & Turek, 1999), or the gustatory system (Matsunami, Montmayeur, & Buck, 2000) could produce proclivities to seek out and/or maintain the environment in a particular configuration or at a particular level.

The model predicts the marked increases in adiposity in modern society (Flegal, 1999; Flegal *et al.*, 2002; Mokdad *et al.*, 1999, 2003; Ogden *et al.*, 2002) in that it predicts that a maintained change in body weight would be associated with a chronic change in the environment. Several such changes have occurred in modern society including a marked decrease in activity levels, an increase in portion sizes and dietary density, and an increase in eating in restaurants. The model predicts that such changes may constitute an "obesigenic" environment (Ravussin &

Bouchard, 2000) and result in a new, higher level for body weight, exactly what is observed.

The model also can explain changes in individual body weights that occur throughout the life span. Sustained body weight changes occur most frequently during the period from the late teens to the late twenties (Pearcey, 2000) when there are large changes in life styles. Such changes in uncompensated factors predict sustained changes in weight, exactly what is observed. In addition, during aging there is a decrease in responsiveness to physiological, compensated factors (Clarkson *et al.*, 1997; Kenney & Chiu, 2001; Rolls, 1989; Rolls & Phillips, 1990) but not uncompensated factors (Blundell, 1988; de Castro, 1993d, 2002b). There also is a reduction of social stimulation in the elderly (Russell, Cutrona, de la Mora, & Wallace, 1997; Tijhuis, De Jong- Gierveld, Feskens, & Kromhout, 1999), in the pleasurable taste of food (Drewnowski, 2000; Schiffman & Graham, 2000), and even in the incidence of restaurant meals (Auty, 1992; Lahue, 2000). As the environment becomes less stimulating, the model predicts a reduction in intake and body weight as has been observed (Drewnowski & Shultz, 2001; Payette, Coulombe, Boutier, & Gray-Donald, 1999). The model suggests that this situation can be prevented or remedied by maintaining or creating a stimulating environment.

The model also is applicable to pathological conditions such as anorexia nervosa that are known to be influenced by inheritance (Bulik, Sullivan, Fear, & Pickering, 2000; Klump, Kaye, & Strober, 2001; Klump, Miller, Keel, McGue, & Iacono, 2001; Lilenfeld & Kaye, 1998). Individuals with anorexia nervosa have higher levels of dietary restraint (Bulik *et al.*, 2000), and dietary restraint is associated with reduced intake (de Castro, 1995; Laessle *et al.*, 1989; Ruderman, 1986; Tuschl *et al.*, 1990) and is influenced by heredity (de Castro & Lilenfeld, 2003). From the perspective of the model, anorexia nervosa appears as an inherited tendency to high levels of, and high responsiveness to, dietary restraint.

The model also may be applicable to fluid intake, which is affected by the compensated factors of plasma osmolarity and volume (Fitzsimons, 1961a, 1961b; Gilman, 1937; Stricker, 1968) and a number of uncompensated factors including the intake of solids that markedly affect the amount and timing of fluid intake (de Castro, 1991a, 1991e). In addition, heredity exerts a considerable influence on fluid intake (de Castro, 1993e). Hence, many of the same variable classes that affect food intake also apply to fluid ingestion, indicating that the model may be applicable here also.

It is realized that the model is more of a template than a theory of intake regulation. Considerable work remains to be done to apply the basic conceptual framework to actual intake variables, physiological systems, and environmental stimuli. In its present state, however, the model is compatible with existing knowledge, parsimonious, applicable to a wide range of conditions, and predicts the phenomena that are occurring in the modern world. The review and the model clearly underscore the fact that the control of intake in free-living humans is a complex phenomenon affected by a wide range of genetic, physiological, psychological, social, and cultural variables, all of which have large individual differences in level and responsiveness.

Acknowledgments

Supported in part by a Georgia State University Research Program Enhancement Grant.

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Body Fluid Homeostasis

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17

Thirst

Edward M. Stricker

Introduction

Thirst is a familiar sensation that can give rise to a strong motivation to seek, to obtain, and to consume water. It derives from a biological need for water in order to maintain bodily hydration. When studied in laboratory animals, the sensation and associated motivation are inferred from observed behavior. Actually, laboratory experiments typically study only the amounts of fluid that are consumed and, less commonly, how long it takes for drinking to begin, because drinking water usually is present in the cage and freely available.

It should be noted at the outset that water consumption motivated by thirst is not the only important response to dehydration. Also vital is secretion of the antidiuretic hormone, vasopressin (VP), which usually occurs concurrently with thirst and complements water ingestion (Stricker & Verbalis, 2002). VP is a peptide that is synthesized in the magnocellular neurons of the supraoptic nuclei (SON) and paraventricular nuclei (PVN) in the hypothalamus, transported along axons projecting to the nearby posterior lobe of the pituitary gland, and secreted from there into the systemic circulation (Figure 1). The hormone's main action is on VP receptors in the kidneys to promote the reabsorption of water from the renal tubules, thereby making urine more concentrated and reducing further water loss. Tiny amounts of VP are needed to create this antidiuretic effect because the hormone is very potent; for example, maximal antidiuresis is achieved in humans when blood levels of VP reach 5–6 pg/ml, which is only a few trillionths of a gram per milliliter above basal levels of 1–2 pg/ml. The importance of VP for body fluid homeostasis is well established. When VP function is impaired, as in diabetes insipidus (a disorder that typically

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Neurobiology of Food and Fluid Intake, 2nd Ed., Volume 14 of *Handbook of Behavioral Neurobiology*, edited by Edward Stricker and Stephen Woods, Kluwer Academic / Plenum Publishers, New York, 2004.

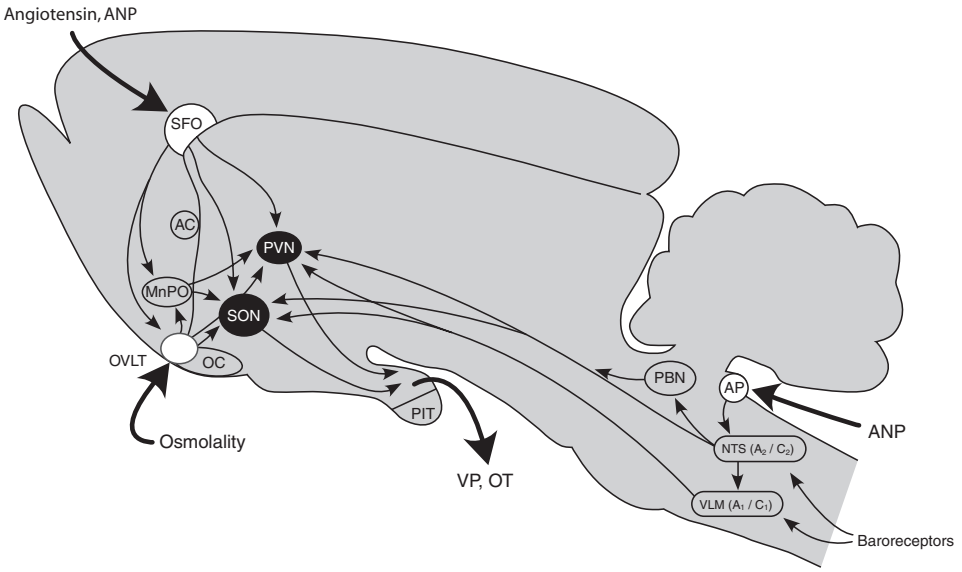

Figure 1. Summary of the main pathways that mediate secretion of vasopressin (VP) and oxytocin (OT) in rats. The vascular organ of the lamina terminalis (OVLT) is especially sensitive to the effective osmolality of plasma. Circulating angiotensin II (AngII) activates neurons of the subfornical organ (SFO), an essential site of AngII action, as well as cells throughout the lamina terminalis. In response to hyperosmolality or AngII, projections from the SFO and OVLT to the median preoptic nucleus (MnPO) activate excitatory and inhibitory interneurons that project to the supraoptic nucleus (SON) and paraventricular nucleus (PVN) to modulate direct inputs to these areas from the circumventricular organs. Baroreceptor-mediated stimuli, such as hypovolemia and hypotension, act on afferents that terminate in the nucleus of the solitary tract (NTS) and area postrema (AP). The major projection to magnocellular VP neurons appears to arise from A1 cells of the ventrolateral medulla (VLM) that are activated by excitatory interneurons from the NTS. Other areas, such as the parabrachial nucleus (PBN), may participate in multisynaptic projections. Circulating atrial natriuretic peptide (ANP) appears to activate neurons in the SFO and AP and thereby inhibit the effect of AngII. Not shown are the sodium-sensitive receptors in the three circumventricular organs, or the likely inhibitory effect of ANP on OVLT neurons. Similar (but not identical) signals and brain sites participate in the central control of thirst and salt appetite. Note that the structure and function of these circumventricular organs have been described more extensively in a recent monograph (McKinley *et al.*, 2003). Abbreviations: AC, anterior commissure; OC, optic chiasm; PIT, anterior pituitary.

results from hypothalamic disease), human patients may excrete 25 L of urine per day and adaptively drink comparable volumes of water in order to maintain water balance. The same disorder in rats results in a daily consumption of water equal in magnitude to their body weight. VP plainly allows animals the freedom to pursue their normal activities without frequent interruption to drink or urinate.

Paralleling the stimulation of thirst and VP secretion in response to dehydration is the inhibition of thirst and VP secretion that occurs when animals become overhydrated. Inhibition of thirst under this circumstance prevents further dilution of body fluids, while inhibition of VP secretion allows excess body water to be excreted in dilute urine. Thus, the two responses again are adaptively coordinated to allow appropriate adjustment to a homeostatic imbalance.

Brief introductory descriptions of body fluid homeostasis, like the one above, invariably prompt questions about the mechanisms underlying the control of thirst and VP secretion. How do animals know when they are dehydrated? What determines how much they drink? Where are the receptors that generate signals of thirst

and satiety? What neural circuits in the brain mediate thirst and VP secretion, and their integration with one another? Such questions have driven research in this field for the past century, and very substantial progress has been made in answering them. Thus, it has been clear for many years that body fluid homeostasis results, in part, from the interrelated regulations of three specific dimensions of body fluids: the osmolality of the fluids, the volume of blood, and the arterial blood pressure (ABP). Changes in each variable are known to influence thirst and VP secretion, and multiple stimuli mediating those responses, both excitatory and inhibitory, have been identified. The stimuli are an interesting mixture of neural signals and blood-borne substrates and hormones.

The present chapter will update this traditional account and, in doing so, will focus on recent findings and unsettled issues that require new speculative formulations regarding the biological bases of thirst and satiety for water. Most attention will be paid to studies in laboratory rats, although prominent results of parallel studies in other species will be included as well. Consideration of VP secretion will be included because, as mentioned above, aspects of kidney function and water consumption play complementary roles in body fluid homeostasis. Two other relevant hormones, oxytocin (OT) and atrial natriuretic peptide (ANP), also will be discussed because of their important roles in the control of fluid intake and urinary excretion, as will salt appetite and its evident integration with the control of thirst. Not considered, however, will be drinking behavior generally or the various reasons that humans drink fluids (e.g., for their flavor or for some ingredient in the beverage, such as alcohol or caffeine). The general perspective taken is analogous to current theories of hunger and satiety for food, as discussed in several chapters in this volume. Specifically, alterations in the osmolality, volume, and pressure of blood appear to provide the signals for responses that promote the long-term stability of body fluids while additionally modulating short-term signals from the gastrointestinal tract that occur during drinking bouts. All these signals appear to act and be integrated in the hypothalamus and brain stem, which therefore are as pertinent to considerations of thirst as they are to discussions of hunger and satiety.

Body Fluid Osmolality

Two variables have dominant influences on the rate of biochemical reactions in body cells. One is the temperature of cells and the other is the concentration of substrates that are involved in cellular reactions. Both variables must be relatively constant for the metabolism of body cells to remain stable. Hence, complex homeostatic mechanisms have evolved to regulate body temperature, while substrate concentrations are regulated largely by controlling the volume of water in which they are dissolved.

Body fluid osmolality is an expression of concentration, representing the ratio of solute to water in the fluid. Osmolality increases either when there is a decrease in the denominator or an increase in the numerator of this ratio. A common example of the former occurs during dehydration resulting from water deprivation. Common examples of the latter involve an osmotic load in the extracellular fluid, whether ingested, administered, or the result of renal Na^+ retention. Under each circumstance, water leaves body cells by osmosis, which has the adaptive effect of blunting the increase in osmolality of extracellular fluid. The reverse effect occurs during overhydration; water enters cells by osmosis, thus blunting the decrease in

extracellular fluid osmolality. Hence, osmosis provides a rapid, intrinsic, first line of homeostatic defense against changes in body fluid osmolality.

When body fluid osmolality is actually altered, extrinsic actions involving various behavioral and physiological responses are stimulated to restore the basal state. Thirst apparently is stimulated by a signal associated with cellular dehydration that results when the osmolality of extracellular fluid is increased. In support of this conclusion are numerous experimental results that drinking is stimulated when systemic infusion of hypertonic NaCl solution causes an osmotic movement of water out of cells. In contrast, much less drinking results from the delivery of equimolar solutions containing urea or glucose, which diffuse freely through cell membranes and therefore do not cause osmosis (Adolph, Barker, & Hoy, 1954; Gilman, 1937). The signal is not necessarily provided by a specific increase in extracellular Na^+ concentration because intravascular infusion of sodium-free hyperosmotic solutions containing nonpermeant solutes such as mannitol, sorbitol, or sucrose are just as effective as equiosmotic NaCl solutions in stimulating thirst (Fitzsimons, 1961a; Holmes & Gregersen, 1950; Schoorlemmer, Johnson, & Thunhorst, 2000). This stimulus increases linearly in proportion to increases in plasma osmolality (pOsm) above a threshold of 1–2% (Fitzsimons, 1963; Wolf, 1950), with intakes proportional to dehydration and sufficient to restore water balance (Adolph *et al.*, 1954; Fitzsimons, 1961a). In addition, the motivational properties of the stimulus in laboratory animals have been established by experiments in which dehydration elicits learned operant behaviors leading to water reinforcements.

Paralleling thirst is neurohypophyseal secretion of VP, which also occurs in response to increases in effective osmolality of blood plasma (Dunn, Brennan, Nelson, & Robertson, 1973; Verney, 1947). The increase in pOsm that provides the threshold stimulus for VP secretion appears to be a little lower than it is for thirst (Robertson, Shelton, & Athar, 1976). This arrangement allows behavior to continue without disruption by thirst until the physiological response of renal water conservation becomes insufficient for dealing with progressive dehydration. Also low is the threshold at which osmotic dilution inhibits VP secretion and thereby allows excess water to be excreted in dilute urine (Baertschi & Pence, 1995). Note that more OT is secreted than VP when pOsm is elevated in rats (Brimble, Dyball, & Forsling, 1978; Stricker & Verbalis, 1986). Like VP, OT is a peptide hormone synthesized in hypothalamic magnocellular neurons and released from the posterior lobe of the pituitary; unlike VP, OT is a potent stimulus of renal Na^+ excretion, and so it contributes to osmoregulation by lowering pOsm (Balment, Brimble, & Forsling, 1980; Huang, Lee, & Sjöquist, 1995; Soares *et al.*, 1999; Verbalis, Mangione, & Stricker, 1991).

The complementary contributions of thirst and neurohypophyseal hormone secretion to osmoregulation can be seen in many experiments that studied both responses to an osmotic load. Thus, for example, a NaCl load elicited a more substantial increase in water intake in rats after renal function was eliminated by bilateral nephrectomy (Fitzsimons, 1961a). Conversely, when daily water intake was limited, ingestion of a salt load by dogs stimulated larger increases in VP secretion, which promoted more urinary water conservation and thereby blunted the induced increase in pOsm (Cowley, Skelton, & Merrill, 1986). Dehydrated rats given only 0.3M NaCl to drink consumed very large amounts and restored pOsm to normal levels by excreting comparable volumes of urine that was more concentrated than 300 mEq Na^+/L. When further challenged with an injected NaCl load, the animals did not increase their fluid intakes but osmoregulated by excreting

more concentrated urine (Stricker *et al.*, 2001). Another example of flexibility in osmoregulation can be seen in the variable responses of individual dogs to a salt load given orally by stomach tube; some animals increased water intake while conserving little urinary water whereas other animals drank little water while excreting very concentrated urine (Holmes & Gregersen, 1950; Kanter, 1953).

It is important to note that the magnitude of water intake stimulated by an osmotic load is quantitatively predictable when it is assumed that the body acts like a perfect osmometer (Wolf, 1950). Thus, by knowing the net osmotic load (usually twice the difference between Na^+ intake and excreted Na^+) and the water balance (usually water intake minus urine volume) in a short-term test, it is easy to compute the amount of water required to dilute elevated body fluid osmolality to normal levels. Those calculated values closely resemble the amounts that animals actually consume in response to systemic injection of hypertonic NaCl solution.

All peripheral body cells lose water by osmosis when the effective osmolality of extracellular fluid increases. Thus, the osmoreceptor cells that stimulate thirst and VP secretion are not unique in their osmosensitivity, unlike the sensitivity of retinal cells to photons. Instead, the osmoreceptors are unique in their neural circuitry. Specifically, the pioneering investigations of Verney (1947) and Andersson (1953) suggested that these cells were located in the anterior hypothalamus, and subsequently osmoreceptors were localized in the vascular organ of the lamina terminalis (OVLT), a midline structure adjacent to the 3rd cerebral ventricle and ventral to the anterior commissure (Figure 1). Like the two other circumventricular organs mentioned below but unlike other brain neurons, the OVLT is highly vascular and lacks a blood–brain barrier, which allows it to respond to changes in systemic pOsm. The cellular mechanism responsible for osmosensitivity appears to involve nonselective cation channels in OVLT cells (Bourque, Oliet, & Richard, 1994). Thus, during conditions of osmotic concentration, the efflux of water shrinks cellular membranes and thereby activates the channels, whereas the reverse happens during conditions of osmotic dilution.

The OVLT sends neural afferents to the magnocellular hypothalamic neurons involved in VP secretion (Camacho & Phillips, 1981; Wilkin, Mitchell, Ganten, & Johnson, 1989), while thirst is mediated by some other neural circuit that has not yet been identified. Thus, experimental damage to the anterior hypothalamus including the OVLT in rats significantly reduced water intake and secretion of VP in response to the administration of hypertonic saline (Buggy & Johnson, 1977; Johnson & Buggy, 1978). Similar observations have been made in goats and sheep (Andersson, Leksell, & Lishajko, 1975; McKinley *et al.*, 1982), in dogs with more discrete surgical lesions of the OVLT (Thrasher & Keil, 1987; Thrasher, Keil, & Ramsay, 1982), and in human patients with brain tumors that destroyed the OVLT and surrounding region (Robertson, Aycinena, & Zerbe, 1982). Complementing these findings are the results of studies showing that damage to the anteroventral hypothalamus impaired VP release from hypothalamo-neurohypophyseal explants in response to osmotic stimulation (Sladek & Johnson, 1983). In addition, an increased expression of Fos (the protein product of the immediate-early gene, *c-fos*, which is a useful marker of cell activation; Hoffman, Smith, & Verbalis, 1993) occurred in OVLT neurons after systemic administration of hyperosmotic solutions (Oldfield, Badoer, Hards, & McKinley, 1994; Oldfield, Bicknell, McAllen, Weisinger, & McKinley, 1991b).

Another circumventricular organ in the brain that influences thirst and VP secretion in response to increases in systemic pOsm is the subfornical organ (SFO), a highly vascularized midline structure that protrudes into the anterior dorsal portion

of the 3rd cerebral ventricle near the interventricular foramen (Figure 1). Thus, neuronal activity in the SFO of rats increases after systemic injection of hypertonic saline (Gutman, Ciriello, & Mogenson, 1988; Oldfield *et al.*, 1991b). Although surgical destruction of the SFO does not eliminate the drinking response of rats to an osmotic load (Lind, Thunhorst, & Johnson, 1984; Simpson, Epstein, & Camardo, 1978), it does raise the threshold for this response (Hosutt, Rowland, & Stricker, 1981; Mangiapane, Thrasher, Keil, Simpson, & Ganong, 1984). Furthermore, damage to efferent fibers from the SFO that project to the ventral portions of the lamina terminalis abolished the drinking response of rats to a low dose of hypertonic saline injected subcutaneously (Lind & Johnson, 1982), which allows the possibility that the SFO sensitizes OVLT neurons to elevations in pOsm. Other efferent fibers from the SFO project to the magnocellular hypothalamic neurons (Lind, Van Hoesen, & Ganten, 1982; Miselis, 1981), and VP secretion in response to an osmotic load is attenuated in rats with SFO lesions (Mangiapane *et al.*, 1984).

The other circumventricular organ of relevance is the area postrema (AP), a neural structure that is located at the dorsal surface of the medulla just ventral to the 4th cerebral ventricle (Figure 1). Neurons from the AP influence the magnocellular cells in the hypothalamus indirectly, via the nucleus of the solitary tract (NTS) (Shapiro & Miselis, 1985; Tribollet, Armstrong, Dubois-Dauphin, & Dreifuss, 1985) and the parabrachial nucleus (Saper & Loewy, 1980). Aspiration lesions of the AP in rats impair VP secretion and renal water conservation in response to systemic injection of hypertonic saline (Arima *et al.*, 1998; Curtis, Huang, Sved, Verbalis, & Stricker, 1999; Huang, Sved, & Stricker, 2000a; Iovino, Papa, Monteleone, & Steardo, 1988; Stricker *et al.*, 2001). In contrast, AP lesions in rats did not affect VP secretion in response to systemic injection of equimolar hypertonic mannitol solution (Huang *et al.*, 2000a). Those startling findings indicate that the neural circuits for control of VP secretion must be more complex than the usual schema, which have focused exclusively on the hypothalamic osmoreceptors. New speculative formulations of the osmoregulatory control of VP secretion likely will include the hypothesis that the rat forebrain contains both osmoreceptors and Na^+-receptors, like goats and sheep (Andersson & Olsson, 1973; McKinley, Denton, & Weisinger, 1978) but unlike dogs (Thrasher, Brown, Keil, & Ramsay, 1980). Consistent with this view is the recent report that voltage-gated sodium channels in the OVLT and SFO (and presumably in the AP, as well) respond to alterations in extracellular Na^+ concentration but not to changes in osmolality (Hiyama *et al.*, 2002). Whatever the mechanism, the central circuit should include a role for the AP as a relay station for neural input from visceral osmo- or Na^+-receptors in the short-term control of VP secretion or water consumption, as discussed below.

Another structure of apparent significance in the central control of osmoregulation is the median preoptic area (MnPO), also known as the ventral or subcommissural portion of the nucleus medianus. This midline structure is located in the lamina terminalis along the rostral border of the 3rd cerebral ventricle (Figure 1), and it receives neural input both from the OVLT and the SFO (Camacho & Phillips, 1981; Miselis, 1981; Saper & Levisohn, 1983). Neurons in the MnPO project to the magnocellular cells in the SON (Oldfield, Miselis, & McKinley, 1991a; Tribollet *et al.*, 1985), and osmoregulatory VP secretion is impaired in rats with MnPO lesions (Gardiner, Verbalis, & Stricker, 1985; Mangiapane, Thrasher, Keil, Simpson, & Ganong, 1983). Intravenous infusion of hyperosmotic solutions increases Fos expression and neuronal activity in the MnPO (McAllen, Pennington, & McKinley, 1990; Oldfield *et al.*, 1991b), while electrolytic lesions of the MnPO in rats attenuate

drinking in response to injections of hypertonic saline (Mangiapane *et al.*, 1983). Note that thirst was disrupted mainly during the light portion of a light–dark cycle (Gardiner & Stricker, 1985), as if the lesions disrupted a circadian rhythm of sensitivity to the osmotic load (although see also Cunningham, Beltz, Johnson, & Johnson, 1992). Furthermore, rats with electrolytic MnPO lesions drank when a salt load injected during the light period was given after an activating dose of caffeine (Gardiner & Stricker, 1985), as if the lesions disrupted the arousal systems that influence drinking behavior.

The posterolateral hypothalamus once was considered to be the "drinking center," based in part on findings that electrolytic lesions of this brain site caused rats to be unresponsive to systemic injection of hypertonic saline (Epstein & Teitelbaum, 1964). Later reports, however, indicated that the hypothalamic lesions disrupted dopaminergic fibers of passage, and that similar effects on behavior were produced by specific, neurotoxin-induced dopamine-depleting brain lesions in rats (Stricker, 1976; Stricker & Zigmond, 1974; Ungerstedt, 1971). It may be concluded that lateral hypothalamic lesions cause behavioral dysfunctions that resemble the impaired ability to initiate movement seen in Parkinson's disease, which also results from the loss of dopaminergic neuronal fibers of passage. More recently, it has become clear that surgical lesions of the lateral hypothalamic area, including the perifornical lateral hypothalamus, additionally damage neurons containing orexin (also known as hypocretin). Those cells project broadly to sites in the forebrain, midbrain, and brainstem, and appear to play an important role in the integrated control of food intake and arousal (Rodgers, Ishii, Halford, & Blundell, 2002; Willie, Chemelli, Sinton, & Yanagisawa, 2001), although there is reason to extend those discussions to include the control of water intake as well (Kunii *et al.*, 1999; Mogenson & Stevenson, 1967).

After surgical elimination of all neural connections between the hindbrain and higher brain areas, the chronic decerebrate rat cannot approach water or initiate appetitive behavior. Nonetheless, thirst can be studied by measuring how much fluid is swallowed when fluid is delivered directly into the mouth via an intraoral cannula. In contrast to neurologically intact control rats, decerebrate rats did not increase their water intake when dehydrated or given hypertonic saline systemically (Grill & Miselis, 1981). These findings are consistent with the common assumption that the controls of osmoregulatory thirst are located in the cerebral hemispheres, and that the failure of decerebrate rats to increase water intake is due to the disconnection of motivational systems in the forebrain from hindbrain systems controlling swallowing. Thus, it is possible that these animals experienced thirst but simply were not able to behave appropriately.

Recent studies of the human brain, using positron emission tomography, allow inquiries into the brain areas involved in the central circuitry mediating the sensation of thirst. This important work has suggested that the cingulate cortex and cerebellum play a role in the genesis of thirst (Denton *et al.*, 1999a, 1999b; Parsons *et al.*, 2000). Because those brain sites have received little attention to date in studies of water ingestion by experimental animals, further investigations are needed to determine their significance.

Blood Volume

Until the early 1960s, the only established stimulus of thirst was cellular dehydration associated with an increase in the effective osmolality of extracellular fluid.

However, it was certainly recognized that elaborate neural and endocrine mechanisms had evolved to maintain blood volume and pressure, among which was the secretion of VP in response to hypovolemia. It was therefore not surprising to discover that an experimental loss of blood volume also stimulated thirst, analogous with the common signal for VP secretion and thirst that is generated when pOsm is elevated.

Several models of hypovolemia were used to investigate this issue. One, hemorrhage (Fitzsimons, 1961b; Oatley, 1964), seems straightforward but actually it is rather complicated and produces inconsistent results. In explanation, small blood loss can be replaced rapidly by the movement of interstitial fluid into the vasculature, whereas large blood loss may produce hypotension and anemia that interfere with drinking behavior. An alternative model involved salt depletion (Cizek, Semple, Huang, & Gregersen, 1951; Holmes & Cizek, 1951), wherein the effective osmolality of extracellular fluid is reduced and water flows into cells by osmosis. However, as explained below, this model actually provides a conflicting mixture of signals, excitation due to hypovolemia and inhibition due to osmotic dilution, and so its short-term effects also were not consistent. A third model involved ligation of the inferior vena cava or suprarenal constriction of the aorta (Fitzsimons, 1969; Fitzsimons & Moore-Gillon, 1980), thereby abruptly reducing venous return to the heart and affecting cardiac baroreceptors that were presumed to provide the relevant signals. However, this model always caused acute arterial hypotension, which itself stimulates drinking behavior (as discussed below). A fourth model, involving traumatic tissue damage (Stricker, 1966, 1980), reduced blood volume as plasma leaked out through damaged capillaries; although such models consistently stimulated water intake, they stopped being used when they no longer conformed to modern sensibilities or to contemporary rules of animal care and use set by the U.S. National Institutes of Health.

A fifth model, involving extravascular administration of a hyperoncotic colloid solution (Fitzsimons, 1961b; Stricker, 1966), has proven to be a benign and reliable treatment with which to induce hypovolemia and thirst in rats. The colloidal solution opposes the equilibrium of fluid movement across the capillary walls near the injection site, so that plasma leaving the blood vessels due to hydrostatic pressure (caused by the contraction of the heart) cannot return readily, and isosmotic extracellular fluid gradually accumulates in the local interstitial fluid. The effect of colloid treatment on plasma volume is orderly; the larger the volume and more concentrated the solution that is injected, the larger the local edema and the greater the plasma volume deficit. This sequestration of fluid prevents the normal restoration of plasma volume from endogenous extravascular reservoirs. Indeed, even ingested fluids are not at first retained in the circulation but are leached into the injection area, and so fluid volumes considerably larger than plasma deficits must be consumed for plasma volume to be restored. Hypovolemia produced in this way lasts for many hours, during which water intake is increased in proportion to the extravascular fluid accumulation (Fitzsimons, 1961b) and the induced plasma volume deficit (Leenen & Stricker, 1974; Stricker, 1968). Beyond a threshold value of ~5%, water consumption is linear up to a plasma volume loss of at least 30%. Comparable intakes are obtained when colloid-treated rats are required to press a lever for water (Stricker, 1968), demonstrating that hypovolemia elicits a motivational state of thirst rather than mere reflexive drinking.

Complementing thirst during hypovolemia is antidiuresis, resulting both from an exponential increase in VP secretion (Dunn *et al.*, 1973; Stricker & Verbalis,

1986) and from a progressive decrease in renal blood flow and glomerular filtration rate. A parallel increase in OT secretion also is stimulated by hypovolemia (Stricker & Verbalis, 1986), although the expected natriuresis does not occur in part because of the dominant sodium-conserving effects of aldosterone (Verbalis *et al.*, 1991), whose secretion is stimulated as well (Stricker, Vagnucci, McDonald, & Leenen, 1979). Instead, circulating OT appears to make several important contributions to cardiovascular homeostasis. For example, elevated plasma OT (pOT) is one of many stimuli in rats that increase renin secretion from the kidneys during hypovolemia or arterial hypotension (Huang, Sjöquist, Skott, Stricker, & Sved, 2000, 2001; Sjöquist *et al.*, 1999), which helps to support ABP in part through the formation of angiotensin II (AngII), a very potent pressor agent.

The identification of hypovolemia as a stimulus of thirst allowed investigations of the interaction between osmoregulatory and volume regulatory controls of water intake and VP secretion. When thirst was stimulated by concurrent treatments that increased pOsm while also decreasing plasma volume, the resultant water consumption suggested an additivity of the two stimuli (Blass & Fitzsimons, 1970; Corbit, 1968; Oatley, 1964; Stricker, 1969). The same was true of VP secretion under these experimental circumstances (Dunn *et al.*, 1973; Quillen & Cowley, 1983; Stricker & Verbalis, 1986). In other experiments, the concurrent treatments were selected to have opposing effects on thirst and VP secretion. For example, in one study, hypovolemic rats were given a gastric water load, which was not excreted rapidly in urine, and the induced osmotic dilution was found to inhibit thirst (Stricker, 1969). The magnitude of this effect was striking: A 5–7% osmotic dilution completely inhibited water intake when the plasma volume depletion was 30%. Indeed, osmotic dilution was found to constrain water intake even when thirst was stimulated by the combination of caval ligation and subcutaneous colloid treatments (Stricker, 1971), an experimental regimen that produced intakes of isotonic saline in excess of 300 ml/24 hr. Osmotic dilution also produced a potent inhibition of VP and OT secretion in hypovolemic rats (Stricker & Verbalis, 1986).

Similar experiments have determined whether acute hypervolemia provides an inhibitory signal in the control of thirst and VP secretion. In contrast to the stimulation of thirst by hypovolemia, expansion of plasma volume by a large intravenous infusion of isotonic saline did not inhibit the water consumption of rats pretreated with an injection of hypertonic saline (Corbit, 1965; Fitzsimons, 1961a). On the other hand, much smaller increases in plasma volume were sufficient to decrease VP secretion in animals with elevated pOsm (Quillen & Cowley, 1983; Robertson *et al.*, 1976). Cumulatively, these findings may be contrasted with the symmetrical arrangement whereby VP secretion was increased when pOsm was elevated and decreased when pOsm was below normal. Although the volume- and osmoregulatory controls of VP and thirst are similar, evidently they are not identical (Stricker & Sved, 2002).

Thirty years ago, Fitzsimons (1969; Fitzsimons & Simons, 1969) discovered that systemic administration of renin or AngII produced a prompt, dose-related increase in water intake in rats. Subsequent investigations revealed that AngII has a potent dipsogenic effect in a great variety of experimental subjects including birds, reptiles, and fish in addition to other mammals. Since the renin–angiotensin system is activated by hypovolemia in proportion to colloid-induced water intakes in rats (Leenen & Stricker, 1974), it was reasonable to ask whether the increased plasma AngII levels were causally related to thirst. Several experiments addressed that question. One approach was to produce hypovolemia in nephrectomized rats.

The rats were found to consume the same amount of water as intact control animals (Fitzsimons, 1961b; Stricker *et al.*, 1979), indicating that the renin–angiotensin system is not necessary for mediating the induced water intake. However, these findings do not reveal whether AngII serves as a dipsogen during hypovolemia in intact rats. Moreover, it was unclear whether the elevated baseline intakes of the anuric rats were additive with thirst due to hypovolemia. If they were, then nephrectomy actually reduced the water intake stimulated by hypovolemia, thus suggesting a contribution of AngII to thirst elicited by colloid treatment in intact rats.

Another approach involved investigations of water intake after colloid treatment in rats with SFO lesions. Although AngII cannot penetrate the brain from the systemic circulation, it can stimulate thirst by acting on AngII receptors in the SFO (Mendelsohn, Quirion, Saavedra, Aguilera, & Catt, 1984; van Houten, Schiffrin, Mann, Posner, & Boucher, 1980) because, as mentioned, the SFO lacks a blood–brain barrier. Drinking in rats can be stimulated by injection of AngII into the SFO in femtomole amounts (Simpson *et al.*, 1978), demonstrating the remarkable potency of the peptide as a dipsogen. In addition, expression of *c-fos* activity is elevated in the SFO when AngII is injected into the circulation (Oldfield *et al.*, 1994; Rowland *et al.*, 1994). Functional studies indicated that drinking elicited by systemic injections of AngII was abolished or severely attenuated by SFO lesions (Abdelaal, Assaf, Kucharczyk, & Mogenson, 1974; Simpson & Routtenberg, 1975) or by administration of AngII receptor blockers into the SFO (Fitts, 1994; Simpson *et al.*, 1978). Thus, when rats with SFO lesions were found to drink little or no water in response to subcutaneous injections of 10% or 20% solutions of polyethylene glycol (PEG), those findings were interpreted to signify that AngII was largely responsible for mediating thirst during hypovolemia (Simpson *et al.*, 1978). Later experiments replicated those findings, but they also showed that rats with SFO lesions responded normally when given subcutaneous injections of 30% PEG solution (Hosutt *et al.*, 1981), which induced larger plasma volume deficits. Collectively, these data are consistent with the possibility that AngII normally plays a role in stimulating thirst during hypovolemia by reducing the volume deficit required to provide stimulation.

The opposite conclusion may be drawn from studies in rats with electrolytic lesions of the septal area, which show an increased sensitivity to the dipsogenic effects of AngII (Blass, Nussbaum, & Hanson, 1974). If AngII provides an important signal for thirst during hypovolemia, then rats with septal area lesions should drink much more water than control rats in response to PEG treatment. In fact they do, but it is interesting to note when this effect occurs; no increase in water intake was seen during the first 8–9 hr after subcutaneous injection of colloid solution (Blass *et al.*, 1974), by which time plasma volume deficits were 25% and plasma renin activities increased to values 20–40 times greater than normal. However, by 12 hr posttreatment those animals drank (and retained) unusually large amounts of water (Stricker, 1978), suggesting that AngII was contributing significantly to thirst then. In other words, it appears from these experiments that AngII normally contributes little to thirst during progressive hypovolemia in intact rats until plasma volume deficits become substantial.

The contributions of hypovolemia and AngII to thirst also have been studied in rats during dehydration. Body fluids are contracted after 1 or 2 days of water deprivation; pulmocutaneous water losses tend to increase pOsm and thereby reduce cell volume, while natriuresis, mediated by OT (Huang, Lee, Arnason, & Sjöquist, 1996), adaptively blunts that increase but reduces extracellular fluid volume (McKinley, Denton, Nelson, & Weisinger, 1983; Schoorlemmer & Evered,

1993) and slightly elevates blood levels of AngII (Mann, Johnson, & Ganten, 1980). Because expansion of plasma volume by an intravenous infusion of isotonic saline or rat serum had little (Ramsay, Rolls, & Wood, 1977) or no impact on the water consumption of rats made thirsty by overnight water deprivation (Corbit, 1967; Corbit & Tuchapsky, 1968), it appeared that cellular losses provide at least two thirds of the stimulus for thirst in rats. On the other hand, pharmacological blockade of the renin–angiotensin system significantly attenuated the drinking response of rats to 1 or 2 days of water deprivation in some studies (Barney, Threatte, & Fregly, 1983; Franci, Kozlowski, & McCann, 1989; Malvin, Mouw, & Vander, 1977), though not invariably (Lee, Thrasher, & Ramsay, 1981), and thus AngII (and hypovolemia) may contribute more to thirst during dehydration than was initially thought.

Baroreceptors located in the relatively distensible great veins and right atrium can sense a fall in blood volume. These receptors do not actually measure blood pressure as their name implies but instead detect changes in the fullness of the vessels, much like stretch receptors located on the outer walls of the bladder and stomach communicate a sense of fullness in those organs. Vagal afferents from cardiac baroreceptors project to the AP and to the lateral portions of the NTS in the caudal brain stem, at the same rostral-caudal level as the AP (Kalia & Mesulam, 1980). Neurons from the NTS, in turn, project to the magnocellular hypothalamic neurons (Ter Horst, deBoer, Luiten, & van Willigen, 1989) and thereby influence VP secretion, and to the MnPO (Saper & Levisohn, 1983) and thereby may influence thirst. Destruction of the atrial baroreceptors in sheep eliminates the drinking response to hypovolemia produced by ultrafiltration of the blood, without affecting osmoregulatory thirst (Zimmerman, Blaine, & Stricker, 1981). A similar loss of the drinking response to hypovolemia was produced by cardiac denervation in dogs (Quillen, Keil, & Reid, 1990). In rats, drinking in response to colloid-induced hypovolemia was abolished by inflating a small balloon in the superior vena cava near the junction of the right atrium, thereby masking the volume deficit from the baroreceptors; the specificity of this procedure was confirmed by control experiments indicating that osmoregulatory thirst was not affected (Kaufman, 1984).

On the other hand, destruction of vagal projection sites to the brain stem by NTS lesions did not diminish either water intake or VP secretion during colloid-induced hypovolemia in rats (Schreihofer *et al.*, 1999; Schreihofer, Stricker, & Sved, 1994). These findings indicate that the neural signal from cardiac baroreceptors is not indispensable for these two responses, and suggest that AngII became critical under those circumstances. However, colloid-induced hypovolemia stimulated normal water intake in rats with NTS lesions, which were given a large dose of captopril to inhibit angiotensin-converting enzyme and block endogenous synthesis of AngII (Schreihofer, Sved, & Stricker, unpublished observations). Furthermore, VP secretion elicited by hemorrhage in rats with NTS lesions was not affected by such captopril treatment, but it was significantly diminished both by renal denervation and by bilateral nephrectomy (Schreihofer, Hoffman, & Sved, 1997). Collectively, these results suggest that thirst and VP secretion can be stimulated by a renal signal of blood volume deficit that does not involve either a neural input through the NTS or blood-borne AngII. In this regard, occlusion of the renal artery or vein excites renal afferent nerves, which appear to communicate a neural signal that courses through the spinal cord and ventrolateral medulla en route to the SFO and the magnocellular hypothalamic neurons, and stimulates VP release (Ciriello, 1997, 1998; Simon, Kasting, & Ciriello, 1989). Additional work is needed to determine whether this neural signal also contributes to the control of thirst during hypovolemia.

Many experimental procedures have been used to alter ABP. Acute arterial hypotension can be produced readily by a large and rapid hemorrhage, which of course reduces blood volume as well. However, diverse treatments can be used to separate hypovolemia from hypotension. For example, subcutaneous colloid treatment causes hypovolemia but not hypotension in rats, whereas drugs that reduce peripheral vascular resistance (e.g., the β-adrenergic agonist isoproterenol) lower ABP without reducing blood volume. Similarly, rapid delivery of isotonic saline into the circulation increases ABP, whereas drugs that constrict peripheral blood vessels (e.g., the α-adrenergic agonist phenylephrine) increase ABP without changing blood volume.

Arterial hypotension in intact rats is known to stimulate thirst (Lehr, Mallow, & Krukowski, 1967), with water intake increasing in proportion to the induced decrease in ABP (Evered, 1990; Hosutt, Rowland, & Stricker, 1978; Hosutt & Stricker, 1981). Renin secretion from the kidneys and elevated blood AngII levels accompany these effects (Johnson, Mann, Rascher, Johnson, & Ganten, 1981; Leenen & McDonald, 1974; Peskar, Meyer, Tauchmann, & Hertting, 1970). Once it was known that intravenous infusion of AngII stimulated thirst, it was reasonable to determine the role of the renin–angiotensin system in thirst during arterial hypotension. In one influential study, surgical removal of the kidneys was found to abolish water intake when rats were treated either with isoproterenol or other hypotensive agents (Houpt & Epstein, 1971). The loss of drinking could not be attributed to anuria because bilateral ligation of the ureters had no effect on the induced water consumption. These findings allowed the conclusion that AngII was necessary for thirst in rats during arterial hypotension. However, AngII is a potent pressor agent, and later studies indicated that the nephrectomized rats were much more hypotensive than intact control rats receiving the same drug treatment (Hosutt *et al.*, 1978; Hosutt & Stricker, 1981). Furthermore, the marked drop in ABP appeared to be debilitating because rats made thirsty by overnight water deprivation before nephrectomy and injection of isoproterenol consumed much less water than comparably treated control rats (Stricker, 1977). Thus, it seemed plausible that the severe hypotension is incompatible with drinking behavior, leaving unsettled the physiological role of AngII in mediating thirst.

Several approaches have been taken to investigate this issue. One group of experiments studied nephrectomized rats and determined the effects of drug-induced decreases in ABP that were not debilitating; in no circumstance did drinking occur (Atkinson, Kaesermann, Lambelet, Peters, & Peters-Haefeli, 1979; Evered, 1990; Evered & Robinson, 1981). Other studies found that the drinking response to arterial hypotension was abolished or severely attenuated in rats with SFO lesions (Fitts, 1994; Simpson *et al.*, 1978). In another approach, water intake was not increased when ABP was lowered by hydralazine, a selective arteriolar vasodilator that stimulates renin secretion but disrupts the formation of AngII and its dipsogenic action (Stocker, Sved, & Stricker, 2000). Thus, neural input into the brain stem from arterial baroreceptors evidently does not elicit thirst independently of blood-borne AngII in rats; instead, it is established by now that AngII mediates thirst in response to arterial hypotension in rats. The interaction of acute arterial hypotension or systemic AngII with other signals for thirst appears to be additive (Fitzsimons & Simons, 1969; Hosutt & Stricker, 1981).

Another early concern about AngII as an endogenous dipsogen derived from observations that intravenous AngII elicited drinking in association with much higher blood levels of AngII than those seen during arterial hypotension (Abraham, Baker, Blaine, Denton, & McKinley, 1975; Pawloski & Fink, 1990; Stricker, Bradshaw, & McDonald, 1976; Van Eekelen & Phillips, 1988). Thus, hypotensive rats did not seem to have enough circulating AngII to account for the large amounts of water that were consumed. To address that issue, Evered and colleagues proposed that the acute increase in ABP that occurs when AngII is administered provides a signal that inhibits thirst, thereby attenuating its dipsogenic effect. In support of this hypothesis, they found that water intake elicited by AngII was elevated progressively when concurrent administration of a vasodilating agent caused graded reductions in the induced hypertension (Evered, 1990; Evered, Robinson, & Rose, 1988; Robinson & Evered, 1987). Similar observations have been reported in dogs (Kucharczyk, 1988). In short, it is now clear that exogenous AngII provides a mixed stimulus for thirst, with both excitatory and inhibitory components, and endogenous AngII can stimulate more water consumption during arterial hypotension because there is no inhibitory component in that circumstance.

Later work confirmed this hypothesis by showing that water intake elicited by AngII was enhanced by surgical lesions of the NTS in rats, which eliminate inputs from arterial and cardiac baroreceptors and thereby prevent the animals from detecting the induced increase in ABP (Schreihofer, Stricker, & Sved, 2000). An increase in AngII-induced water consumption also was observed after complete surgical destruction of the sinoaortic baroreceptors in dogs (Klingbeil, Brooks, Quillen, & Reid, 1991) or rats (Stocker, Stricker, & Sved, 2002), for the same reason. In addition, thirst elicited by intracerebroventricular AngII was enhanced by intravenous infusion of a vasodilator to lower ABP (Thunhorst & Johnson, 1993, 2001). It therefore seems likely that arterial hypotension increases water intake not only by activating the renin–angiotensin system but also by removing all inhibitory input from baroreceptors.

An inhibitory effect of acute hypertension on water intake also has been shown to occur in rats made thirsty by intravenous hypertonic saline or subcutaneous PEG solution (Stocker, Stricker, & Sved, 2001). This inhibition of drinking was proportional to the induced increase in ABP up to 160 mmHg, beyond which further decreases in water intake did not occur (thus resembling the limits of acute hypertension that produce a reflexive reduction in heart rate). Furthermore, the latency to drink was lengthened in proportion to the induced increase in ABP, which suggests that the lower intakes resulted from diminished thirst rather than from enhanced inhibitory signals produced by water consumption.

A full account of the drinking response to AngII would consider both circulating AngII levels and the number of AngII receptors in the SFO, not to mention other post-receptor factors. This consideration is complicated by the fact that the number of receptors is plastic; for example, it increases significantly when rats consume and retain large amounts of NaCl (De Nicola, Seltzer, Tsutsumi, & Saavedra, 1993). Thus, the drinking response of rats to acute arterial hypotension was not impaired by pretreatment maintenance on high salt diet, even though renin secretion was markedly diminished; the induced water intake was abolished by systemic treatment with captopril and therefore appears to reflect an increase in the dipsogenic potency of AngII (Stocker, Smith, Kimbrough, Stricker, & Sved, 2003). Conversely, the drinking response to intracerebroventricular AngII was diminished in rats maintained on salt-deficient diet, as was the number of AngII receptors in the SFO (Mann *et al.*, 1980; Ray, Ruley, & Saavedra, 1990).

Expression of *c-fos* activity is elevated in the SFO, OVLT, and MnPO when AngII is injected into the circulation (Oldfield *et al.*, 1994; Rowland *et al.*, 1994). Thirst is abolished or severely attenuated in response to systemic AngII in rats with lesions of these brain areas (Eng & Miselis, 1981; Fitts, 1994; Lind & Johnson, 1982; Mangiapane *et al.*, 1983; Simpson *et al.*, 1978), although OVLT lesions have little effect on drinking in response to arterial hypotension (Fitts, 1994). It is important to note that AngII is a neurotransmitter in efferent neural pathways from the SFO (Lind, Swanson, & Ganten, 1985); evidently, renin, angiotensinogen, and angiotensin-converting enzyme all are present in the brain (Wright & Harding, 1997). This arrangement has several implications with regard to thirst. First, in addition to its action as a blood-borne hormone, AngII likely is dipsogenic as a neurotransmitter released in the brain when AngII of renal origin acts in the SFO. Second, if angiotensinergic efferent neurons projecting from the SFO do play a role in mediating thirst, then AngII receptors in the OVLT, MnPO, or elsewhere in the brain (including the SFO) likely are activated when intracerebroventricular AngII stimulates water intake (Oldfield *et al.*, 1994). Third, the presence of angiotensin-converting enzyme in the brain provides an explanation for paradoxical observations that systemic administration of captopril in rats may increase water intake during hypovolemia or arterial hypotension (Evered & Robinson, 1984; Fitzsimons & Elfont, 1982; Lehr, Goldman, & Casner, 1973; Stricker *et al.*, 1976). That is, the drug given in low doses blocks the conversion of angiotensin I to AngII in the periphery but not in the SFO (or elsewhere in the brain), where production of AngII increases water intake (Lehr *et al.*, 1973; Schiffrin & Genest, 1982; Thunhorst, Fitts, & Simpson, 1989).

An acute decrease in ABP also stimulates VP secretion in rats (Knepel & Meyer, 1980; Schiltz, Hoffman, Stricker, & Sved, 1997). Although arterial hypotension is known to stimulate renin secretion (Leenen & McDonald, 1974; Peskar *et al.*, 1970), AngII is known to stimulate VP secretion (Ferguson & Renaud, 1986), and direct neural connections between the SFO and the magnocellular neurons in the SON and PVN are known to help mediate VP secretion in response to systemic AngII administration (Ferguson & Renaud, 1986; Iovino & Steardo, 1984; Mangiapane *et al.*, 1984), nonetheless AngII does not play a crucial role in stimulating VP secretion during arterial hypotension, as it does in stimulating thirst (Knepel & Meyer, 1980). Instead, neural input from arterial baroreceptors is presumed to be a major factor in this effect. Also unlike the drinking response, VP secretion elicited by AngII was not enhanced either by chronic cardiac and arterial baroreceptor denervation in dogs (Brooks, Klingbeil, Quillen, Keil, & Reid, 1989) or by surgical lesions of the NTS in rats (Schreihofer *et al.*, 2000).

Acute arterial hypertension increases secretion of ANP from the cardiac atria. ANP is a volume-regulatory hormone whose secretion from storage granules in atrial myocytes is increased by volume expansion and decreased by volume contraction (Chiu *et al.*, 1987; Palkovits, Bahner, & Geiger, 1995). Accordingly, ANP inhibits secretion of renin and aldosterone (Atlas & Maack, 1987). Like OT, ANP has potent natriuretic properties (Soares *et al.*, 1999). In fact, circulating OT stimulates ANP secretion, and therefore at least part of OT's action to increase urinary Na^+ excretion appears to be indirect (Haanwinckel *et al.*, 1995).

ANP is also released from hypothalamic neurons and acts as a neurotransmitter in the brain. These neurons are stimulated by neural input from arterial baroreceptors and renal afferents in response to blood volume expansion in rats (Antunes-Rodrigues *et al.*, 1992). One of their functions is to mediate cardiac ANP

secretion (Antunes-Rodrigues, Picanço-Diniz, Favaretto, Gutkowska, & McCann, 1993; Antunes-Rodrigues *et al.*, 1991; Rauch, Callahan, Buckalew, & Morris, 1990). Another is to inhibit water intake during acute hypervolemia or hypertension by opposing the actions of AngII (McCann, Gutkowska, & Antunes-Rodrigues, 2003; Saavedra, 1990). Consistent with this hypothesis, intracerebroventricular injection of ANP was found to attenuate the drinking response of rats to intracerebroventricular AngII (Antunes-Rodrigues, McCann, Rogers, & Samson, 1985) while central administration of ANP antiserum enhanced the drinking response (Katsuura *et al.*, 1986). Similarly, pretreatment with ANP in the SFO blunted the dipsogenic effects of AngII administered in the SFO (Ehrlich & Fitts, 1990). Note that the SFO and AP have high numbers of ANP receptors (Quirion, Dalpé, & Dam, 1986; Saavedra, 1987) but contain sparse ANP-positive nerve terminals (Skofitsch, Jacobowitz, Eskay, & Zamir, 1985), which suggests that these two circumventricular organs may respond to circulating ANP. In this regard, dehydration-induced thirst in rats was significantly attenuated whether ANP was given by intravenous or intracerebroventricular infusion (Antunes-Rodrigues *et al.*, 1985).

Signals Associated with Drinking Bouts

A simple negative feedback system provides the most familiar schema for the control of water intake. According to this view, water ingested by dehydrated animals dilutes pOsm and ultimately removes the excitatory signal for thirst that was generated by cerebral osmoreceptors, thereby causing satiety. However, rehydration actually does not occur rapidly enough for this mechanism to work. Because dehydrated animals do not drink water in amounts exceeding need, thirst appears to be inhibited by some early stimulus in anticipation of rehydration.

In an outstanding series of studies by Ramsay, Thrasher, and colleagues, dehydrated dogs were found to replace their water deficits precisely even though water ingestion ended within a few minutes, which was well before a decrease in pOsm became apparent (Thrasher, Nistal-Herrera, Keil, & Ramsay, 1981). That early signal, which simultaneously inhibited VP secretion, also occurred when osmotic dilution was precluded either by having dogs drink isotonic saline or by having the ingested water drain through an open gastric fistula (Thrasher *et al.*, 1981). Moreover, such rapid inhibitory effects did not occur when a water load was delivered directly to the stomach (Thrasher *et al.*, 1981). Indeed, the early inhibitory effects occurred even when dogs drank concentrated saline solution, although, as might be expected, the dogs became even thirstier and secreted more VP once the saline was absorbed and pOsm was elevated (Appelgren, Thrasher, Keil, & Ramsay, 1991). Ramsay and Thrasher (1990) concluded from these and other experiments that when dehydrated dogs drink water, an early inhibitory signal is temporary and is supplemented and then replaced by a postgastric satiety signal associated with rehydration. The initial inhibitory effects appear to be mediated by oropharyngeal signals associated with the rhythmic pattern of swallowing that occurs during drinking (Thrasher, Keil, & Ramsay, 1987).

The rapid inhibition of thirst and VP secretion by water consumption, mediated by pre-systemic signals, also has been observed to occur during rehydration in sheep, in humans, and in nonhuman primates (Bott, Denton, & Weller, 1965; Denton *et al.*, 1999a; Geelen *et al.*, 1984; Rolls *et al.*, 1980; Wood, Maddison, Rolls, Rolls, & Gibbs, 1980). In dehydrated sheep and humans, as in dogs, the early effects on VP secretion

occurred independently of the composition of the ingested fluid (Blair-West, Gibson, Woods, & Brook, 1985; Geelen, Greenleaf, & Keil, 1996; Seckl, Williams, & Lightman, 1986). Thus, under normal circumstances, water intake evidently provides in these animals a very early stimulus that in essence signals the brain that rehydration is imminent. Such an effect resembles the adaptive feed-forward reflexes found in the control of numerous autonomic functions, such as insulin secretion in response to the taste of food prior to its digestion and assimilation.

In contrast to these established effects, a somewhat different picture emerged when the subjects used in related experiments were laboratory rats (Huang, Sved, & Stricker, 2000b). When rats infused with hypertonic NaCl solution for 220 min were allowed to drink water for 5 min, a substantial fall in plasma VP (pVP) was observed 5 min later even though the osmolality of systemic blood had not yet decreased. These results demonstrate that, as with dogs, a rapid inhibitory signal is evident when thirsty rats drink water. However, unlike the case in dogs, no effect on pVP was seen when similarly treated rats consumed comparable quantities of isotonic saline solution for 5 min. Thus, oropharyngeal signals associated with the act of drinking and swallowing do not appear to provide an early signal inhibiting VP secretion in rats.

This experiment in rats used a different model of plasma hyperosmolality than that used in the studies of dehydrated dogs, and the induced increase in pVP in rats was much greater. In order to determine whether these differences accounted for the different effects of drinking on VP secretion in dogs and rats, another investigation was conducted recently in which rats drank water or isotonic saline after overnight water deprivation (Stricker & Hoffmann, 2003b). The results again indicated that water intake caused a very rapid fall in pVP whereas the intake of saline had no such effect. In contrast, no change in pVP was observed when water ingested by dehydrated rats drained through an open gastric fistula (Stricker & Hoffmann, unpublished observations). Thus, the mechanism by which VP secretion is suppressed during rehydration appears to be different in rats than in dogs and other species studied to date.

In considering the nature of this mechanism in rats, one possibility is that visceral osmoreceptors inhibit VP secretion when diluting fluids pass through the intestines. That hypothesis was proposed by Baertschi and Pence (1995), who reported that gastric water loads rapidly decreased systemic pVP in dehydrated rats. As an important control in those experiments, osmotic dilution of systemic pOsm was prevented by concurrent intravenous infusion of hypertonic saline. These results suggest the participation of visceral sensors, distinct from cerebral osmoreceptors, which detect some rapid, presystemic consequence of the ingested water. The sensors in question might be located in the small intestines, hepatic portal vein, or liver (or, indeed, at each of these sites) and thus be well positioned to sample solutions and influence ongoing behavioral and physiological responses before significant absorption of the gastric loads into the general circulation allowed detection by cerebral osmoreceptors. Because all of the putative postgastric receptor sites are known to send vagal afferent projections to overlapping areas of the caudal brain stem (Norgren & Smith, 1988), it seems plausible that receptors in each site may contribute to a collective signal.

The results of parallel studies suggest that an early signal of hydration also can inhibit thirst in rats. Those other studies presumed that the vagus carried the signals from splanchnic receptors to the brain stem, at least in part, and examined the effects of disrupting vagal input on water intake in rats. Three approaches have

been taken. In one, peripheral sensory fibers were destroyed nonselectively by systemic injection of the neurotoxin, capsaicin. Rats pretreated with capsaicin were found to ingest excessive amounts of water when thirst was stimulated by injections of intraperitoneal hypertonic saline or subcutaneous PEG solution (Curtis & Stricker, 1997). In another approach, visceral signals were disrupted by AP lesions and identical effects on water ingestion were obtained (Curtis, Verbalis, & Stricker, 1996). Rats with AP lesions also drank increased amounts of water when they were treated systemically with AngII (Edwards & Ritter, 1982), as did rats with chronic lesions of the NTS/AP in response to PEG treatment (Schreihofer *et al.*, 1999). Finally, surgical vagotomy has been reported to blunt the inhibitory effects of water infused into the hepatic portal vein on drinking by dehydrated rats (Kobashi & Adachi, 1993). In each of these studies, the thirsty rats behaved as if they were not receiving an early inhibitory signal of hydration but ultimately stopped drinking when ingested water was absorbed into the circulation and detected by cerebral osmoreceptors.

Recent experiments indicate that water ingested by dehydrated rats empties from the stomach very rapidly, passes deep into the intestines, and begins to dilute systemic pOsm while the animal is still drinking (Stricker & Hoffmann, 2003b). Because dilution of body fluids evidently occurs more rapidly than when dehydrated dogs drink water, it seems likely that cerebral osmoreceptors contribute to the observed satiation of thirst and inhibition of VP secretion in rehydrating rats, perhaps by potentiating the effects of the splanchnic receptors. Note that more rapid rehydration occurs when thirsty pigs drink water, precluding the need for early receptors to mediate satiety in this species (Houpt, Yang-Preyer, Geyer, & Norris, 1999).

In addition to signals generated by osmoreceptors in the viscera and forebrain, other signals may inhibit thirst and VP secretion in rats during rehydration. One such signal may derive from gastric distension, although the volumes required to inhibit the water intake of thirsty rats are relatively large (Davis & Sayler, 1997; Hall & Blass, 1977). However, because much smaller volumes can be detected (Mathis, Moran, & Schwartz, 1998; Renaud, Tang, McCann, Stricker, & Verbalis, 1987), they may contribute to the inhibition of thirst in combination with other signals. Distension of the small intestines may provide one such signal. In this regard, special terminal structures of vagal afferent fibers, which might have this function, have been identified along the entire length of the gastrointestinal tract in rats (Berthoud, Patterson, Neumann, Neuhuber, 1997). Furthermore, intestinal distension is pronounced in rats drinking isotonic saline after overnight water deprivation (Hall & Blass, 1975). Note that these animals stop drinking well before their kidneys have extracted needed water from the ingested load, and gastric distension is not very large because saline empties from the stomach even more rapidly than water (Stricker & Hoffmann, 2003b).

Just as cerebral osmoreceptors respond both to increases and decreases in systemic pOsm, splanchnic osmoreceptors may respond to concentrations of intestinal fluids that are above or below isotonic. In fact, pVP was observed to increase after rats were given a NaCl load by gavage (Carlson, Beitz, & Osborn, 1997; Choi-Kwon & Baertschi, 1991; Choi-Kwon, McCarty, & Baertschi, 1990), at which time pOsm was elevated in the hepatic portal blood but not in systemic blood (Carlson *et al.*, 1997). In addition, superfusion of the hepatic portal vein with hypertonic NaCl solution stimulated VP secretion without elevating pOsm in systemic blood, an effect abolished by the administration of a local anesthetic (Baertschi & Vallet, 1981). A gastric

NaCl load also was found to stimulate drinking by rats before systemic pOsm was observed to increase (Kraly, Kim, Dunham, & Tribuzio, 1995). In short, rats apparently respond to an early signal of imminent dehydration both by increasing VP release and by consuming water.

In considering the site of the receptors that stimulate these effects, and the nature of the stimulus, it may be relevant that infusion of hypertonic NaCl solution into the hepatic portal vein of rats increased activity in vagal afferent nerves and NTS neurons (Adachi, Niijima, & Jacobs, 1976; Kobashi & Adachi, 1985), whereas infusion of equiosmotic hypertonic mannitol solution had no such effect (Kahrilas & Rogers, 1984; Morita *et al.*, 1997). Furthermore, neurons in the NTS and AP are reported to increase activity in response to gastric or intraperitoneal NaCl loads in rats (Kobashi, Ichikawa, Sugimoto, & Adachi, 1993; Olson *et al.*, 1993). In addition, infusion of hypertonic saline into the portal vein induced *c-fos* in NTS and AP neurons, and in hypothalamic magnocellular cells, and subdiaphragmatic vagotomy eliminated those effects (Morita *et al.*, 1997). Although the functional significance of these findings remains to be determined, collectively they suggest that Na^+-receptors located in the hepatic portal vein of rats, rather than osmoreceptors, can detect hypertonic fluid and communicate that information to the brain via the vagus nerve. On the other hand, stimulation of VP secretion by intragastric NaCl loads was not affected either by subdiaphragmatic vagotomy (Carlson & Osborn, 1998; Choi-Kwon & Baertschi, 1991) or by complete hepatic denervation (Carlson & Wyss, 1999). In one study, the induced VP secretion was blunted by lesions of the major splanchnic nerves to the spinal cord (Choi-Kwon & Baertschi, 1991), which innervate the upper small intestines and the hepatic portal area, whereas in another study those nerves were damaged in the hepatic denervation that failed to affect VP secretion (Carlson & Wyss, 1999). In short, it is not yet certain whether the visceral receptors in question are located in the hepatic portal vein or elsewhere (or both), whether they are osmoreceptors or Na^+-receptors (or both), and whether the same sensory system responds both to dilute and concentrated fluid.

In all the experiments just mentioned, the animals were euhydrated before being given a gastric salt load, which means that cerebral osmoreceptors detected hydration of body fluids while visceral receptors detected hyperosmolar fluid. In order to eliminate the conflict in signals, the gastric NaCl load was given to dehydrated rats (Stricker, Callahan, Huang, & Sved, 2002). As expected, the load increased pVP before further increases in pOsm were noticeable, but the magnitude of stimulation was much larger than that which occurred when the load was given to euhydrated rats. A potentiating effect of dehydration also was seen when water intake was measured after a gastric NaCl load (Stricker *et al.*, 2002). Collectively, these recent findings suggest that splanchnic receptors in rats can provide an early signal of concentrated fluid passing through the gastrointestinal tract, which is especially effective in stimulating thirst and VP secretion when the animals are already dehydrated. In other words, the system operates as if there was a gating mechanism that inhibits the peripheral signals when cerebral osmoreceptors detect euhydration and disinhibits them when cerebral osmoreceptors detect dehydration. Whatever the mechanism, the adaptive significance of this arrangement is plain. When rats are dehydrated and consume concentrated NaCl solution, it is useful for them not to wait for pOsm to further increase before augmenting VP secretion and water intake.

Recent experiments have re-examined these issues in rats fed a high salt diet (Stricker, Hoffmann, Riccardi, & Smith, 2003), which is a more natural way of

administering a salt load and activating splanchnic osmoreceptors than gavage. Those rats consumed much more water each day than they did when maintained on standard laboratory chow. The elevated daily water intake resulted from increases in the size and number of drinking bouts. Of considerable interest were observations that drinking bouts always occurred within 5 min after the end of feeding bouts, and water intakes during ingestive episodes were proportional in size to the food consumed in the episodes. Those findings suggest that the consequences of eating a high salt diet were detected rapidly. To explore this issue further, rats were deprived of high salt diet overnight, given food to eat the next morning, and killed as soon as they began to drink water. At this time of evident thirst, ~40% of the ingested food had left the stomach and moved deep into the intestines, and both pVP and systemic pOsm were significantly elevated. However, when the experiments were repeated but this time animals were killed before thirst became evident, pVP again was increased but systemic pOsm was not yet elevated (Stricker & Hoffmann, 2003a). Those observations are consistent with the possibility that visceral osmo- or Na^+-receptors actually do contribute to VP secretion when rats eat high salt diet.

But do they also contribute to thirst? To address that question, other experiments were initiated recently which proceeded from the observation that gastric chyme was very compressed and had the same low water content whether rats ate high salt diet or standard laboratory chow (Stricker *et al.*, 2003). These findings allowed the possibility that the embedded NaCl could not be detected by putative duodenal osmoreceptors until after the chyme had emptied from the stomach and been digested in the intestines. To test that hypothesis, salivary flow was surgically interrupted in rats so they had to consume large amounts of water at frequent intervals in order to swallow the dry food, thereby preventing the formation of compacted chyme. Those animals ate food very slowly and drank much more water than the amounts ingested either by intact rats eating high salt diet or saliva-less rats eating standard chow. More to the point, the Na^+ concentration of their gastric fluid was isotonic with plasma (Stricker, Spicer, & Hoffmann, unpublished observations). In other words, the rats behaved as if duodenal osmoreceptors were able to detect the high salt concentration in gastric fluid, presumably because the pyloric sphincter remained open throughout the prolonged period of eating and generated an excitatory signal to drink until sufficient water was consumed to remove the osmotic stimulus for thirst.

Salt Appetite

Although water is the appropriate fluid for animals to drink and thereby dilute body fluids to normal when thirst results from an osmotic load and cellular dehydration, water consumption is not very effective in repairing plasma volume deficits. Ingested water is distributed as body fluids are distributed, which means that about two thirds of the water moves into cells by osmosis while most of the remainder stays in the interstitial space. Instead of water, hypovolemic animals need isotonic saline, which remains extracellular and therefore can repair the fluid deficits effectively. Their drinking behavior is, in fact, appropriate to that need. Thus, when given isotonic saline to drink in a one-bottle test, PEG-treated rats consumed it in large amounts sufficient to restore plasma volume without the inhibition of thirst that results when they drink and retain water (Stricker & Jalowiec,

1970). Furthermore, and most remarkably, when given water and a concentrated saline solution to drink in a two-bottle test, hypovolemic rats alternately ingested the two fluids in the amounts required to concoct an isotonic saline solution (Stricker, 1981; Stricker, Gannon, & Smith, 1992a). In short, hypovolemia appears to stimulate both thirst and salt appetite in rats, and the two allied motivations together direct the animal to consume the fluid mixture suitable for repairing the plasma volume deficit.

The signals that stimulate and inhibit salt appetite are comprehensively discussed in Chapter 18 in this volume (by Weisinger & colleagues), and elsewhere (Fitzsimons, 1998; Johnson & Thunhorst, 1997), and consequently they will not be considered in detail here. Instead, this section will summarize the main findings of numerous investigations of saline ingestion by rats during hypovolemia, and consider their relevance to, and interaction with, the multiple controls of thirst. A good place to begin is with the key point that the stimulation of thirst and salt appetite by subcutaneous colloid treatment is strongly influenced by the diet on which the animals had been maintained. Thus, when rats are fed standard laboratory chow, salt appetite usually develops many hours after colloid treatment, well after thirst appears (Stricker & Jalowiec, 1970). In contrast, when rats are fed sodium-deficient diet, salt appetite becomes evident before thirst (Stricker, 1981). Since colloid treatment produces similar plasma volume deficits regardless of which diet had been consumed, an obvious question arises as to why the induced effects on drinking were different. The apparent answer to this question draws attention to the two signals that seem to be most relevant to the control of salt appetite after colloid treatment in rats: increases in an excitatory signal that appears to derive from blood-borne AngII, and decreases in an inhibitory signal that is associated with neurohypophyseal OT secretion. Both developments must occur before salt appetite becomes manifest.

Although acute systemic administration of AngII does not reliably stimulate salt appetite in sodium-replete rats, perhaps because of the induced elevation in ABP (as with thirst), nonetheless many noteworthy observations are consistent with the hypothesis that salt appetite in rats is stimulated by AngII, both blood-borne and acting as a neurotransmitter in the brain. (1) Renin secretion, which increased progressively over time after colloid treatment when rats were maintained on standard chow, was considerably elevated in parallel with enhanced salt appetite when rats were fed sodium-deficient diet (Stricker *et al.*, 1979). Conversely, bilateral nephrectomy eliminated renin secretion and abolished salt appetite in PEG-treated rats (Stricker *et al.*, 1979). (2) Hypovolemic rats began to drink saline immediately and in enhanced amounts when given a low dose of captopril (Stricker, 1983). The same treatment similarly enhanced the salt appetite of sodium-depleted rats (Moe, Weiss, & Epstein, 1984; Weisinger, Denton, Di Nicolantonio, & McKinley, 1988). Larger doses of captopril suppressed salt appetite in sodium-depleted rats (Moe *et al.*, 1984; Thunhorst & Fitts, 1994), but NaCl intake was restored by intravenous AngII (Fitts & Thunhorst, 1996). (3) Salt appetite of sodium-depleted or colloid-treated rats was suppressed by interference with the central actions of AngII (Buggy & Jonklaas, 1984; Sakai, Nicolaidis, & Epstein, 1986; Weisinger *et al.*, 1988; Weiss, Moe, & Epstein, 1986), whereas it was enhanced when AngII was administered directly into the brain of PEG-treated rats (Fitts, Thunhorst, & Simpson, 1985a). Furthermore, SFO lesions severely reduced NaCl intake in sodium-depleted rats (Thunhorst, Beltz, & Johnson, 1999; Weisinger *et al.*, 1990). (4) The pronounced salt appetite in rats after bilateral adrenalectomy (Richter, 1936), which is caused

by the uncontrolled loss of Na^+ in urine following removal of aldosterone, is associated with a substantial increase in circulating AngII levels (Sakai & Epstein, 1990). Salt appetite in these animals was blunted by administration of an AngII receptor antagonist intracerebroventricularly (Sakai & Epstein, 1990). Systemic injection of captopril increased their salt appetite when given in small doses but attenuated NaCl intake when given in large doses (Elfont & Fitzsimons, 1985; Sakai & Epstein, 1990), yet it was restored almost completely by an intravenous infusion of AngII (Schoorlemmer *et al.*, 2001). (5) Intracerebroventricular infusion of renin in the rat caused a pronounced increase in NaCl intake that was not secondary to renal Na^+ loss (Avrith & Fitzsimons, 1980); this effect was abolished by intracranial injection of captopril or an AngII receptor blocker (Avrith & Fitzsimons, 1983). A role for the OVLT and/or MnPO area in mediating this phenomenon is suggested by the stimulating effect of AngII injected locally (Avrith & Fitzsimons, 1983; Fitts & Masson, 1990).

It is well known that sodium depletion increases renin secretion, and that even a day or two of sodium deprivation increases the steroidogenic effects of AngII in the rat owing to an increase in the number and affinity of AngII receptors in the adrenal glomerulosa cells and in the capacity of these cells for aldosterone production. One popular hypothesis to account for the salt appetite seen during sodium depletion, and for its enhancement in sodium-deprived rats, is that the steroid hormone itself provides an excitatory signal, and complements its potent sodium-conserving properties by creating a synergy with AngII in stimulating salt appetite (Fluharty & Epstein, 1983). Consistent with this proposal, intracerebroventricular administration of AngII increased NaCl intake rapidly in rats maintained previously on sodium-deficient diet (Buggy & Fisher, 1974). This AngII treatment had a similar effect on NaCl intake when rats were pretreated systemically with desoxycorticosterone (DOC), whose sodium-conserving properties prevented the usual AngII-induced natriuresis (Fluharty & Epstein, 1983). Furthermore, salt appetite in sodium-depleted rats was blunted by the administration of drugs that blocked either AngII synthesis or central mineralocorticoid receptors, and combined treatment eliminated the induced NaCl intake (Sakai *et al.*, 1986). Because DOC treatment sufficient to induce salt appetite in rats increased AngII binding in the SFO and MnPO (De Nicola *et al.*, 1993), it seems plausible that the lipid-soluble mineralocorticoids, which enter the brain readily, act by modifying the central actions of AngII. In this regard, salt appetite induced by subcutaneous PEG treatment was not enhanced by dietary sodium deprivation in hypophysectomized rats, which could augment secretion of renin but not aldosterone (Stricker, 1983). Conversely, a low dose of captopril produced an especially large increase in NaCl intake when intact rats had been maintained on sodium-deficient diet before subcutaneous PEG treatment (Stricker, 1983).

In considering the control of salt appetite in rats, several noteworthy observations are also consistent with the hypothesis that increased pOT is associated with an inhibitory signal. Indeed, to identify many of the conditions that inhibit salt appetite, it is only necessary to identify many of the treatments that stimulate OT secretion in male rats. Some of these treatments also stimulate VP secretion and have been mentioned above in this context. For example, hyperosmolality (whether associated with hypernatremia or not), hypovolemia, and arterial hypotension each elicits OT secretion (Huang *et al.*, 2000a; Stricker, Hosutt, & Verbalis, 1987), and each inhibits salt appetite as well (Fitzsimons & Wirth, 1978; Hosutt & Stricker, 1981; Stricker, 1981; Stricker & Verbalis, 1987). Similarly, anuria associated with nephrectomy provides a potent stimulus for OT secretion (Stricker *et al.*, 1987) and inhibition of salt appetite

in PEG-treated, sodium-deficient, or adrenalectomized rats (Fitzsimons & Stricker, 1971; Fitzsimons & Wirth, 1978; Stricker *et al.*, 1979). Other treatments that substantially increase OT secretion in rats include systemic injection of lithium chloride or copper sulfate (Verbalis, McHale, Gardiner, & Stricker, 1986), both of which inhibit salt appetite (Stricker & Verbalis, 1987). In addition, blood volume expansion increases pOT to very high levels (Haanwinckel *et al.*, 1995), and that treatment presumably is mimicked by acute arterial hypertension, which may inhibit NaCl intake (Fitts & Thunhorst, 1996).

Conversely, saline ingestion appears to be most prominent when pituitary OT secretion is low. For example, in rats drinking water after subcutaneous PEG treatment, OT secretion was inhibited by progressive osmotic dilution resulting from renal retention of ingested water (Stricker & Verbalis, 1986); that dilution appears to release salt appetite from inhibition while also removing thirst as a competing drive (Stricker, 1981). Similarly, pretreatment maintenance of rats on sodium-deficient diet decreased OT secretion after subcutaneous PEG treatment in rats (Stricker, Schreihofer, & Verbalis, 1994; Stricker *et al.*, 1987) and was associated with enhanced salt appetite (Stricker, 1981). Likewise, basal pOT is depressed in adrenalectomized rats; furthermore, and especially striking, their NaCl ingestion abruptly ceased after injection of hypertonic saline solution and resumed only after the induced increase in pOT had subsided (Stricker & Verbalis, 1987). In addition, adrenalectomized rats drank concentrated NaCl solution ad libitum in numerous but very short bouts (Stricker, Gannon, & Smith, 1992b), as if each bout generated a rapid inhibitory signal (which, plausibly, might be associated with OT secretion; Stricker *et al.*, 2002).

OT secretion was also inhibited by systemic treatment either with alcohol or with the mineralocorticoid DOC, and both agents enhanced AngII-induced ingestion of saline but not water (Blackburn, Stricker, & Verbalis, 1994; Roesch, Blackburn-Monro, & Verbalis, 2001). In this regard, it has long been known that pharmacological doses of mineralocorticoids elicit a dose-related increase in salt appetite in rats (Fregly & Waters, 1966; Rice & Richter, 1943; Wolf, 1965). In addition to modulating the central effects of AngII, mineralocorticoids likely act by inhibiting basal OT secretion and thereby releasing NaCl intake from tonic inhibition, with a phasic inhibition associated with stimulation of neurohypophyseal OT secretion returning each time the animals ingest concentrated saline solution. That indirect mechanism of action would allow mineralocorticoids to enhance NaCl ingestion even when plasma AngII levels were low, as occurs during DOC treatment (Pettinger, Marchelle, & Augusto, 1971). This view is supported by observations that DOC treatment significantly lowers basal pOT in rats (Stricker & Verbalis, 1987), and that, like adrenalectomized rats, DOC-treated rats consume saline ad libitum in numerous but very short bouts (Stricker *et al.*, 1992b). Furthermore, DOC-treated rats drank excessive amounts of saline after peripheral sensory fibers were destroyed nonselectively by systemic injection of capsaicin (Curtis & Stricker, 1997).

Like adrenalectomized rats, DOC-treated rats may consume up to 30 ml per day of 0.5M NaCl solution. In contrast, rats with focal AP lesions ingested up to 50–100 ml of 0.5M NaCl daily, which means they drank the equivalent of 3–6 times their total body Na^+ content each day (Curtis *et al.*, 1999). The basis of this prodigious intake may be an elevation in the basal excitatory tone for salt appetite, presumably set by central AngII, which results from the loss of inhibitory effects generated by the action of systemic ANP on its receptors in the AP. An additional

factor may be the absence of a rapid inhibitory signal generated by visceral osmo- or Na^+ receptors each time the animals drink saline. In this regard, rats with AP lesions have impaired secretion of OT in response to an administered NaCl load, as mentioned above (Curtis *et al.*, 1999; Huang *et al.*, 2000a). It is reasonable to assume that they also have impaired OT secretion in response to an ingested NaCl load; since that load normally causes a rapid secretion of OT (Stricker *et al.*, 2002) and inhibition of NaCl intake, rats with AP lesions would be expected to consume concentrated NaCl solution ad libitum in drinking bouts that are relatively large. In fact, that is exactly what they do (Stricker, Curtis, Peacock, & Smith, 1997).

Despite the many circumstances in which pOT is inversely related to salt appetite in rats, such findings do not demonstrate that changes in pOT secretion cause reciprocal changes in NaCl intake. In fact, a direct test of the hypothesis indicated that it was incorrect. Intravenous infusion of OT even in pharmacological amounts did not inhibit NaCl intake in hypovolemic rats, and NaCl intake was not enhanced by intravenous infusion of an OT receptor antagonist (Stricker & Verbalis, 1987). Thus, elevated pOT does not cause the inhibition of salt appetite but merely is a correlate of that effect. The real cause of inhibition seems to be central release of OT (acting in the brain as a neurotransmitter) from a subset of parvocellular hypothalamic neurons, which is thought to occur in parallel with activation of magnocellular OT-containing neurons (Stricker & Verbalis, 1990). Since subcutaneous PEG treatment and intracerebroventricular AngII each stimulate OT secretion in addition to NaCl ingestion (Lang *et al.*, 1981; Stricker & Verbalis, 1986), it is important to note that salt appetite is proposed to occur only when the inhibitory component of these treatments is suppressed relative to the excitatory component. In support of this hypothesis, administration of OT directly into the cerebral ventricles of rats eliminated the increased NaCl intake normally seen after PEG treatment (Blackburn, Stricker, & Verbalis, 1992b), while intracerebroventricular pretreatment with an OT receptor antagonist enhanced NaCl intake in AngII-treated rats (Blackburn *et al.*, 1992a). Moreover, and most persuasively, salt appetite in PEG-treated rats was abolished by systemic injection of the opioid antagonist naloxone, which blocks the receptors of endogenous opioids and thereby removes their basal inhibition of OT secretion; the inhibitory effect of naloxone of NaCl intake was prevented by intracerebroventricular pretreatment with an OT receptor antagonist (Blackburn *et al.*, 1992b).

Also striking are the results of studies using rats injected intracerebroventricularly with OT conjugated to the toxic A chain of the plant cytotoxin ricin (rA-OT), which was intended to destroy brain neurons that contained OT receptors. These animals then were made hypovolemic by subcutaneous PEG treatment before being injected systemically either with hypertonic mannitol solution, which raised pOsm but lowered plasma Na^+ concentration, or with equimolar saline solution, which raised both pOsm and plasma Na^+ concentration. At issue was whether injection of the hypertonic fluids would reduce the stimulated NaCl intake as it does in control hypovolemic rats. The results indicated that rats with rA-OT lesions did not show inhibition of NaCl intake in response to injection of hypertonic mannitol solution, but did inhibit NaCl intake normally when the saline solution was injected (Blackburn, Samson, Fulton, Stricker, & Verbalis, 1993, 1995). Those findings indicate that there is differential sensing of pOsm and Na^+ concentration in rats, and suggest that central OT mediates the inhibition of salt appetite stimulated by cerebral osmoreceptors but not by Na^+-receptors. Recall that the existence of central Na^+-sensing mechanisms in rats was proposed earlier in this chapter to explain the

control of disrupted VP secretion in rats with AP lesions. Furthermore, mutant mice deficient in the gene for voltage-gated sodium channels in the circumventricular organs consumed concentrated NaCl solution in significantly greater amounts when sodium-depleted than did control animals (Watanabe *et al.*, 2000), which suggests a possible role of central Na^+-receptors in the inhibition of salt appetite.

Another intriguing result was obtained when the cytotoxin-treated rats were given systemic injections of the hypertonic fluids without having been made hypovolemic. Control animals adaptively increased consumption of water but not of concentrated saline solution, as would be expected since NaCl intake would aggravate the induced hyperosmolality. However, rats with rA-OT lesions increased their intakes of both fluids after injection of hypertonic mannitol solution, although they drank only water after injection of hypertonic saline (Blackburn *et al.*, 1993). These results were replicated when rats were pretreated with an OT receptor antagonist intraventricularly rather than with rA-OT (Blackburn *et al.*, 1993). Those unexpected observations suggest that hyperosmolality provides a mixed stimulus for salt appetite in addition to providing a stimulus for thirst. The latent excitatory component of the stimulus for salt appetite was not seen before, nor likely even was suspected; it was revealed only under the unusual circumstance in which hypertonic mannitol solution was injected and the inhibitory effect of cerebral OT was eliminated.

It is interesting to note that rats with rA-OT lesions did not increase basal NaCl intakes (Blackburn *et al.*, 1993, 1995). Similarly, mutant mice in which the gene for OT had been deleted did not have a spontaneous salt appetite, although they did show elevated intakes of NaCl solution after overnight water deprivation (Amico, Mantella, & Vollmer, 2003; Amico, Morris, & Vollmer, 2001). Thus, it appears that central oxytocinergic pathways normally do not inhibit salt appetite tonically but instead provide inhibition only when body fluid homeostasis is challenged and stimuli for thirst and salt appetite are present.

These findings collectively suggest that thirst and salt appetite generally are activated by overlapping stimuli, and how they affect central OT release influences which motivation emerges. Thus, neurologically normal rats made hypovolemic by subcutaneous PEG treatment drink saline when OT release is inhibited by osmotic dilution or mineralocorticoids (because pOsm is low), and they drink water when OT release is stimulated (because pOsm is not low). The same alternating pattern of water and saline intakes are observed when rats are given central injections of AngII (Fluharty & Epstein, 1983), presumably for the same reasons.

The continued inhibition of salt appetite by injection of hypertonic saline solution in rats with rA-OT lesions indicates the presence of another inhibitory system influencing salt appetite, linked to the Na^+-sensing mechanism. In this regard, no inhibition of salt appetite was observed after systemic injection of either hypertonic mannitol or saline solution in hypovolemic rats that had been injected intracerebroventricularly with ANP conjugated to ricin, which was intended to destroy brain neurons that contained ANP receptors (Blackburn *et al.*, 1995). Those results suggest that central ANP is necessary both for Na^+- and osmolality-mediated inhibition of salt appetite. Consistent with a role of ANP in inhibiting salt appetite, NaCl ingestion was reduced in sodium-depleted rats after central ANP administration (Antunes-Rodrigues, McCann, & Samson, 1986; Fitts, Thunhorst, & Simpson, 1985b) or modest elevation in ABP (Thunhorst & Johnson, 1994, 2001), and it was blunted similarly in DOC-treated rats when a small balloon was inflated in the right atrium (Toth, Stelfox, & Kaufman, 1987). In addition, pharmacological disruption of central serotonergic pathways, which interferes with ANP secretion in response

to blood volume expansion (Reis *et al.*, 1994), produces a very substantial increase in NaCl intake in response to intracerebroventricular AngII or sodium depletion (Menani, De Luca, & Johnson, 1998; Menani, Thunhorst, & Johnson, 1996).

Summary and Conclusions

For the past 30 years it has been recognized that thirst is stimulated by three separate but related signals: increased pOsm, decreased plasma volume, and increased circulating levels of AngII resulting from arterial hypotension. It is also well known that decreased pOsm and acute hypertension provide potent inhibitory signals of thirst. The present discussion updated those familiar elements in the control of thirst, affirmed their significance, and added other elements for consideration that derived from recent studies of fluid processing in the alimentary canal, of salt appetite, and of multiple endocrine responses to alterations in body fluids. Three principles emerge that deserve emphasis. First, each of the three systemic signals for thirst additionally stimulates salt appetite as well, although NaCl intake may not become manifest because of overriding inhibitory factors. Second, central AngII helps to mediate thirst and salt appetite in rats, while central ANP opposes those effects and central OT helps to mediate inhibition of salt appetite. Third, many treatments that affect thirst or salt appetite actually provide mixed signals containing both excitatory and inhibitory components.

Consideration of these general issues and many more specific ones makes the overall formulation rather complex, involving multiple excitatory and inhibitory systems in the control of two motivated behaviors that help to regulate three dimensions of body fluid homeostasis. In order to simplify presentation of a schematic summary of these issues, the relevant schema proposed to operate in the regulation of blood osmolality, volume, and pressure in rats are depicted separately in Figures 2–4. Note that the working models depicted in these figures were based in part on recent experimental findings and in part on surmise. Future experimental results are expected to modify these hypothetical arrangements just as previous formulations have been modified by new data.

The control of water and NaCl intake in the regulation of blood osmolality is considered in Figure 2. The three main points of this figure are that separate mechanisms mediate osmolality-and Na^+-responsive signals, that the same excitatory signals for thirst also stimulate salt appetite, and that central OT- and ANP-containing neurons inhibit salt appetite when pOsm or plasma Na^+, respectively, have been increased. The components and operation of these putative elements in the control of water and NaCl intake become clear when the behavioral effects of the prototypical treatments that stimulate "osmoregulatory thirst" are described. For example, administration of hypertonic mannitol solution raises pOsm and thereby stimulates thirst but provides a mixed signal for salt appetite, so that NaCl intake does not increase unless the function of central OT neurons is blocked. Similarly, administration of hypertonic saline solution stimulates thirst while providing a mixed signal for salt appetite, so that NaCl intake does not increase unless the functions of central ANP and OT neurons are blocked. Note that the effects of osmotic dilution to inhibit stimulated thirst and to release salt appetite from oxytocinergic inhibition are also shown; these actions are relevant to the behavioral contributions to the regulation of blood volume and pressure shown in Figures 3 and 4.

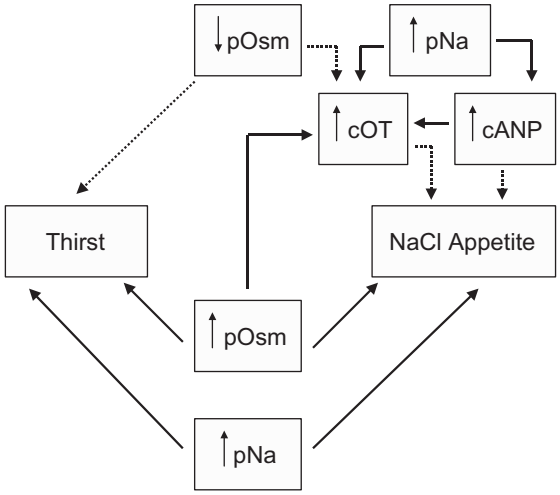

Figure 2. Schematic arrangement of the signals that stimulate (unbroken arrows) or inhibit (dashed arrows) thirst and salt appetite in rats in response to changes in plasma osmolality (pOsm) or plasma Na^+ concentration (pNa). (Note that to simplify the presentation, increased pNa appears twice in this figure, and signals associated with increased pOsm and decreased pOsm are spatially separated.) Thirst is stimulated by increased pOsm whether or not increased pNa is present. Increased pOsm additionally provides a mixed stimulus of salt appetite, as does increased pNa, with inhibition mediated by central systems in which oxytocin (cOT) and atrial natriuretic peptide (cANP) are neurotransmitters. ANP receptors present in the subfornical organ are presumed to detect blood-borne hormone, whereas ANP receptors are presumed to be present on OT-containing neurons that provide additional inhibition of salt appetite. Decreases in pOsm inhibit thirst and the oxytocinergic inhibition of salt appetite.

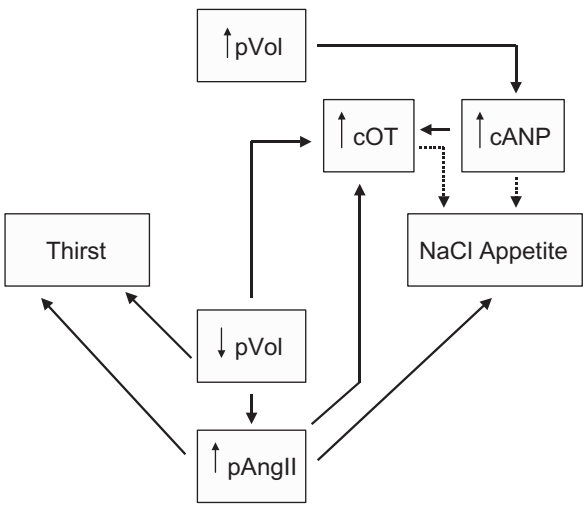

Figure 3. Schematic arrangement of the signals that stimulate (unbroken arrows) or inhibit (dashed arrows) thirst and salt appetite in rats in response to changes in plasma volume (pVol). (Note that to simplify the presentation, signals associated with increased pVol and decreased pVol are spatially separated.) Thirst is stimulated by a neural input from cardiac baroreceptors resulting from a decrease in pVol and from an increase in plasma levels of angiotensin II (pAngII). Salt appetite is also stimulated by the increase in pAngII. However, both hypovolemia and increased pAngII activate central OT-containing neurons, which inhibit salt appetite. Increased pVol is presumed to inhibit the angiotensinergic stimulus of salt appetite by the activation of central ANP-containing neurons.

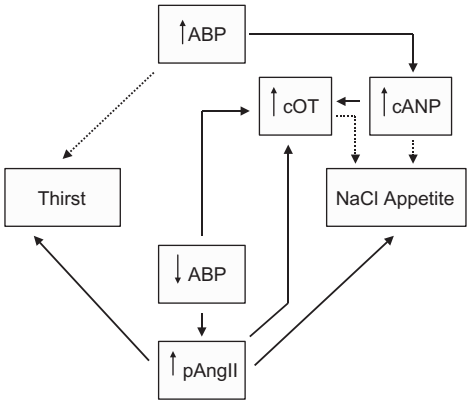

Figure 4. Schematic arrangement of the signals that stimulate (unbroken arrows) or inhibit (dashed arrows) thirst and salt appetite in rats in response to changes in arterial blood pressure (ABP). (Note that to simplify the presentation, signals associated with increased ABP and decreased ABP are spatially separated.) Both thirst and salt appetite are stimulated by an increase in pAngII. However, the decrease in ABP and the increased pAngII both produce a strong activation of central OT-containing neurons, which inhibit salt appetite. Increased ABP is presumed to inhibit the angiotensinergic stimulus of thirst and salt appetite both by the activation of central ANP systems and (not shown) by the detection of blood-borne ANP in the subfornical organ and area postrema.

The control of water and NaCl intake in the regulation of blood volume is considered in Figure 3. There are several important points contained in this formulation. One is that multiple signals mediate thirst during hypovolemia, including a neural stimulus from cardiac baroreceptors and the blood-borne stimulus of AngII; not shown is a second neural signal, presumably mediated by renal afferent nerves. A second point is that AngII also provides an excitatory signal of salt appetite, whereas no evidence as yet indicates that the neural signal for thirst does the same. A third point is that activation of central OT- and ANP-containing neurons inhibits salt appetite, as was shown in Figure 2. The prototypical treatment that stimulates "hypovolemic thirst" in rats is subcutaneous injection of PEG solution, and the behavioral responses obtained depend on the magnitude and pattern of excitatory and inhibitory signals induced in the brain. It seems likely that central ANP (as might be stimulated by blood volume expansion) mediates inhibition of the component of thirst and salt appetite that is stimulated by AngII, hence the inclusion of this putative effect in the figure.

This figure deals only with the consequences of changes in blood volume. When the hypovolemic rat consumes water and/or concentrated saline solution, Figure 2 describes the effects of the induced change in pOsm. Specifically, water consumption dilutes pOsm, which inhibits thirst and disinhibits salt appetite, whereas the subsequent consumption of hypertonic saline removes the inhibition of thirst while reinstating the oxytocinergic inhibition of salt appetite.

The control of water and NaCl intake in the regulation of blood pressure is considered in Figure 4. The two main points of this figure are that thirst is stimulated by AngII but not by a neural signal from arterial baroreceptors, and that arterial hypertension inhibits thirst and, perhaps, salt appetite as well. Central OT- and ANP-containing neurons are presumed to be important in inhibiting salt appetite during increases in plasma AngII and in ABP. The prototypical treatment that stimulates "hypotensive thirst" is systemic injection of a vasoactive drug that causes acute

arterial hypotension. This treatment stimulates thirst but salt appetite is not evident, perhaps because inhibitory oxytocinergic neurons are intensely activated. Note that systemic administration of AngII raises ABP and thereby provides a mixed signal for thirst. In consequence of this mixed input, plasma AngII levels are much higher when a given amount of water intake is stimulated by exogenous AngII than by hypovolemia or arterial hypotension.

Many important factors were not included in these three figures in order to simplify the presentation. They are as follows. (1) Water deprivation provides all three excitatory signals for thirst (i.e., increased pOsm, decreased blood volume, and increased plasma AngII). In consequence, dehydration is very effective in stimulating thirst and in resisting inhibition. (2) Surgical ligation of the inferior vena cava provides multiple signals for thirst, with elements of hypovolemia and arterial hypotension. Osmotic dilution that results from the renal retention of ingested water inhibits the induced thirst. (3) Circulating AngII stimulates thirst and salt appetite by acting on AngII receptors in the SFO. Neural projections from the SFO use AngII as a neurotransmitter, which allows AngII receptors located in target areas at other brain sites to mediate the two motivated behaviors. (4) Central ANP acts, in part, to inhibit the stimulating effects of AngII on thirst and salt appetite. The presence of ANP receptors in the SFO, OVLT, and AP allows the possibility that neurons in these circumventricular organs respond to ANP in the circulation. (5) Central OT secretion is strongly activated by some consequence of anuria. Thus, it is very difficult to stimulate NaCl ingestion in rats after nephrectomy, severe hypovolemia, or arterial hypotension. (6) Central OT can be inhibited by mineralocorticoids, whether of endogenous or exogenous origin. These steroids may have a direct excitatory effect on salt appetite as well. (7) Maintenance of rats on high salt diet increases the binding of AngII to its receptors in the SFO, whereas maintenance of rats on low salt diet has the opposite effect while also decreasing central ANP. Consequently, the same increase in blood levels of AngII may stimulate thirst or salt appetite depending on the pretreatment dietary maintenance. (8) Finally, thirst and salt appetite are affected by visceral signals associated with the passage of fluid through the alimentary canal. These signals provide early information of imminent hydration or dehydration, which supplement and sometimes precede the appearance of systemic signals.

Figures 2–4 together contain many signals that are known or suspected to participate in the control of thirst, in contrast to the single osmoregulatory stimulus that was recognized to elicit thirst 40 years ago. The increased complexity of the systems in the present formulation represents substantial progress in this field, which has profited greatly from the rapid development of techniques and accretion of information within the neurosciences during the past generation or so of scientific investigation. Indeed, studies of thirst and related topics have provided a wonderful subject for understanding the brain's control of motivated ingestive behavior generally, and for comprehending the integrative influence on the brain of various hormones, neural messages, and substrates. However, the multiple vexing issues that remain unsettled indicate how much additional research remains to be done to further refine our understanding.

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Neurobiology of Sodium Appetite

Richard S. Weisinger, John R. Blair-West, Peta Burns, Nora Chen, and Harrison S. Weisinger

General Introduction

Sodium (Na^+) is crucial for the maintenance of extracellular fluid (ECF) volume, blood circulation, neuronal function, and reproductive success (Aumann & Emlen, 1965; McBurnie, Blair-West, Denton, & Weisinger, 1999; McBurnie, Denton, & Tarjan, 1988). The mechanisms involved in the regulation of body sodium homeostasis are complex, and rely heavily upon receptor-mediated events. Central nervous system mechanisms are needed to coordinate information regarding body fluid and electrolyte status with the appropriate physiological and behavioral responses. While Na^+ is ingested in the form of a salt (most commonly NaCl), in this chapter, we will refer to the appetite for, and the intake of, the ion as "sodium" appetite and intake.

Evidence suggests that increased sodium preference occurs in humans subsequent to salt loss (Beauchamp, Bertino, Burke, & Engelman, 1990; Leshem, Abutbul, & Eilon, 1999). Human salt appetite is perhaps best demonstrated by African desert dwellers, where highly developed salt trades have developed. The report (Wilkins & Richter, 1940) of a young boy with an adrenal tumor that caused salt wasting, who manifested a craving for salt and thereby kept himself alive,

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Neurobiology of Food and Fluid Intake, 2nd Ed., Volume 14 of *Handbook of Behavioral Neurobiology*, edited by Edward Stricker and Stephen Woods, Kluwer Academic / Plenum Publishers, New York, 2004.

is a classic example of innate sodium appetite and is supported by more recent reports (Ingram, Wallace, Collier, Fraser, & Connell, 1996).

Many instances of sodium appetite in the wild have been documented, across many different animals, including rabbits, elephants, and sheep. Attraction of wild gorillas and chimpanzees to natural salt sources also has been reported (Goodall, 1986; Schaller, 1963).

After more than 50 years of research, it would be nice to be able to report the fully characterized neural systems that are responsible for the regulation of sodium intake in mammals. Though we are somewhat closer to that goal now than when the pioneering work of Curt Richter commenced in the 1930s, much is still unknown.

The variety of species studied (e.g., rodents, ruminants, and primates), in diverse ecological and nutritional circumstances, has resulted in a diversity of candidate mechanisms being identified. Prominent among these are:

1. The hormones associated with sodium deficiency, for example, angiotensin (ANG) II, aldosterone.
2. Changes in [Na^+] in blood, cerebrospinal fluid (CSF), and brain intracellular fluid (ICF) and ECF, and involvement of sodium channels and transporters.
3. Neurotransmitters and brain peptides, for example, oxytocin (OT), somatostatin (SOM).
4. The hormones associated with stress, for example, adrenocorticotrophic hormone (ACTH), corticotrophin-releasing factor (CRF).
5. The sympathetic nervous system (SNS).
6. The hormones of pregnancy and lactation, for example, estrogen, progesterone, ACTH, prolactin, OT.

Any or all of these factors may be involved. The interactions between them are extensive.

It is also clear that a complex neurochemical organization within the brain underlies the appetite. This complexity is reflected in the range of brain regions

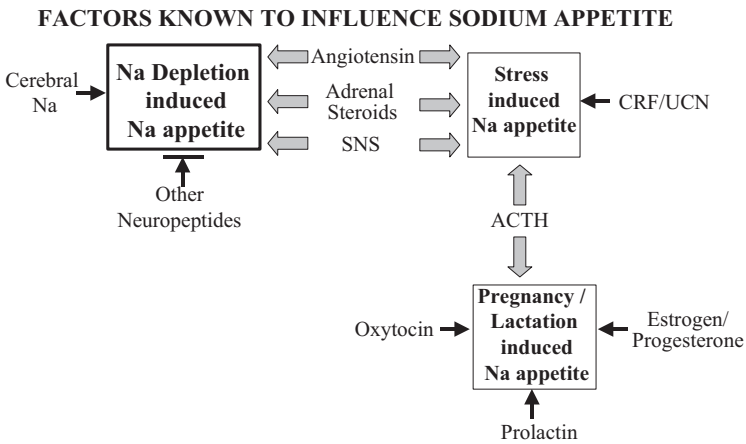

Figure 1. Schematic diagram of factors influencing salt appetite induced by sodium depletion, stress, and that which occur during pregnancy and lactation. The different causes of sodium appetite share common elements of control. For example, ANG II and adrenocorticosteroid hormones are common to both sodium depletion and stress-induced sodium appetite.

and the diversity of neurotransmitters that subserve the behavior. There appears to be spatial organization of the neural systems such that some elements respond to ionic change while others located elsewhere respond to steroids or peptides. Destruction of one area by lesion may destroy one element of response to stimuli while leaving other responses intact. However, even though there are different causes of sodium appetite, for example, sodium deficiency, stress, or reproduction, there seems to be some elements of commonality in their central control (see Figure 1).

In this review, we will simplify findings where possible, but importantly, highlight the inconsistencies and anomalies that continue to make this one of the more difficult and challenging, but also exciting, fields of behavioral neurobiology today.

Angiotensin II, Adrenocorticosteroids and Sodium Appetite

Much research over the past 20 years has concentrated on the roles of ANG II and the adrenal steroids in the genesis of sodium appetite. In large part, Epstein, Sakai, and their colleagues have stimulated this research. They stated that "salt appetite is aroused by a synergy of ANG II and aldosterone, a mineralocorticoid hormone primarily involved in minimizing renal sodium loss. This simple theory unites the behavioral and renal contributions to sodium homeostasis in the same hormonal network" (Epstein, 1984; Sakai, Nicolaidis, & Epstein, 1986) with "cerebral, rather than blood-borne, ANG as the agent that participates in this synergy" (Epstein, 1982, 1991; Yang & Epstein, 1991). The influence of ANG II and adrenal hormones on sodium appetite will now be considered.

Angiotensin II

The Renin–Angiotensin System. The biologically active component of the renin–angiotensin system (RAS), ANG II, is an 8-amino acid peptide that is formed in the peripheral circulation (see Figure 2). In the blood, the enzyme, renin, acts on its substrate, angiotensinogen, to form ANG I which is then converted to the biologically active ANG II by the action of angiotensin converting enzyme (ACE). All components of the RAS are also found in the brain, and ANG II can be generated in the brain via a pathway involving renin and ACE. In addition, ANG II can be formed in the brain directly from angiotensinogen by the action of cathepsin G and tonin (Lippoldt, Paul, Fuxe, & Ganten, 1995). The formation of ANG II from ANG I is prevented by ACE inhibitors (Ondetti, Rubin, & Cushman, 1977; Song & White, 2002).

The activities of ANG II are mediated primarily by two ANG II receptor subtypes, type 1 (AT1) and type 2 (AT2), with AT1 receptors being the primary mediator of sodium appetite and thirst (Fitzsimons, 1998; McKinley *et al.*, 1996).

Brain Angiotensin System. ACE (Chai, McKinley, & Mendelsohn, 1987), ANG II (Oldfield, Ganten, & McKinley, 1989), and ANG II receptors (McKinley, Allen, Clevers, Denton, & Mendelsohn, 1986) have been identified in a number of different brain structures in both rats and sheep. These structures, which contain predominantly AT1 receptors (Gehlert, Gackenheimer, & Schober, 1991; McKinley *et al.*, 1996), include the lamina terminalis which is located in the front wall of the

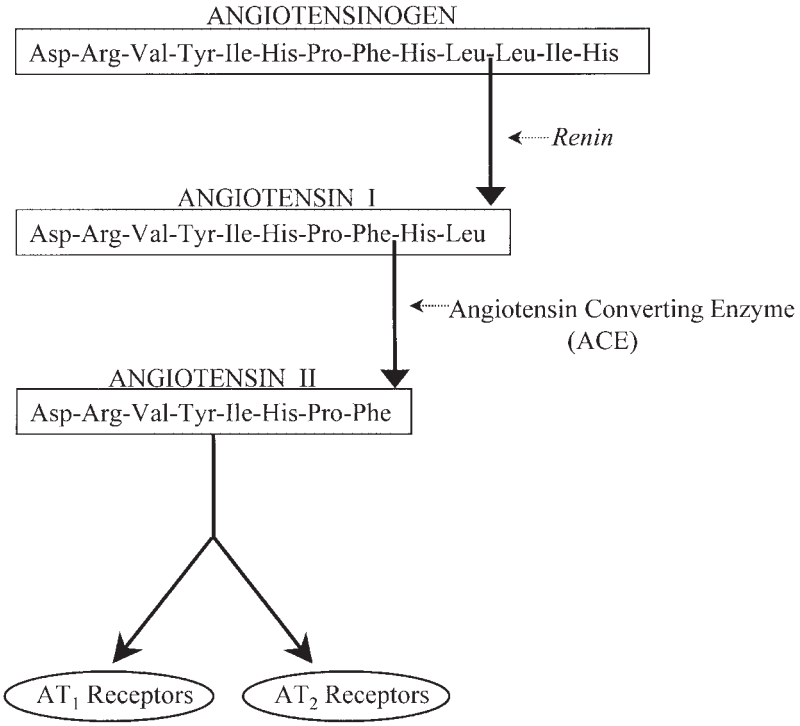

Figure 2. The renin–angiotensin system. Agents that block or prevent the formation of angiotensin II can be used to examine the contribution of each component of the system to the generation of sodium appetite. The formation of angiotensin I can be prevented using renin inhibitors. A number of angiotensin converting enzyme inhibitors (e.g., Captopril, Perindopril, Lisinopril) can be utilized to block the cleaving of angiotensin I to angiotensin II. Receptor antagonists are available to selectively block either the AT1 receptor (e.g., Losartan, Irbesartan, Candesartan) or the AT2 receptor (e.g., PD123319, PD123177). Both receptor subtypes also can be blocked by nonspecific angiotensin II analogues such as, for example, Saralasin and Sarthran.

third cerebral ventricle and consists of the subfornical organ (SFO), median preoptic area (MnPO), and organum vasculosum of the lamina terminalis (OVLT). Other brain areas that contain ANG II receptors include the arcuate nucleus (ARC), supraoptic nucleus (SON), amygdala, bed nucleus of the stria terminalis (BNST), area postrema (AP), nucleus of the solitary tract (NTS), paraventricular nucleus of the hypothalamus (PVN), and dorsal motor nucleus of the vagus nerve (DMV) (McKinley *et al.*, 1986). ANG II immunoreactivity has been observed in nerve terminals located in SFO, MnPO, OVLT, BNST, amygdala, lateral septum, lateral hypothalamus (LH), PVN, SON, lateral parabrachial nucleus (LPBN), AP, and NTS (Lind, Swanson, & Ganten, 1985; Oldfield *et al.*, 1989). ANG II may act as a neurotransmitter or neuromodulator in these brain areas; an angiotensinergic pathway between the SFO and the PVN has been documented (Ferguson & Washburn, 1998; Li & Ferguson, 1993).

Peripheral Administration of ANG II. Peripheral administration of ANG II increases intake and excretion of sodium in rats and sheep (Findlay & Epstein, 1980; Weisinger, Denton, McKinley, Muller, & Tarjan, 1986a; Yang & Epstein, 1991). Systemic administration of ANG II can cause increased sodium intake independent of sodium loss. For example, ANG II-induced sodium appetite, in the absence of

increased sodium excretion, has been reported in deoxycorticosterone (DOC)-treated adrenalectomized (ADX) rats (Dalhouse, Langford, Walsh, & Barnes, 1986). Furthermore, in sheep (Weisinger *et al.*, 1987a), cows (Blair-West, Denton, McKinley, & Weisinger, 1988), rats (Fitts & Thunhorst, 1996; Weisinger *et al.*, 1996), rabbits (Tarjan, Ferraro, May, & Weisinger, 1993), and mice (Weisinger *et al.*, 1990a), peripheral administration of ANG II increases sodium intake of sodium-depleted animals treated with captopril. In this situation, sodium appetite is decreased by the captopril treatment, and subsequently, is increased or restored to the appropriate level by ANG II.

Central Administration of ANG II. ANG II as well as other components of the RAS cause increased intake and excretion of sodium when administered into the brain tissue directly or into the CSF of the brain ventricular system, of rats, sheep, mice, and baboons (Avrith & Fitzsimons, 1980; Blair-West, Carey, Denton, Weisinger, & Shade, 1998; Camargo *et al.*, 1994; Denton *et al.*, 1990; Fluharty & Manaker, 1983; Weisinger *et al.*, 1986a). However, as with peripherally administered ANG II, sodium appetite induced by centrally administered ANG II, independent of sodium loss, has been reported in rats, mice, and baboons (Avrith & Fitzsimons, 1980; Blair-West *et al.*, 1998; Denton *et al.*, 1990; Fitts, Thunhorst, & Simpson, 1985; Yang & Epstein, 1991). Furthermore, in rats, increased sodium intake, independent of sodium loss, has been produced by administration of ANG II directly into the SFO (Araujo Almeida, Antunes, Abrao Saad, & de Arruda Camargo, 1999), MnPO (da Silva, Saad, Renzi, Menani, & Camargo, 1995; do Vale *et al.*, 1997), or OVLT (Fitts & Masson, 1990).

In contrast, considerable evidence suggests that the intake of sodium resulting from ICV administration of ANG II in sheep is secondary to increased sodium loss because: (1) increased sodium intake occurs only with doses of ANG II that increase sodium loss (Weisinger *et al.*, 1989); (2) in initially sodium-replete sheep, the increase in sodium intake caused by ICV infusion of ANG II for 24–48 hr is eliminated by preventing the ANG II-induced sodium loss (Weisinger *et al.*, 1986a); (3) in sodium-depleted sheep, ICV infusion of ANG II does not increase sodium loss and subsequently, causes little, if any, change in sodium intake (Weisinger *et al.*, 1986a); and (4) ICV administration of ANG II does not restore the sodium appetite of the captopril-treated sodium-depleted sheep (Weisinger *et al.*, 1987a).

Brain Structures involved in ANG II-Induced Sodium Appetite

SFO, MnPO, and OVLT. Sodium intake caused by administration of components of the RAS into the lateral preoptic area or into a brain ventricle in rats is decreased by lesion of the SFO (Buggy & Fisher, 1976) or the anteroventral third ventricle (AV3V), a brain area including the ventral portion of the MnPO and the OVLT (De Luca, Galaverna, Schulkin, Yao, & Epstein, 1992; Vivas & Chiaraviglio, 1992).

Intake of sodium caused by administration of ANG II into the MnPO is decreased in animals with lesion of the LH (da Silva *et al.*, 1995) and enhanced in animals with lesion of the ventromedial nucleus of the hypothalamus (VMH) (do Vale *et al.*, 1997). In initially sodium-replete sheep, sodium appetite induced by 24-hr intravenous (IV) infusion of ANG II is unchanged in sheep with lesion of the AV3V (Weisinger *et al.*, 1996).

BNST, Amygdala. Rats with lesion of the central nucleus of the amygdala (CeA) or BNST show reductions in sodium appetite induced by ICV administration of renin (Galaverna, De Luca, Schulkin, Yao, & Epstein, 1992).

AP. In rats, lesion of the AP enhances sodium intake caused by SC administration of ANG II (Watson, 1986).

Brain Structures Activated by ANG II. Immunohistochemical techniques have been used to detect the presence of Fos, the protein product of the proto-oncogene c-fos, which serves as a marker of increased neural activity (Dragunow & Faull, 1989; Sagar, Sharp, & Curran, 1988). Systemic administration of ANG II causes increased Fos in the SFO, MnPO, OVLT, SON, PVN, BNST, CeA, AP, and NTS (Lane, Herbert, & Fitzsimons, 1997; McKinley, Badoer, & Oldfield, 1992; McKinley, Badoer, Vivas, & Oldfield, 1995). The increase in Fos in the SFO and OVLT does not occur with IV infusion of phenylephrine, a drug that increases blood pressure, suggesting that an increase in blood pressure caused by ANG II was not responsible for the increase in Fos in these areas (McKinley *et al.*, 1992).

ICV administration of ANG II elicits increased Fos in SFO, MnPO, OVLT, SON, PVN, BNST, amygdala, NTS, and LPBN (McKinley *et al.*, 1995; Rowland, Li, Rozelle, & Smith, 1994; Xu & Herbert, 1994). The increase in activity is blocked in most areas by administration of losartan (Rowland *et al.*, 1994), indicating that AT1 receptors mediate the increase in brain activity.

The increase in activity in the PVN and SON occurs in cells co-labeled for arginine vasopressin (AVP) and OT, and activity is decreased in these neurons subsequent to water intake (Xu & Herbert, 1994). Thus, these nuclei appear to be primarily concerned with body water balance.

Although similar brain structures are activated by the two routes of administration (McKinley *et al.*, 1995), comparison shows that administration of ANG II into the circulation predominantly activates the SFO and OVLT whereas administration of ANG II into the CSF activates primarily the MnPO. In addition, administration of ANG II into the circulation caused activation throughout the SFO whereas after administration into the CSF, activation occurred in cells adjacent to the ventricular surface. This evidence is consistent with a CSF brain barrier (Krisch, Leonhardt, & Buchheim, 1978).

Adrenal Hormones

Peripheral administration of mineralocorticoid hormones, aldosterone or DOC, causes an increase in sodium intake in rats (Rice & Richter, 1943; Richter, 1956; Tordoff, Hughes, & Pilchak, 1993a; Weisinger & Woods, 1971). Due to their lipophilic nature, steroid hormones readily cross the blood–brain barrier such that an increase in peripheral levels will be reflected by increased brain levels. In intact rats, the action of DOC on sodium appetite appears to be mediated by mineralocorticoid receptors (MRs) but not by glucocorticoid receptors (GRs) (Ma, Itharat, Fluharty, & Sakai, 1997; Vallee *et al.*, 1995). MRs are located in SFO, MnPO, OVLT, AP, periventricular brain regions, and some amygdaloid nuclei, but not in PVN or SON (de Kloet, Oitzl, & Joels, 1993; McEwen, Lambdin, Rainbow, & De Nicola, 1986; Pietranera *et al.*, 2001; Vallee *et al.*, 1995).

Some evidence suggests that the amygdala is important to the manifestation of steroid-induced sodium appetite. The amygdala has both MR and ANG terminals and is the terminus for major gustatory projections from the NTS/PBN (Lind *et al.*, 1985; Stumpf & Sar, 1979). Administration into the amygdala of aldosterone or DOC increases sodium intake in rats (Sakai, McEwen, Fluharty, & Ma, 2000). Interference with MR, but not GR, formation in the amygdala (by antisense

treatment) interferes with the expression of DOC-induced sodium appetite (Sakai, Ma, Zhang, McEwen, & Fluharty, 1996; Sakai *et al.*, 2000). In addition, sodium intake in rats is increased by 3β, 5β tetrahydroaldosterone (or 5α-tetrahydro-DOC), an A-ring reduced form of aldosterone (or DOC), steroids that bind to GABA-ergic membrane receptors but not to intracellular MRs (Sakai *et al.*, 2000). Thus, mineralocorticoids can act on brain nuclei, particularly in the amygdala, to influence sodium intake through both genomic mechanisms (involving cytosolic MR) and nongenomic mechanisms, via GABA-A receptors.

DOC treatment causes an increase in the mRNA for enkephalin in brain structures associated with reward (Lucas, Pompei, & McEwen, 2000). The increased sodium intake caused by DOC may be mediated, in part, by changes in the reward value of salt taste.

Mineralocorticoid administration increases sodium intake in other species, including mice (Blair-West, Denton, McBurnie, Tarjan, & Weisinger, 1995), rabbits (Denton & Nelson, 1970), hamsters (Fitts, Yang, Corp, & Simpson, 1983), and sheep (Hamlin, Webb, Ling, & Bohr, 1988).

Administration of glucocorticoids (i.e., cortisol or corticosterone) increases salt appetite in rabbits (Blaine, Covelli, Denton, Nelson, & Shulkes, 1975) and mice (Blair-West *et al.*, 1995). Glucocorticoid hormones also potentiate the sodium appetite induced by mineralocorticoid hormones in rats (Ma, McEwen, Sakai, & Schulkin, 1993; Shelat, King, Flanagan-Cato, & Fluharty, 1999), and administration of hormones with both mineralocorticoid and glucocorticoid activity (e.g., fludrocortisone) causes a greater sodium appetite in mice than DOC (Underwood, McCutcheon, & Dudek, 1993). Interestingly, administration of glycyrrhizic acid, a compound found in licorice, causes an increase in circulating glucocorticoid levels and an increase in salt intake of rats (Cooney & Fitzsimons, 1996). Glycyrrhizic acid inhibits the action of 11βOHSD, an enzyme that converts the active corticosterone to an inactive form, allowing aldosterone to occupy MRs. With the inhibition of 11βOHSD, excessive glucocorticoid hormone, acting on MRs, causes an increase in salt intake. IV infusion of a steroid cocktail including both glucocorticoid and mineralocorticoid hormones increases sodium appetite of sheep to a greater extent than administration of a mineralocorticoid hormone alone (Weisinger *et al.*, 1980).

Brain Structures involved in Steroid-Induced Sodium Appetite

SFO, MnPO, OVLT. Sodium appetite induced by treatment with DOC is enhanced, rather than depressed, in rats with lesion of the OVLT (Fitts, 1991; Fitts, Tjepkes, & Bright, 1990) and is unchanged in rats with lesion of the SFO (Fitts, 1991) or AV3V (De Luca *et al.*, 1992).

BNST, Amygdala. Rats with lesion of the medial amygdala (MeA) have impaired aldosterone- or DOC-induced sodium appetite, and the corticosterone potentiation of aldosterone-induced salt appetite is eliminated (Nitabach, Schulkin, & Epstein, 1989; Schulkin, Marini, & Epstein, 1989; Zhang, Epstein, & Schulkin, 1993).

Rats with lesion of the CeA or BNST show deficits in sodium appetite induced by DOC (Galaverna *et al.*, 1992; Reilly, Maki, Nardozzi, & Schulkin, 1994).

Other Brain Structures. Rats with lesion of the gustatory subnucleus of the ventral posterior complex of the thalamus (Wolf & DiCara, 1974) or LH, but not septal nucleus (Wolf & Quartermain, 1967) or hippocampus (Magarinos, Coirini,

De Nicola, & McEwen, 1986), show deficits in sodium appetite induced by DOC. Most of the aldosterone receptors in the brain are located in the hippocampus, and rats with lesion of the hippocampus have an 80% decrease in MRs (Magarinos *et al.*, 1986).

Brain Structures Activated by Steroid Hormones. In rats, DOC treatment increases neural activity, as measured by Fos immunoreactivity, in many of the same areas that are activated by ANG II, including the MnPO, OVLT, BNST, amygdala, SON, and PVN (Lane *et al.*, 1997; Pietranera *et al.*, 2001).

Interaction of Adrenal Hormones and ANG II

Experimental representation of the synergy hypothesis involves concurrent administration of ANG II centrally and DOC or aldosterone systemically at doses that, individually, do not stimulate NaCl intake. Using this model, NaCl appetite has been reported in rats (Camargo *et al.*, 1994; Fluharty & Epstein, 1983; Zhang, Stellar, & Epstein, 1984) and pigeons (Massi & Epstein, 1990), but could not be demonstrated in sheep or rabbits (Weisinger *et al.*, 1996).

Recent experiments have demonstrated synergy between ANG II and aldosterone in a primate (Shade *et al.*, 2002a). While sodium intake was not altered by administration of a low dose of either ANG II or aldosterone, sodium intake increased ~10-fold when these agents were administered together in baboons. It is therefore possible that this synergy may be the basis of the salt appetite caused by sodium depletion in the baboon, as has been suggested for the rat. Further experiments in Na-replete baboons have shown no effect on salt intake of the systemic administration of aldosterone alone at rates equal to the rates of secretion in sodium-deficient baboons (unpublished observations). Taken together, these results indicate that the contribution of aldosterone may be limited to its potentiation of central ANG mechanisms in primates.

Mechanism of Synergistic Action of Aldosterone and ANG II. The synergy between the two major hormones of sodium deficiency might occur by any of several mechanisms, including an interaction between brain sites that are separately sensitive to ANG II (e.g., lamina terminalis) and aldosterone (e.g., amygdala), as proposed by Schulkin (Schulkin, 1991); interactions at the same brain site (e.g., by aldosterone upregulating ANG II receptors); increasing ANG II binding to its receptors (De Nicola, Seltzer, Tsutsumi, & Saavedra, 1993; Shelat *et al.*, 1999); or inhibition of a central inhibitory mechanism such as oxytocinergic neurons (Stricker & Verbalis, 1996; Verbalis, Blackburn, Hoffman, & Stricker, 1995).

Summary

Peripherally administered ANG II can stimulate sodium intake without increasing sodium excretion. This is especially evident in Na-depleted animals treated with captopril. Evidence suggests that a brain structure without a blood–brain barrier such as the SFO or OVLT mediate sodium appetite induced by peripheral ANG II.

Peripheral administration of steroid hormones can stimulate sodium intake. Lines of evidence suggesting that brain structures in the AV3V are involved in sodium appetite induced by steroids are that MRs are located there and that increased detection of Fos has been reported in the MnPO and OVLT in

DOC-treated rats. Lines of evidence suggesting that brain structures in the AV3V are not involved in sodium appetite induced by steroids are that sodium appetite induced by treatment with DOC is unchanged or even enhanced in rats with lesion of the SFO, OVLT, or AV3V. Other evidence suggests that the amygdala and BNST have a role in steroid-induced sodium appetite: (1) interference with MR formation in the amygdala (e.g., by antisense treatment) interferes with the expression of DOC-induced sodium appetite; and (2) rats with lesion of the MeA or CeA have impaired mineralocorticoid-induced salt appetite.

Central administration of ANG II can stimulate sodium intake without increasing sodium excretion in rats, mice, and baboons. Evidence suggests that brain structures located on the front wall of the third cerebral ventricle (e.g., SFO, MnPO, and OVLT) mediate sodium appetite induced by central ANG II. The failure of centrally administered ANG II to elicit increased sodium intake in the absence of an increase in sodium loss in sheep or cows suggests that a brain ANG II system is not involved in the central mechanism controlling sodium appetite of ruminants.

A synergistic action of mineralocorticoid and ANG II seems to stimulate sodium appetite in rats, pigeons, and baboons, but not in sheep or rabbits. The mechanism responsible for this synergy remains to be determined.

Sodium Depletion-Induced Sodium Appetite

Depletion of body sodium, as would occur with diarrhea or profuse sweating, results in a number of physiological changes including renal, gastrointestinal, and other peripheral mechanisms geared to minimize further loss of body sodium. For instance, secretion of aldosterone is increased and acts on the kidney to ensure that urinary excretion of sodium is minimized, by maximizing the reabsorption of sodium. Losses of sodium in faeces, sweat, and other secretions also are reduced. Sodium deficits also occur in animals living in salt poor areas of the world, for example, the interiors of continents where sodium content in rain water is very low (Blair-West *et al.*, 1968). Importantly, sodium-depleted animals manifest an appetite for sodium-containing solutions or substances. Several experimental models of sodium depletion-induced sodium appetite have been investigated. These include: (1) maintenance on a very low sodium diet (Stricker, Thiels, & Verbalis, 1991). Loss of sodium occurs over the first few days, whereas an increase in sodium intake is observed after 7–10 days. (2) Adrenalectomy (Richter, 1936). Loss of sodium occurs due to the removal of hormones that enable reabsorption of sodium in the kidney, intestines, rectum, and sweat glands. An increase in sodium intake in rats is observed within a few days. (3) Peritoneal dialysis (Chiaraviglio & Perez Guaita, 1986; Falk, 1966). Loss of sodium occurs rapidly due to its withdrawal from the body. An increase in sodium intake is observed within a day. (4) Furosemide treatment (Jalowiec, 1974; Weisinger, Denton, Di Nicolantonio, & McKinley, 1988). Loss of sodium in urine occurs within hours. An increase in sodium intake usually occurs within a day. (5) Loss of sodium in parotid saliva (Denton, 1966; Denton & Sabine, 1961). The surgical exteriorization of the duct of the parotid gland causes a continuous and substantial loss of body sodium (a technique used primarily in ruminants). An increase in sodium intake usually occurs within a day. (6) Total water deprivation over 24–48 hr (Weisinger, Denton, McKinley, & Nelson, 1985b). Loss of sodium occurs in urine. An increase in sodium intake usually occurs within a day.

The sodium appetite induced in these models is commensurate with (Denton, 1966; Denton & Sabine, 1961; Weisinger *et al.*, 1985b), or greater than (Falk, 1966; Stricker *et al.*, 1991; Weisinger *et al.*, 1988), the sodium loss. For example, in rats, during a 24-hr period of water deprivation, a sodium deficit of 0.6–1.0 mmol develops. Presumably, the increase in sodium excretion occurs in response to the elevation of plasma sodium concentration. When NaCl solution is made available, sodium intake is increased such that Na balance is restored to pre-dehydration levels (Weisinger *et al.*, 1985b).

In sheep, there is evidence that taste factors are important in the correction of sodium deficit. Sodium-deficient sheep commensurately replaced their deficit when the sodium concentration of $NaHCO_3$ solution presented was varied from 119 to 952 mmol/L. Thus, the sheep behaved as if it could multiply volume by concentration in order to restore sodium balance (Denton, 1966; Denton & Sabine, 1961). In a subsequent experiment, sheep were offered one concentration of $NaHCO_3$ solution (300 or 900 mM) for the initial 100–150 ml of intake and then the other concentration of $NaHCO_3$ solution for subsequent drinking. Significant underdrinking occurred when the second concentration was lower than the first. This failure of sheep to respond appropriately to the decrease in $NaHCO_3$ concentration was attributed to taste adaptation (Osborne, Denton, & Weisinger, 1987b).

Angiotensin II in Sodium Depletion: Central and Peripheral Origins

Intact Animals. Sodium depletion causes an increase in circulating levels of ANG II (Sakai & Epstein, 1990) as well as brain levels of various components of an ANG II system. For example, sodium depletion increased CSF [ANG II] but IV infusion of ANG II did not (Abraham, Baker, Blaine, Denton, & McKinley, 1975). Also, sodium depletion enhanced the expression of AT1 receptor mRNA in the SFO, MnPO, and PVN (Charron, Laforest, Gagnon, Drolet, & Mouginot, 2002). As noted earlier, central and peripheral administration of ANG II can stimulate sodium appetite. However, controversy exists as to the relative contribution of circulating and brain ANG II to sodium depletion-induced sodium appetite.

Peripheral administration of ACE inhibitors such as captopril or enalapril decrease the sodium intake of sodium-depleted rats (Fitts & Thunhorst, 1996; Moe, Weiss, & Epstein, 1984; Thunhorst & Fitts, 1994; Weisinger *et al.*, 1988, 1996), sheep (Weisinger *et al.*, 1987a), cows (Blair-West *et al.*, 1988), rabbits (Tarjan *et al.*, 1993), mice (Weisinger *et al.*, 1990a), and baboons (Blair-West *et al.*, 1998). As ACE inhibitors can readily cross the blood–brain barrier, the relative contribution of peripheral and central ANG II cannot always be ascertained. In a study aimed at evaluating the relative contributions of each, sodium intake of sodium-depleted rats was decreased when captopril was administered peripherally at a dose that blocked the peripheral but not the central conversion of ANG I to ANG II (Thunhorst & Fitts, 1994). Furthermore, as noted earlier, the sodium appetite of the systemic captopril-treated sodium-depleted animal is restored to the appropriate level by peripheral, but not by ICV, administration of ANG II. As blood pressure of a sodium-depleted animal is, in part, maintained by ANG II, administration of an ACE inhibitor such as captopril also decreases blood pressure (Fitts & Thunhorst, 1996; Tarjan *et al.*, 1993; Thunhorst & Fitts, 1994; Weisinger *et al.*, 1987a). This decrease in blood pressure is not the cause of the decrease in sodium appetite since the appetite is not restored by peripheral administration of phenylephrine, at a dose that restores blood pressure to normal (Fitts & Thunhorst, 1996).

Since ANG II does not readily cross the blood–brain barrier (Epstein, 1981; Reid, 1984; Schelling, Hutchinson, Ganten, Sponer, & Ganten, 1976), this line of evidence suggests that sodium intake in sodium-depleted animals is mediated by peripherally generated ANG II acting in brain areas without a blood–brain barrier (Weisinger *et al.*, 1996).

On the other hand, ICV infusion of ANG II antagonists decreases sodium appetite (Buggy & Jonklaas, 1984; Sakai, Chow, & Epstein, 1990; Weiss, Moe, & Epstein, 1986), consistent with a role of brain ANG II in the sodium appetite of rats. Furthermore, in direct contrast to the results of Thunhorst and Fitts (1994), Sakai *et al.* (1990) have shown that peripheral administration of antagonists of the RAS which completely block the peripheral but not the central actions of ANG II, do not influence sodium intake. Only when the central actions of ANG II are blocked is sodium appetite decreased or abolished. In baboons, the central infusion of the selective non-peptide AT1 receptor antagonist ZD7155 inhibits the high sodium intake caused by sodium deficiency (Blair-West *et al.*, 1998). ICV administration of sarile decreases the sodium intake of sodium-depleted pigeons (Massi & Epstein, 1987). However, ICV administration of ANG II receptor antagonists or ACE inhibitors does not decrease sodium intake caused by sodium depletion in sheep (Coghlan *et al.*, 1981; Weisinger, Blair-West, Denton, & Tarjan, 1997a, 1997b) or cows (Blair-West *et al.*, 1988). Hence, the inhibition of central ANG II alone may be sufficient to block sodium appetite in rats, pigeons, and baboons, but does not appear to be sufficient in sheep or cows.

With regard to the synergistic action of ANG II and aldosterone, Sakai *et al.* (1986) demonstrated that the sodium appetite of the sodium-depleted rat is reduced by central, but not peripheral, administration of an aldosterone receptor antagonist, RU28318, as well as by peripheral administration of captopril. However, the greatest reduction in appetite occurred with concurrent administration of the two antagonists. A more recent study, however, reported that the sodium appetite of the sodium-depleted rat was not decreased by administration of metyrapone, an inhibitor of adrenal steroid synthesis (Rowland & Morian, 1999). Clearly there is still controversy surrounding the combined roles of aldosterone and ANG II in sodium appetite under physiological conditions.

Role of ANG II in Water Deprivation-Induced Sodium Appetite. Water deprivation results in both water and sodium deficits (McKinley, Denton, Nelson, & Weisinger, 1983), and, consequently, causes increased intakes of both water and sodium (De Luca *et al.*, 2002; Weisinger *et al.*, 1985b). The increase in sodium intake is blocked either by peripheral administration of captopril or by central administration of ANG II receptor antagonists (Sato, Yada, & De Luca, 1996).

Adrenalectomized Animals. The importance of the steroids and catecholamines secreted from the adrenal glands for survival and well-being has been known for at least 150 years. Mineralocorticoids are crucial to sodium homeostasis while glucocorticoids are crucial to metabolism and response to stress. Catecholamines are involved in both body fluid and energy homeostasis. Nonetheless, ADX rats, but not sheep, can be kept alive merely by providing them with extra NaCl in their food or drink. That is, the ADX rat not only has a need but also a voracious appetite for NaCl solutions (Bare, 1949; Richter, 1936, 1956). In contrast, in sheep, sodium appetite is not stimulated by adrenalectomy. Furthermore, with minimal replacement therapy, sodium intake of ADX sheep,

as in non-ADX sheep, is commensurate with the sodium deficit caused by loss of saliva from a parotid fistula (Denton, Orchard, & Weller, 1969b).

The appetite for sodium of ADX rats is due to the increased loss of sodium in the urine; when urinary sodium loss is prevented by administration of mineralocorticoid hormones (either aldosterone or DOC), the appetite for NaCl is reduced. Given the increase in sodium intake caused by aldosterone or DOC in intact rats, it is clear that a U-shaped function relates mineralocorticoid level and intake of sodium (Fregly & Waters, 1966; Tordoff *et al.*, 1993a). The decrease in sodium appetite of ADX rats treated with mineralocorticoid hormone is attenuated by the central administration of RU28318 (McEwen *et al.*, 1986). This suggests that central actions of aldosterone influence sodium appetite in addition to its known peripheral action on retention of filtered sodium. Also, as noted earlier, the sodium appetite of sodium-depleted intact rats is decreased by ICV administration of RU28318 (Sakai *et al.*, 1986). Taken together, these results suggest that central actions of aldosterone may mediate both increases and decreases in sodium appetite. Administration of glucocorticoid hormones does not reduce the NaCl intake of ADX rats (De Nicola *et al.*, 1993; Fregly & Waters, 1966; McEwen *et al.*, 1986).

The sodium appetite of the ADX rat is dependent upon ANG II. As in intact rats, sodium intake of ADX rats is decreased by peripheral administration of a large dose of captopril (Elfont & Fitzsimons, 1985), ZD7155 (Weisinger *et al.*, 2000b), or losartan (Tordoff, Pilchak, & Hughes, 1993b). Sodium intake is also decreased by peripheral administration of captopril at a dose that blocks the peripheral but not the central conversion of ANG I to ANG II, and this attenuated appetite is restored to normal by systemic administration of ANG II (Schoorlemmer, Johnson, & Thunhorst, 2001).

On the other hand, the sodium appetite of the ADX rat is decreased by ICV infusion of sarile or losartan, or AT1 receptor antisense, but not by the selective AT2 receptor antagonist PD123319 (Galaverna *et al.*, 1996; Ma *et al.*, 1997; Rowland, Rozelle, Riley, & Fregly, 1992; Sakai & Epstein, 1990). Thus, as is the case with intact sodium-deplete rats, a controversy exists as to whether the sodium appetite of the ADX rat is dependent on blood-borne ANG II, brain ANG II, or both.

Modification of Brain ANG System by Adrenalectomy. The levels of angiotensinogen mRNA are reduced by 50–60% in the OVLT and MnPO of ADX rats and are restored to normal by treatment with RU28362, a glucocorticoid agonist, or dexamethasone, but not by treatment with aldosterone at a dose that restores sodium intake to control levels (Deschepper & Flaxman, 1990; Riftina, Angulo, Pompei, & McEwen, 1995). In addition, the level of ANG II binding is unaltered in the SFO and MnPO and reduced in the PVN. Treatment of the ADX rat with aldosterone, which decreases the need for, and intake of, sodium, did not alter ANG II binding in the MnPO but increased ANG II binding in the SFO and the PVN (De Nicola *et al.*, 1993). Thus, the changes in sodium intake of ADX rats do not seem to be clearly related to the observed changes in the brain ANG II system.

Cerebral Sodium

Research over the past 60 years has demonstrated the existence of sensors in the brain that respond to changes in the body's sodium content or osmolality (Andersson & Olsson, 1973; Denton, McKinley, & Weisinger, 1996; McKinley, Denton, & Weisinger, 1978). Debate on the precise contribution of these cerebral

sodium sensors and osmoreceptors to sodium appetite and thirst continues today. Evidence reviewed below suggests that cerebral sodium sensors but not osmoreceptors contribute to the control of sodium appetite whereas both sodium sensors and osmoreceptors contribute to the control of thirst.

The hypothesis that the sodium appetite of sodium-deplete sheep is caused by changes in the ICF [Na^+] of cells specifically subserving sodium appetite was proposed over 30 years ago (Denton, 1966; Denton, Kraintz, & Kraintz, 1969a; Denton & Sabine, 1961) and was supported by experimental evidence 15–20 years later (Weisinger, Considine, Denton, & McKinley, 1979; Weisinger, McKinley, Muller, & Tarjan, 1985a; Weisinger *et al.*, 1982).

It was observed that increasing the [Na^+] of CSF and brain ECF decreased the sodium intake of sodium-deplete sheep. Sodium intake of sodium-depleted sheep was decreased by ICV infusion of a solution of artificial CSF with [Na^+] = 500 mM, which increased CSF [Na^+] by 15–25 mM, or by infusion of artificial CSF with [Na+] = 300 mM, which increased CSF [Na^+] by 10–15 mM. However, sodium intake was not altered by infusion of artificial CSF with [Na^+] = 200 mM, which increased CSF [Na^+] by 6–9 mM (Weisinger *et al.*, 1989).

The ability of increasing CSF [Na^+] to decrease sodium intake has also been observed in sodium-replete sheep with a high need-free intake of sodium and in sodium-replete sheep infused ICV with ANG II over 24 hr (Weisinger, Denton, McKinley, Simpson, & Tarjan, 1986b). Increased cerebral sodium has been shown to decrease sodium appetite of sodium-deplete rats (Chiaraviglio & Perez Guaita, 1986) with a concomitant decrease in sodium depletion-induced Fos in both the OVLT and the SFO (Vivas, Pastuskovas, & Tonelli, 1995).

In contrast, it was observed that decreasing the [Na^+] in the CSF and brain ECF could increase the sodium intake of sodium-deplete and sodium-replete sheep. Sodium intake was increased by infusion of a solution of 700 mM mannitol in artificial CSF with [Na^+] = 150 mM, which is equi-osmotic with artificial CSF with [Na^+] of 500 mM, and by infusion of a solution of 270 mM mannitol with [Na^+] = 0 mM. Both of these infusions decreased CSF [Na^+] by 15–25 mM while the former but not the latter infusion raised CSF osmolality. Furthermore, sodium intake of sodium-replete sheep was enhanced during ICV infusion of solutions that decreased CSF [Na^+] by as little as 4–6 mM (Weisinger, Denton, McKinley, Osborne, & Tarjan, 1987b; Weisinger *et al.*, 1982; 1985a; 1989). ICV infusion of an equi-osmotic solution of 340 mM mannitol in artificial CSF with [Na^+] = 330 mM, which raised CSF osmolality but did not alter CSF [Na^+], did not cause any change in sodium intake. Interestingly, infusions that decreased CSF and brain ECF [Na^+] also increased aldosterone secretion (Coghlan *et al.*, 1980) while those that increased CSF and brain ECF [Na^+] decreased aldosterone secretion (Abraham *et al.*, 1976).

Experimentally induced changes in CSF [Na^+] are, in general, greater than those observed in the physiological situation. For example, in rats, CSF [Na^+] decreases 5–11 mmol/L during the first 4 hr after intraperitoneal dialysis (Chiaraviglio & Perez Guaita, 1986), while in sheep, Na depletion via loss of parotid saliva decreases CSF [Na^+] by 3–4 mmol (Weisinger *et al.*, 1987a, 1987b). It is conceivable that the neural regions involved in responding to such experimental changes are some distance from the ventricles such that the change in [Na^+] in these regions would be much smaller than that in CSF. Clearly, the evidence suggests that experimentally induced changes in CSF and thus brain ECF [Na^+] impinge on the neural mechanisms involved in the initiation and/or satiation of sodium appetite.

There is evidence to suggest that the specific sodium sensors involved in the control of sodium appetite are some distance from the brain ventricles and respond to changes in $[Na^+]$ of ECF rather than CSF. That is, the sodium appetite of sodium-depleted sheep is enhanced by ICV infusion of hypertonic mannitol, sucrose, L-fucose, or L-glucose, but not by D-glucose, D-mannose, 2-deoxy-D-glucose, or 3–O-methyl glucose (Weisinger *et al.*, 1985a). CSF $[Na^+]$ is decreased by all of these treatments. Substances that do not readily penetrate into cells and capillaries (e.g., mannitol) would reach and influence the sensor while substances that do diffuse into cells (e.g., D-glucose) would not have this effect.

There is other evidence supporting the proposition that the sodium appetite of sodium-depleted sheep is controlled, at least in part, by changes in ICF $[Na^+]$ of cerebral sodium sensors. First, the sodium appetite of sodium-depleted sheep is decreased by administration of drugs that increase ICF $[Na^+]$, for example, by inhibiting Na^+-K^+-ATPase, an enzyme that pumps sodium out of the cell (ouabain, ethacrinic acid, vanadate), or by opening sodium channels (monensin). Using a "push-pull" perfusion procedure that restricts the changes to relatively specific brain regions, sodium appetite of a sodium-depleted sheep was decreased with perfusion of 200 mmol/L NaCl, ouabain, or monensin (Denton *et al.*, 1969a; Tarjan, Cox, Denton, McKinley, & Weisinger, 1986). Further, this approach suggested that the sodium sensors in sheep are situated in the vicinity of the anterior wall of the third ventricle (Tarjan, Denton, & Weisinger, 1989). Second, ICV infusion of phlorizin increased the sodium appetite of sodium-depleted sheep and prevented the decreased sodium intake caused by ICV infusion of hypertonic NaCl (Weisinger *et al.*, 1985a). Phlorizin is a drug that blocks sodium-coupled glucose transport; it has been proposed that it decreases ICF $[Na^+]$ of cerebral sodium sensors when given ICV, and it prevents increases in ICF $[Na^+]$ of cerebral sodium sensors when given together with hypertonic NaCl.

Similar evidence has been reported in the rat. For example, the sodium appetite of sodium depleted rats is decreased by ICV administration of ethacrinic acid, vanadate, or ouabain, and is increased by diphenylhydantoin, a drug that inhibits cellular sodium influx (Vivas & Chiaraviglio, 1987). In ADX rats, the decrease in sodium intake during administration of mineralocorticoid is associated with decrease in Na^+-K^+-ATPase activity in various brain regions associated with sodium appetite including the amygdala and hypothalamus (Grillo, Coirini, McEwen, & De Nicola, 1989).

ICV infusion of artificial CSF with 700 mM mannitol ($[Na^+]$ = 150 mM) increased the sodium intake of cows (Blair-West *et al.*, 1987) but not of rabbits (Denton *et al.*, 1984b), mice (Osborne *et al.*, 1990), pigeons (Massi & Epstein, 1990), or rats (Denton *et al.*, 1984b; Epstein *et al.*, 1984; Osborne, Denton, & Weisinger, 1987a).

The influence of changes in $[Na^+]$ and osmolality of blood perfusing the brain also has been investigated. Intracarotid infusion of 4 M NaCl decreased sodium intake in sodium-replete and sodium-depleted sheep, and in sodium-replete sheep infused ICV with ANG II over 24 hr (Muller, Denton, McKinley, Tarjan, & Weisinger, 1983; Weisinger *et al.*, 1986b). This systemic infusion raised the $[Na^+]$ and osmolality of blood and CSF and, in sodium-depleted sheep, replaced up to 40–70% of the sodium deficit. Interestingly, the decrease in sodium intake caused by this infusion is similar to that caused by ICV infusion of hypertonic NaCl-CSF, though this latter infusion did not alter $[Na^+]$ of blood.

Further evidence that increases in cerebral sodium are important in causing the decrease in sodium intake during intracarotid infusion of hypertonic NaCl is that the inhibitory effect of intracarotid hypertonic NaCl on sodium intake was blocked by ICV infusion of hypertonic mannitol (Muller *et al.*, 1983). In contrast, the water intake caused by intracarotid infusion of 4 M NaCl was increased by the concurrent ICV infusion of hypertonic mannitol.

The Sodium Sensor. In rats, the cells of the OVLT are sodium sensitive. Using a brain slice preparation, changes in $[Na^+]$ of the bathing medium surrounding neural cells from the OVLT altered firing rates, as did application of substances that alter ICF $[Na^+]$ such as ouabain or diphenylhydantoin (Vivas, Chiaraviglio, & Carrer, 1990).

A sodium channel (Nax) thought to be the sodium sensor has been identified in mice. The sodium channel Nax has been identified in several brain areas including the SFO and OVLT. Evidence obtained showed that the neural cells responded to increases in ECF $[Na^+]$ but not to increases in osmolality or chloride concentration or to changes in ICF $[Na^+]$ (Hiyama *et al.*, 2002).

Cerebral Sodium Sensors: Role of ANG II. Although the sodium intake of sodium-depleted sheep is not decreased by ICV administration of losartan, losartan paradoxically blocks the decrease in sodium intake caused by systemic or ICV infusion of hypertonic NaCl (Weisinger *et al.*, 1997b) and the increase in sodium intake caused by the ICV infusion of hypertonic mannitol. Captopril did not block the increase in sodium appetite caused by hypertonic mannitol. Therefore, the formation of ANG II by the sodium sensor cells involved in sodium appetite appears not to be dependent on ACE.

One possible explanation for the paradoxical actions of losartan is that the brain angiotensinergic neurons, activated by reduced brain ECF $[Na^+]$, are located in brain areas more readily accessible to antagonists infused into the CSF than is the brain angiotensinergic system normally activated by sodium depletion. Under conditions of moderate sodium depletion, given that losartan readily penetrates the blood–brain barrier (Song, Zhuo, & Mendelsohn, 1991), the angiotensinergic system involved in sodium appetite may be too distant from the brain ventricular system to be adequately blocked by losartan. Therefore, the role of brain ANG II in sodium-depleted sheep has not been revealed as yet.

One explanation for the observation that losartan prevents the decrease in sodium intake caused by administration of hypertonic NaCl, is that hypertonic NaCl, via an angiotensinergic pathway, stimulates the release of some factor, for example, SOM (see following section), which inhibits sodium intake. Blocking the action of ANG II with losartan prevents the release of this inhibitory factor (Weisinger & Burns, 1999).

Factors that Inhibit Sodium Appetite

A decrease in activity of excitatory neurons or an increase in activity of inhibitory neurons could mediate termination of sodium appetite subsequent to sodium intake. Brain activity, as measured by Fos expression, is decreased, subsequent to sodium intake, in the OVLT and to a lesser extent, the SFO (Vivas *et al.*, 1995), while sodium intake after sodium depletion has been shown to increase Fos in the PVN (Franchini & Vivas, 1999) or NTS (Houpt, Smith, Joh, & Frankmann,

1998). These observations suggest that the SFO, OVLT, PVN, and NTS are involved in satiation. Drinking sodium after sodium depletion also increases the activity and the expression of OT in the PVN (Franchini & Vivas, 1999) and activity and the expression of serotonin in the raphe nucleus (Franchini, Johnson, de Olmos, & Vivas, 2002). These results suggest that oxytocinergic cells in the PVN and serotonergic cells in the raphe may be involved in satiation or inhibition of sodium intake. The latter observation is consistent with the evidence that serotonergic mechanisms can inhibit sodium appetite. The administration of fenfluramine or dexfenfluramine, drugs that enhance serotonergic transmission, decreases sodium intake of sodium-depleted rats (Badaue-Passos, Ventura, Silva, Olivares, & Reis, 2003; Rouah-Rosilio, Orosco, & Nicolaidis, 1994). Pharmacological blockade of the inhibitory serotonergic system also increases the sodium intake of sodium-depleted rats. Interestingly, injecting losartan into the SFO does not block the sodium intake of sodium-depleted rats but blocks the additional sodium intake of sodium-depleted rats treated with methysergide (Menani, Colombari, Beltz, Thunhorst, & Johnson, 1998a; Menani, De Luca, & Johnson, 1998b).

A number of neuropeptides, including OT, interfere with the expression of sodium appetite subsequent to sodium depletion. In some instances, ANG II, presumably in the neural system subserving thirst, stimulates the release of a neuropeptide that inhibits the expression of sodium appetite (Blackburn, Demko, Hoffman, Stricker, & Verbalis, 1992a). The effects of ANG II itself are also subject to inhibitory factors such as increased blood pressure, via baroreceptor-mediated mechanisms (Evered, Robinson, & Rose, 1988; Thunhorst & Johnson, 1993; Thunhorst, Lewis, & Johnson, 1993) and some neuropeptides. Given that angiotensinergic mechanisms are involved in the mechanisms subserving both thirst (Blair-West *et al.*, 1994; Weisinger *et al.*, 1997b) and sodium appetite (Sakai & Epstein, 1990; Sakai *et al.*, 1990; Weisinger *et al.*, 1997b), it is possible that specificity of intake is achieved by the interaction of angiotensinergic mechanisms with other inhibitory peptidergic systems. For example, in the situation of a body fluid deficit, excitation of angiotensinergic mechanisms involved in thirst could cause ingestion of water and the release of neuropeptides that inhibit or delay sodium intake (Weisinger, Blair-West, Burns, Denton, & Purcell, 2001; Weisinger & Burns, 1999).

This section will document the actions of various neuropeptides that might fill such inhibitory roles. The influence of OT, SOM, atrial natriuretic peptide (ANP), and several of the tachykinin (TK) family of peptides (e.g., substance P, neurokinin A, and neurokinin B) on intakes induced by centrally administered ANG II as well as those occurring in physiological situations will be described. The evidence leads us to the conclusion that neuropeptides, both excitatory (ANG II) and inhibitory (OT, SOM, ANP, and TKs), have a role in the maintenance of body fluid homeo-stasis. Interestingly, except for ANP (Chinkers *et al.*, 1989; Goeddel, & Schulz, 1989), the receptors for these neuropeptides are members of the seven transmembrane-G protein coupled receptor family (OT, Barberis, Mouillac, & Durroux, 1998; SOM, Raulf, Perez, Hoyer, & Bruns, 1994; and TKs, Patacchini & Maggi, 1995).

Oxytocin. OT is a 9-amino acid peptide synthesized in the magnocellular and parvocellular neurons of the hypothalamic PVN or SON that project either to the neurohypophysis or to sites within the brain (Insel, 1992). OT receptors are found in the BNST, NTS, and DMV.

Evidence suggestive that brain OT may be one of the main inhibitory factors limiting the expression of sodium appetite (Stricker, Hosutt, & Verbalis, 1987;

Stricker & Verbalis, 1986, 1987) has been obtained. ICV but not systemic administration of OT decreases the sodium appetite of hypovolemic rats (Blackburn, Stricker, & Verbalis, 1992b; Stricker & Verbalis, 1987). IV administration of an OT-receptor antagonist blocks the increase in sodium loss that occurs during water deprivation (Huang, Lee, Arnason, & Sjoquist, 1996), suggesting that peripheral OT is involved in the control of sodium output.

Administration of an OT antagonist does not influence need-free sodium intake but potentiates ANG II-induced sodium intake (Blackburn *et al.*, 1992a). Presumably, centrally administered ANG II stimulates water intake and the release of OT, and OT acts to inhibit sodium intake. The expression of sodium appetite is inversely related to central levels of OT (Stricker & Verbalis, 1986, 1987; Stricker *et al.*, 1987) and it has been shown that OT receptor knockout mice have increased sodium intake but not water intake (Amico, Morris, & Vollmer, 2001). One explanation for the influence of mineralocorticoid hormones, alone or in synergy with ANG II, on sodium appetite is that they inhibit central OT secretion (Stricker & Verbalis, 1996; Verbalis *et al.*, 1995).

Considerable evidence is consistent with the role of OT as an inhibitory peptide controlling sodium appetite. A novel technique utilized to evaluate the role of neurons with OT receptors in sodium appetite was to couple the A-chain of ricin (a cytotoxic agent) to OT, such that cells with OT receptors would be eliminated. It was observed that sodium intake of hypovolemic rats treated with hyperosmotic stimuli or of normovolemic rats treated with ICV injection of ANG II was potentiated (Blackburn, Samson, Fulton, Stricker, & Verbalis, 1995). In another experiment, the sodium appetite observed after 8 days of sodium deprivation was greater in male than in female rats (Stricker *et al.*, 1991). Furthermore, the sodium appetite of female rats was enhanced by gonadectomy and was decreased in both male and female gonadectomized rats by treatment with estrogen. Estrogen increases the number of OT receptors in rat brain (de Kloet, Voorhuis, Boschma, & Elands, 1986), thus, these results were interpreted as evidence in favor of an inhibitory oxytocinergic system involved in the control of sodium appetite.

ICV administration of ANG II (Blackburn *et al.*, 1992a) or peripheral administration of hypertonic NaCl (Giovannelli, Shiromani, Jirikowski, & Bloom, 1990) increases Fos expression in OT-expressing neurons in the PVN and SON. In addition, ICV administration of ANG II, or direct injection of ANG II into the SFO, increases secretion of OT (Ferguson & Wall, 1992; Keil, Rosella-Dampman, Emmert, Chee, & Summy-Long, 1984). Thus, brain angiotensinergic mechanisms subserving thirst can influence sodium homeostasis by influencing central oxytocinergic pathways, involved in the inhibition of sodium appetite, and peripheral OT secretion, involved in the stimulation of sodium excretion.

Somatostatin. SOM, a 14- or 28-amino acid peptide, was originally isolated from the ovine hypothalamus (Brazeau *et al.*, 1973). Somatostatinergic neurons (Crowley & Terry, 1980; Krisch & Leonhardt, 1980) and SOM binding sites (Leroux & Pelletier, 1984; Patel, Baquiran, Srikant, & Posner, 1986) have been identified in the circumventricular organs (CVOs) as well as other areas implicated in sodium appetite. SOM has been identified in pathways connecting the PBN to the BNST (Moga, Saper, & Gray, 1989) and amygdala (Moga & Gray, 1985) as well as in pathways connecting the parvocellular PVN to the dorsal vagal complex (Swanson & Sawchenko, 1980).

ICV infusion of SOM-28 decreases the sodium intake of sodium-depleted sheep (Weisinger, Blair-West, Denton, & Tarjan, 1991) and completely blocks the enhanced sodium intake caused by lowered cerebral sodium in sodium-depleted sheep. Thus, SOM-28 appears to act centrally as an inhibitory factor impinging on the central mechanism controlling the initiation of sodium intake in sodium-depleted sheep.

SOM-28 does not influence the decreased sodium intake of the sodium-depleted sheep infused with hypertonic NaCl, either systemically or ICV. The failure of SOM to act additively with the elevated CSF and ECF [Na^+] to further reduce the sodium intake of sheep is consistent with the proposition that hypertonic NaCl maximally activates the somatostatinergic system. As noted earlier, ICV administration of losartan reduced the osmotic inhibition of sodium intake suggesting a role for brain angiotensinergic mechanisms (Weisinger *et al.*, 2001).

At present, there is no direct evidence that brain ANG II can stimulate SOM release. However, there is evidence that ANG II acting via AT1 receptors can stimulate the formation and release of dopamine from neurons in the nigrostriatal pathway (Mendelsohn, Jenkins, & Berkovic, 1993) and ARC (Johren, Sanvitto, Egidy, & Saavedra, 1997) and that dopamine injected into the striatum stimulates the release of SOM (Asan, 1997). Thus, at least in the striatum, ANG II can stimulate dopamine release and dopamine can stimulate somatostatinergic transmission. Furthermore, somatostatinergic neurons in the amygdala are innervated by dopaminergic afferents (Asan, 1997). Although clearly speculative, it is conceivable that osmotic stimulation of angiotensinergic mechanisms in the thirst system would stimulate water intake and somatostatinergic mechanisms that inhibit sodium appetite.

Atrial Natriuretic Peptide. ANP (Kawata *et al.*, 1985; Saper *et al.*, 1985), a 28-amino acid peptide, and its receptors (Mendelsohn, Allen, Chai, Sexton, & Figdor, 1987) are distributed widely throughout the brain including many of the areas thought to be involved in body fluid homeostasis (e.g., lamina terminalis, SON, PVN, and septum). Indeed, many of the brain areas that contain ANG II terminals and/or ANG II receptors also contain ANP terminals and/or ANP receptors (Mendelsohn *et al.*, 1987).

In rats, ICV administration of ANP decreases sodium intake caused by central administration of ANG II (Ehrlich & Fitts, 1990; McCann, Gutkowska, Franci, Favaretto, & Antunes-Rodrigues, 1994) and sodium depletion (Stellar & Epstein, 1991). ICV administration of ANP decreased the sodium intake of sodium-depleted rabbits, as well as both sodium and water intake of sheep infused ICV with ANG II (Weisinger, Blair-West, Denton, & Tarjan, 1992). At present, there is no evidence that ANP can influence the sodium intake of sodium-depleted sheep (Parkes, Coghlan, Weisinger, & Scoggins, 1988).

Tachykinins. The TK peptides are biologically active peptides sharing the common carboxy (COOH) terminal sequence: Phe-X-Gly-Leu-Met-NH2 (Saria, 1999). Four TKs, substance P (11 amino acids), Neurokinins A and B (10 amino acids), and Neuropeptide K (36 amino acids), and three receptors (NK1, 2, and 3) have been identified in the mammalian brain. These peptides and their receptors are widely distributed in the brain including those areas involved in sodium appetite and thirst (Dam, Martinelli, & Quirion, 1990; Larsen, Jessop, Chowdrey, Mikkelsen, & Lightman, 1992).

Central administration of TKs reduced sodium appetite. ICV administration of NK3 receptor agonists decreased sodium depletion-induced sodium intake in sheep, rabbits, rats, and cows (Flynn, Smith, & Bieber, 1999; Tarjan *et al.*, 1990; Weisinger & Burns, 1999). ICV administration of NK1 or NK2 receptor agonists, at molecular doses higher than that needed with NK3 agonists, also decreased sodium intake caused by sodium depletion, DOC, or adrenalectomy in rats (Massi & Epstein, 1989; Massi, Polidori, Perfumi, Gentili, & de Caro, 1991). ICV administration of Neurokinin A (Massi & Epstein, 1989) or kassinin (Massi, Perfumi, de Caro, & Epstein, 1988) decreased DOC-induced sodium appetite as well as sodium appetite induced by other stimuli.

Administration of NK3 receptor agonists into the MeA (Massi, Gentili, Perfumi, de Caro, & Schulkin, 1990) or BNST (Pompei, Tayebaty, De Caro, Schulkin, & Massi, 1991) decreased sodium intake caused by sodium depletion. Furthermore, administration into the MeA also decreased sodium appetite caused by ICV administration of renin but not that caused by DOC. This result suggests that the MeA is involved in ANG II and sodium depletion-induced sodium appetite and that activation of NK3 receptors, in this area, inhibits the ANG II-component of physiological sodium appetite. Paradoxically, lesion of the MeA in rats interferes with only steroid-induced sodium appetite.

Other evidence suggesting that these endogenous TKs are involved in the expression of sodium appetite is that the genes encoding for the TKs are decreased in the amygdala and/or the BNST in sodium-depleted animals (Pompei, Lucas, Angeletti, Massi, & McEwen, 1997).

Substance P is another TK that inhibits sodium intake. ANG II (Barnes, Diz, & Ferrario, 1991; Diz, Westwood, Bosch, Ganten, & Ferrario, 1998) as well as osmotic stimulation increases levels of substance P and/or substance P binding sites as well as neurokinin A in various brain regions including the PVN, SON, and SFO (Larsen, Jessop, Lightman, & Chowdrey, 1993). Increased substance P immunoreactivity in the LH and ARC has been shown to occur with chronic osmotic stimulation, and substance P-containing neurons are known to project to the PVN (Larsen *et al.*, 1993). Thus, it appears that several TKs, including substance P, could be involved in the inhibitory control of sodium intake.

Brain Structures involved in Sodium Depletion-Induced Sodium Appetite

SFO, MnPO, OVLT. As noted earlier, ANG II does not readily cross the blood–brain barrier (Epstein, 1981; Reid, 1984; Schelling *et al.*, 1976). Thus, the role of circulating ANG II in salt appetite most likely is mediated by its influence on brain structures lacking a blood–brain barrier, such as the CVOs: SFO, OVLT, and AP (Krisch *et al.*, 1978). The sodium appetite of sodium-depleted rats is decreased or abolished by lesion of the SFO (Thunhorst, Beltz, & Johnson, 1999; Thunhorst, Ehrlich, & Simpson, 1990; Weisinger *et al.*, 1990b), the OVLT (Chiaraviglio, 1984a; Fitts *et al.*, 1990), or the AV3V (De Luca *et al.*, 1992; Vivas & Chiaraviglio, 1992). The evidence is consistent with sodium depletion-induced sodium appetite being mediated by the action of circulating ANG II on AT1 receptors in the SFO or OVLT.

Sodium appetite caused by furosemide and low dose of captopril is decreased in rats with lesion of the OVLT (Fitts *et al.*, 1990), but is unaltered in rats with lesion of the SFO (Thunhorst, Fitts, & Simpson, 1987; Weisinger *et al.*, 1990b). Furthermore, sodium intake induced by sodium depletion or low-dose captopril is

blocked by administration of antagonists of the ANG system into the OVLT but not by their administration into the SFO (Fitts & Masson, 1989, 1990). Since neural pathways connecting the SFO, OVLT, and MnPO are well documented (McKinley *et al.*, 1989), the explanation for this difference is not clear at present.

In contrast to rats, sheep with lesion of the AV3V have impaired osmotic thirst but do not have impaired sodium appetite induced by sodium depletion (Weisinger *et al.*, 1993b). The enhanced sodium-intake of sodium-depleted sheep induced by ICV infusion of hypertonic mannitol-CSF is umimpaired, as is the decreased sodium intake caused by intracarotid infusion of hypertonic NaCl (Weisinger *et al.*, 1993b). The results indicate that different brain areas are involved in the control of sodium appetite in sheep, compared with rats.

AP, NTS. The AP, located in the hindbrain, lacks a blood–brain barrier. The AP and the adjacent NTS receive neural afferents from arterial and cardiac baroreceptors (Ciriello, Hochstenbach, & Roder, 1994; Ferguson & Lowes, 1994) and sensory inputs from the abdominal area. Projections from the AP go to the PBN, PVN, DMV, and forebrain areas such as the SFO (Shapiro & Miselis, 1985; van der Kooy & Koda, 1983). Sodium appetite of sodium-deplete rats with lesion of the AP/medial NTS was not altered (Edwards, Beltz, Power, & Johnson, 1993). Sodium appetite of sodium-depleted rats with lesion of the rostral NTS, an area receiving taste inputs, however, was reduced (Grigson, Shimura, & Norgren, 1997). Sodium and water intake of hypovolemic rats with lesion of the medial NTS, an area receiving cardiac and arterial baroreceptor inputs, was not impaired (Schreihofer *et al.*, 1999).

BNST, Amygdala. Sodium depletion-induced sodium appetite is unaltered in rats with lesion of the MeA (Black, Weingarten, Epstein, Maki, & Schulkin, 1992; Nitabach *et al.*, 1989; Schulkin *et al.*, 1989; Zhang *et al.*, 1993).

The CeA receives synaptic input from several sources, especially the PBN, BNST, and the hypothalamus (Price, Russchen, & Amaral, 1987). Rats with lesion of the CeA or BNST show deficits in sodium appetite induced by sodium depletion (Galaverna *et al.*, 1992; Reilly *et al.*, 1994; Zardetto-Smith, Beltz, & Johnson, 1994). The deficit appears to be due to disruption of consummatory behavior and not to a disruption of gustatory function (Galaverna *et al.*, 1993; Seeley, Galaverna, Schulkin, Epstein, & Grill, 1993).

It has been argued that the MeA is involved in steroid-induced sodium appetite while the CeA and BNST are involved in both steroid- and sodium depletion-induced sodium appetite (Schulkin, 1991).

Other Brain Structures. Lesion of the PBN decreased sodium depletion-induced sodium appetite (Scalera, Spector, & Norgren, 1995; Spector, Scalera, Grill, & Norgren, 1995). This result is interesting in that blocking serotonergic output from the PBN enhances sodium intake in response to a variety of stimuli (Menani, Thunhorst, & Johnson, 1996; Menani *et al.*, 1998b). Evidence also shows that sodium depletion-induced sodium appetite is decreased in rats with lesion of the LH (Wolf, 1967; Wolf & Quartermain, 1967), anterior hypothalamus (Mercer, Mogenson, & Paquette, 1978), anterior medial hypothalamus (Covian & Antunes-Rodrigues, 1963), or septal nuclei (Chiaraviglio, 1969).

Changes in Need-Free Sodium Intake with Lesion. The results of the studies in which sodium depletion-induced sodium appetite is altered by lesion

must be viewed cautiously because in some instances, basal, need-free, or hedonic sodium intake is also altered, although not necessarily in the same direction. For example, basal sodium intake increased in rats with a lesion of the AP (Contreras & Stetson, 1981; Curtis, Huang, Sved, Verbalis, & Stricker, 1999; Watson, 1986) or AP/caudal medial NTS (Hyde & Miselis, 1984). The increase in sodium intake was not mediated by ANG II (Watson, 1986) or urinary sodium loss (Curtis *et al.*, 1999). Lesion of the septal nuclei (Chiaraviglio, 1969; Saad, de Arruda Camargo, Antunes-Rodrigues, & Simoes, 1998) and the ventral MnPO (Gardiner, Jolley, Vagnucci, & Stricker, 1986) also increased need-free sodium intake. On the other hand, need-free sodium intake decreased in rats with lesion of the MeA (Nitabach *et al.*, 1989), CeA, BNST (Galaverna *et al.*, 1992; Reilly *et al.*, 1994; Zardetto-Smith *et al.*, 1994), anterior hypothalamus (Mercer *et al.*, 1978), PBN (Hill & Almi, 1983), or AV3V (Bealer & Johnson, 1979; De Luca *et al.*, 1992).

Evidence from electrical stimulation studies suggests a role for hypothalamic/limbic brain regions in salt appetite of sheep (McKenzie & Denton, 1974). In rats, stimulation of the pre-limbic area in rats enhanced sodium intake caused by sodium depletion while stimulation of the anterior cingulate decreased this effect (Chiaraviglio, 1984b).

Brain Structures Activated by Sodium Depletion

In intact rats, sodium depletion as a result of furosemide treatment or intraperitoneal dialysis results in activation in the SFO, MnPO, OVLT, and, in some reports, AP, NTS, and LPBN (Franchini & Vivas, 1999; Han & Rowland, 1995; Houpt *et al.*, 1998; Lane *et al.*, 1997; Pastuskovas & Vivas, 1997; Vivas *et al.*, 1995). Fos is observed in the midline area of the OVLT and central region of the SFO (Han & Rowland, 1995). Sodium depletion-induced activation of the SFO, MnPO, and OVLT is blocked by systemic administration of captopril or ZD7155 but not by ICV administration of sarile (Pastuskovas & Vivas, 1997; Vivas *et al.*, 1995; Weisinger & Denton, 2000; Weisinger *et al.*, 2000b). Fos does not appear to be present in SON, PVN, or CeA (Han & Rowland, 1995) in sodium-depleted animals.

Water deprivation causes activation of the SFO, MnPO, OVLT, SON, and PVN (De Luca *et al.*, 2002; McKinley, Hards, & Oldfield, 1994; Xu, Lane, Zhu, & Herbert, 1997), brain structures also activated by furosemide or peritoneal dialysis. A high proportion (30%) of the Fos-labeled neurons in the MnPO and OVLT project to the SON (McKinley *et al.*, 1994). In an experiment in which animals were allowed to drink water after 24 hr of water deprivation, but prior to sodium access, Fos levels were observed to be reduced in the OVLT, MnPO, and SON; however, they were maintained in the SFO, suggesting that the increase in sodium intake that occurs subsequent to water deprivation is mediated by activity in the SFO (De Luca *et al.*, 2002).

Increased Fos activity is observed (Weisinger *et al.*, 2000b) in the SFO and OVLT in ADX rats with or without access to sodium during the previous 24 hr, and in the MnPO when sodium is not available (Weisinger *et al.*, 2000b). Activity in the SFO and OVLT is decreased by DOC in ADX rats only when sodium is available, suggesting that the activity of the SFO and OVLT is increased when there is a sodium deficit and restored to basal activity with the restoration of sodium balance. In contrast to the conclusions of McEwen *et al.* (1986) and Sakai *et al.* (1986), activity in the SFO and OVLT does not appear to be dependent on just the presence or absence of DOC. In addition, neural activity in the SFO of ADX or intact

sodium-depleted rats is decreased by peripheral administration of ZD7155 (Weisinger *et al.*, 2000b), consistent with a role for ANG II.

Summary

The controversy regarding the relative contributions of peripheral and central ANG II to sodium depletion-induced sodium appetite is yet to be resolved. Indeed, as shown in Figure 3, both peripheral and central ANG II are likely to have a role in sodium depletion-induced sodium appetite. As sodium depletion increases, both peripheral and, presumably, central levels of ANG II increase. Sodium appetite would then be influenced by peripheral ANG II acting on AT1 receptors in CVOs, and by brain ANG II acting at AT1 receptors in brain structures with a blood–brain barrier (e.g., the MnPO and amygdala) or at the ventricular surface of the CVOs, brain areas without a blood–brain barrier but with a CSF–brain barrier.

There is good evidence that peripheral ANG II is involved in sodium depletion-induced sodium appetite. In sheep, cows, rabbits, rats, and mice, sodium appetite of the captopril-treated sodium-depleted animal is reduced and can be restored by IV administration of ANG II (a peptide that does not readily cross the blood–brain barrier). The appetite of the captopril-treated sodium-depleted animal is not restored by preventing the captopril-induced decrease in blood pressure with phenylephrine (rats). In rats, lesion of the SFO or OVLT decreases sodium depletion-induced sodium appetite. In sheep, however, other brain areas must be involved or recruited since lesion of the AV3V does not interfere with sodium depletion-induced sodium appetite.

The CVO neurons with the AT1 receptors, activated by peripheral ANG II, may or may not use ANG II as a neurotransmitter. Evidence that sodium depletion-induced sodium appetite is decreased by ICV administration of an ANG II receptor antagonist (in rats, pigeons, and baboons) is consistent with an angiotensinergic system of peripheral or central origin. Evidence that sodium depletion-induced sodium appetite is not decreased by ICV administration of an ANG II receptor antagonist (in sheep and cows) is consistent with the terminals of the peripheral ANG II-activated CVO neurons being non-angiotensinergic or with the involvement of a non-angiotensinergic system of central origin. However, the possibility that a brain ANG system is involved in sodium appetite in sheep and cows, but is not accessed by the centrally administered antagonists, cannot be dismissed. Furthermore, the inability of ICV administration of antagonists to ANG II to decrease sodium appetite in sheep and cows may be explained by a role of ANG II in both excitatory and inhibitory mechanisms influencing sodium appetite.

Adrenocorticosteroid hormones also may contribute to sodium appetite, either directly or indirectly, for example, by enhancing the central actions of ANG II. Although the mechanisms by which these hormones influence sodium appetite are still to be defined, it would appear that the amygdala is involved.

Evidence obtained in experiments in sheep and cows suggests that sodium appetite also is controlled by cerebral sodium sensors. These sensors are located some distance from the ventricle, are spatially different from those subserving thirst (Park, Denton, McKinley, Pennington, & Weisinger, 1989; Weisinger *et al.*, 1993b), and may utilize ANG II as a neurotransmitter. In addition, cerebral sodium sensors may indirectly contribute to the control of sodium appetite by influencing aldosterone secretion. Thus far, there is no evidence that changes in cerebral sodium

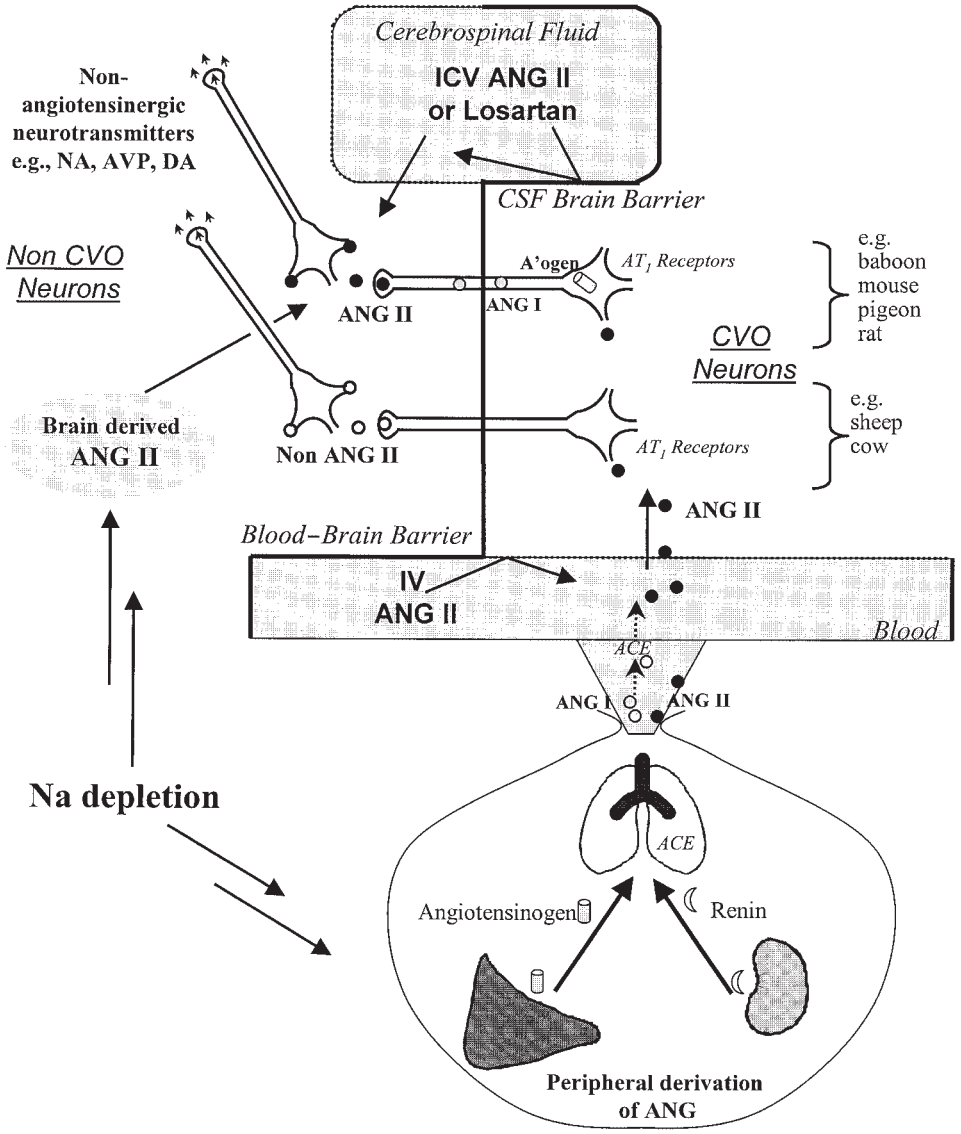

Figure 3. Illustration of mechanisms involved in sodium appetite induced by sodium depletion. Sodium depletion-induced rise in blood angiotensin II activate AT1 receptors in circumventricular organs (CVOs), which lack a blood–brain barrier. Subsequently, angiotensinergic and non-angiotensinergic pathways, behind the blood–brain barrier, would be activated. Similarly, ANG II infused into the circulation (IV ANG II) would affect neurons in the CVOs. Sodium depletion-induced changes in brain ANG II activate angiotensinergic pathways behind the blood–brain barrier. Similarly, ANG II or antagonists of the renin–angiotensin system administered into the brain ventricles would activate or inhibit angiotensinergic pathways, respectively, behind the blood–brain barrier.

can influence the sodium intake of baboons, rabbits, or mice, and the evidence obtained in rats is mixed.

Inhibitory factors are important to the control of sodium appetite. OT and SOM-28 are neuropeptides with some specificity with regard to sodium intake. The TKs and ANP, on the other hand, appear to have an inhibitory role in both sodium appetite and thirst.

Figure 4 presents a schematic of the HPA axis. The perception of a threat (stress) causes increased release of CRF/urocortin (UCN), which in turn causes release of ACTH from the pituitary. ACTH acts on its receptors in the adrenal gland to cause the release of adrenocortical hormones (Axelrod & Reisine, 1984; Rivier & Vale, 1983b).

STRESS-INDUCED SODIUM APPETITE

One of the first demonstrations of stress-induced sodium appetite was made while attempting to fit wild rabbits with jackets designed for carrying small infusion pumps. This restraint of movement caused the rabbits to become agitated, and over the course of several days sodium intake was increased from <1 mmol to 15 mmol per day (Denton *et al.*, 1984a). Immobilized or restrained mice also manifest an increase in sodium appetite (Denton *et al.*, 1999; Kuta, Bryant, Zabik, & Yim, 1984).

An "intruder" stress caused increased sodium intake in SHR, WKY, and hybrid cross rats (Bourjeili, Turner, Stinner, & Ely, 1995; Ely, Thoren, Wiegand, & Folkow, 1987). Clonidine or reserpine blocked the increase in sodium intake, suggesting that the increase in intake was due to stimulation of the SNS. The mechanism by which stimulation of the SNS stimulates sodium appetite is not known, but is possibly mediated by its action on the RAS. Crowding, a type of imposed social stress, has been shown to cause increased intake of hypertonic NaCl solution (Weisinger & Denton, 2000).

ADRENOCORTICOTROPHIC HORMONE (ACTH)

The hormonal consequences of stress (primarily the release of ACTH and the adrenocorticosteroids) elicit sodium appetite. The influence of the adrenocorticosteroids has already been discussed and the influence of ACTH and CRF/UCN will be discussed here.

Peripheral administration of ACTH causes a large increase in sodium intake in several species including sheep (Weisinger *et al.*, 1980), rabbits (Blaine *et al.*, 1975), rats (Weisinger, Denton, McKinley, & Nelson, 1978), and mice (Denton *et al.*, 1999). The appetite for sodium caused by ACTH is entirely (Weisinger *et al.*, 1978, 1980) or partially (Blaine *et al.*, 1975) eliminated by adrenalectomy (i.e., it is entirely or partially due to adrenal hormones). In all of the animals tested with a choice of hypertonic solutions of NaCl, KCl, $CaCl_2$, and $MgCl_2$, the appetite caused by ACTH administration has been shown to be predominantly for NaCl solution. In intact sheep, a large increase in sodium intake occurs after 2–3 days of ACTH administration (Weisinger *et al.*, 1980). In ADX sheep, administration of a cocktail of hormones that are secreted from the adrenal gland during stimulation with ACTH (i.e., aldosterone, corticosterone, cortisol, 11-deoxycortisol, and DOC) causes an increase in sodium appetite which is similar to that induced by ACTH in intact animals. In intact rabbits, concurrent administration of cortisol and corticosterone, in doses that increase plasma levels to those observed in ACTH-treated animals, increases appetite by about half as much as that caused by ACTH. ACTH-induced sodium appetite was clearly demonstrated in ADX rabbits. These rabbits were

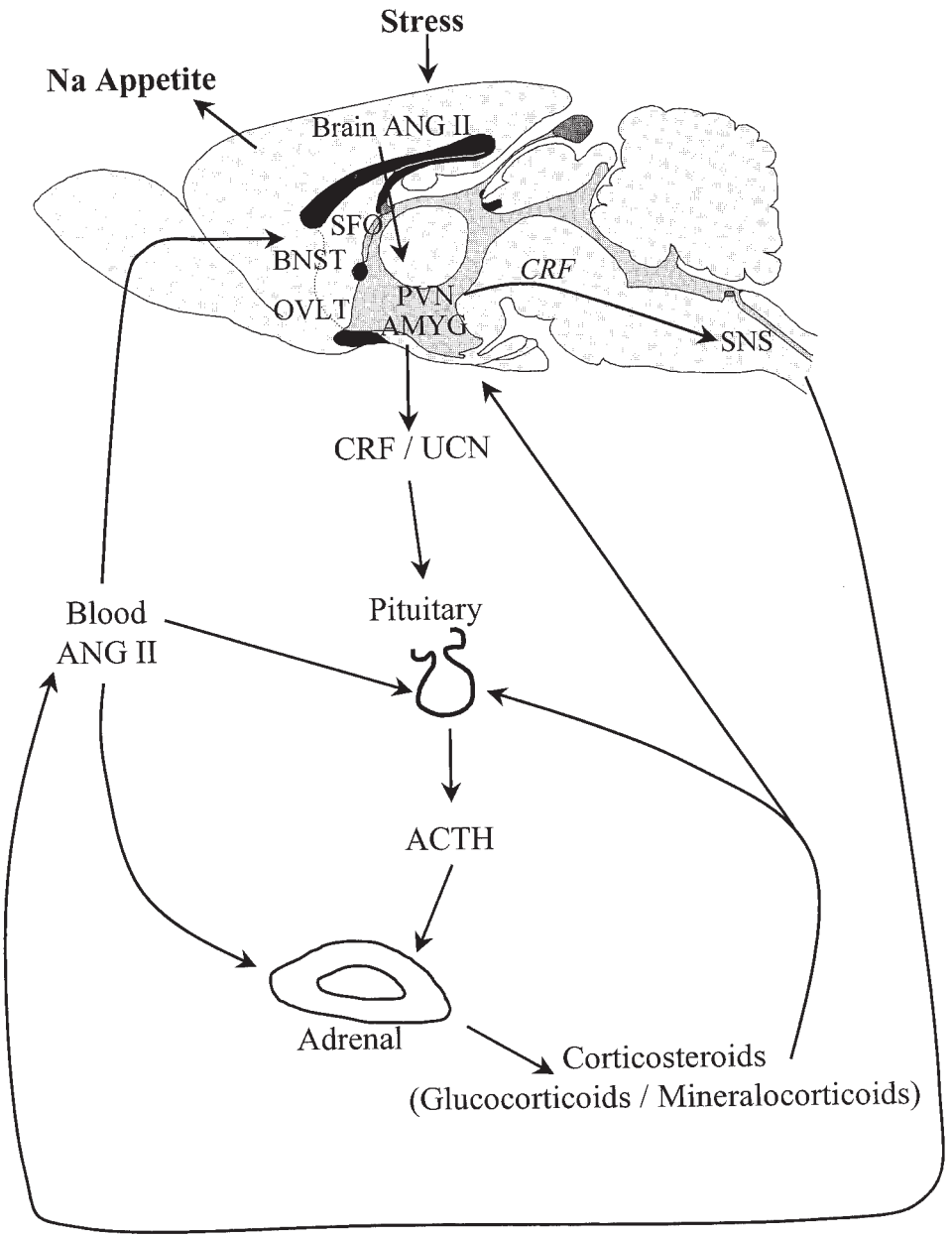

Figure 4. Illustration of mechanisms involved in sodium appetite induced by stress. Stress-induced rise in brain ANG II activates angiotensinergic pathways behind the blood–brain barrier, as shown in Figure 3, and also stimulates increased brain CRF/UCN. Increased CRF/UCN stimulates ACTH release from the pituitary, which causes the release of adrenocorticosteroid hormones. These adrenocorticosteroid hormones stimulate sodium appetite directly or via their facilitating influence on the actions of ANG II. Stress, via brain ANG II and CRF/UCN, also stimulates the SNS, which causes increased blood ANG II and thereby stimulates sodium appetite, as shown in Figure 3.

maintained in good health with a combined mineralocorticoid–glucocorticoid treatment. The mineralocorticoid treatment was such that urinary sodium loss was minimized while the glucocorticoid treatment was such that blood ACTH was maintained at a basal level (i.e., blood glucocorticoids have a negative feedback control of ACTH secretion). When glucocorticoid treatment was reduced such that blood ACTH was no longer maintained at basal level, sodium intake was increased (Denton *et al.*, 1984a). This evidence is consistent with the ability of ACTH to stimulate sodium appetite independent of steroid hormones, and is not due to sodium loss.

In rats, by the fifth day of treatment, 24 hr sodium intake (and output) was approximately equal to the rat's total body sodium (Weisinger *et al.*, 1978). ACTH also increased the sodium intake of both the SHR and WKY rats. In SHR, the increase was in addition to their already high sodium intake (Ely *et al.*, 1987). Intramuscular injections of porcine ACTH or synthetic ACTH for 5 days in baboons did not affect daily intake of NaCl intake although the doses were sufficient to increase cortisol secretion (Shade *et al.*, 2002b). Thus, it appears that one of the primary hormones associated with stress might not stimulate sodium appetite in primates.

Corticotrophin-Releasing Factor (CRF)/Urocortin (UCN)

CRF, a 41-amino acid peptide, is expressed in neurons or terminals throughout the limbic system (e.g., hypothalamus and amygdala) as well as in systems associated with autonomic function (e.g., PBN, locus coeruleus, DMV). CRF is involved in the initiation of the behavioral and physiological responses to stress. UCN, a 41-amino acid peptide, has a 45% homology with CRF and binds with high affinity to CRF receptors. UCN is expressed in many of the same brain areas as CRF, including those involved in body fluid and electrolyte homeostasis, and it may contribute to responses previously attributed to CRF (Spina *et al.*, 1996; Vaughan *et al.*, 1995; Weisinger *et al.*, 2000a).

ICV administration of ovine CRF increased sodium intake in rabbits. In addition, the ICV infusion caused a sustained increase in both cortisol and corticosterone (Tarjan & Denton, 1991; Tarjan, Denton, Ferraro, & Weisinger, 1992; Tarjan, Denton, & Weisinger, 1991). In recent experiments in sheep, however, prolonged ICV or IV administration of CRF or UCN failed to stimulate salt appetite although secretion of ACTH was increased (Weisinger *et al.*, 2000a). Prolonged ICV infusions of CRF and UCN decreased food intake and need-free salt intake in sheep (Weisinger *et al.*, 2000a), mice (Sinnayah *et al.*, 2003), and baboons (Shade *et al.*, 2002b). The decrease in sodium intake in baboons was transient but the decrease in food intake was sustained throughout the infusion period.

Administration of CRF/UCN would be expected to increase intake of sodium due to their stimulatory influence on secretion of ACTH (Weisinger *et al.*, 1980) or activity of the SNS (Bourjeili *et al.*, 1995; Thunhorst, Kirby, & Johnson, 1996). At present, the explanation for the inhibitory influence of CRF/UCN is not known. Presumably, the inhibition of sodium appetite is caused by the influence of CRF on systems other than those influencing ACTH secretion or sympathetic nervous activity, such as OT secretion (Bruhn, Sutton, Plotsky, & Vale, 1986; Olson, Drutarosky, Stricker, & Verbalis, 1991). Clearly the inhibitory actions of CRF and UCN need further exploration.

Stress-Induced Sodium Appetite: Role of ANG II. The increase in sodium intake of restrained mice is prevented by treatment with captopril (Kuta *et al.*, 1984). This finding suggests that the appetite is induced by activation of the RAS, which is known to be elevated in stressful situations (Aguilera, Kiss, & Luo, 1995; Castren & Saavedra, 1988). It has been suggested that both peripherally as well as centrally generated ANG II can stimulate the secretion of ACTH, the latter via its influence on CRF release from the PVN (Gaillard & Al-Damluji, 1987; Rivier & Vale, 1983a). Increased sodium intake in mice observed during cold stress also has been attributed to increased activity of the RAS (Dejima, Fukuda, Ichijoh, Takasaka, & Ohtsuka, 1996). In mice, the increase in sodium intake caused by ACTH is not altered by treatment with captopril (Denton *et al.*, 1999). The observation that stress-induced (Kuta *et al.*, 1984) but not ACTH-induced sodium intake is blocked by captopril suggests that stress-induced ANG II precedes the increase in ACTH.

Brain Structures Activated by Stress or the Hormones Associated with Stress

In crowded rats, Fos is increased in a number of brain areas including the SFO, PVN, and amygdala. Increased Fos in the PVN, SON, locus coeruleus, BNST, midbrain raphe, NTS, MeA, CeA, cortex, lateral septum, lateral preoptic area, anterior hypothalamus, LH, Barringtons nucleus, DMV, A1 and A5 noradrenergic fibers, BNST, and striatum has been reported in rats subjected to stress (Imaki, Shibasaki, Hotta, & Demura, 1993; Martinez, Phillips, & Herbert, 1998).

Summary

Overall, the evidence suggests that stress can cause sodium appetite. Although there is evidence in the rabbit for a direct effect of ACTH on sodium appetite, in rats and sheep the increased intake caused by ACTH appears to be mediated by its influence on the secretion of adrenocortical hormones. These adrenocorticosteroid hormones presumably act on receptors located in the amygdala and BNST to cause sodium appetite. At present, however, a role for adrenocortical hormones acting on receptors located in the lamina terminalis cannot be ruled out. An increase in activity of the SNS and the RAS caused by stress also may stimulate sodium intake. The failure of CRF/UCN to stimulate sodium appetite is paradoxical, and the mechanisms responsible remain to be discovered.

Sodium Appetite during Pregnancy and Lactation

Sodium intake is essential for reproductive success. In both rats and humans, plasma [Na^+] is decreased during pregnancy (Durr, Stamoutsos, & Lindheimer, 1981) due to the actions of the hormone, relaxin, on the osmotic threshold for thirst and AVP secretion (Weisinger, Burns, Eddie, & Wintour, 1993a). Retention of sodium is increased during pregnancy, with over 55% of the sodium found in the products of conception (Churchill, Bengele, & Alexander, 1980). The reproductive process is a powerful stimulus of specific sodium appetite in several species, including rats, rabbits, sheep, and mice (Denton & Nelson, 1971, 1980; McBurnie *et al.*, 1988; Richter & Barelare, 1938). Increased sodium intake occurs in pregnancy, and then it is greatly augmented above that elevated level during lactation. In rabbits,

the increase in Na appetite appears to be caused by the hormones associated with reproduction. ACTH seems to be part of the hormone complement necessary to induce the appetite. The very large increase in sodium intake during lactation is mimicked by treating rabbits with estradiol and progesterone initially, which produces a small increase in sodium appetite, and then treating the rabbits with prolactin, ACTH, and OT (Denton & Nelson, 1980). The increase in Na intake occurs even though the rabbits are maintained on a diet containing an adequate amount of sodium. Thus the behavior is primarily hormone-induced, as opposed to it being determined by sodium deficiency (caused by sequestration of sodium in the products of conception or by loss of sodium in milk). In rats, the increase in Na intake during pregnancy occurs in animals maintained on normal rat chow, a chow typically containing normal or high sodium. Furthermore, peripheral administration of losartan from the second to the nineteenth day of gestation in rats did not alter sodium intake. Thus, ANG II does not appear to have a role in the increase in water and sodium intake observed in pregnant rats (Butler, Pak, Midgely, & Nemati, 2002). This evidence is consistent with the Na appetite that occurs during pregnancy not being due to Na depletion. On the other hand, there is some evidence that the increase in Na intake that occurs during lactation may be due to Na depletion (Thiels, Verbalis, & Stricker, 1990).

Conclusions

ANG II has a clear role in sodium appetite caused by sodium depletion (see Figure 5). Depletion of body sodium causes increased production of ANG II. Subsequently, increased blood levels of ANG II stimulate the secretion of aldosterone. When the formation or action of ANG II is blocked, sodium appetite is decreased. The stimulation of sodium intake that occurs in sodium-depleted animals could be mediated in the CVOs located on the front wall of the third brain ventricle (e.g., SFO or OVLT) responsive to the high blood levels of ANG II. Sodium depletion could directly cause an increase in ANG II formed in the brain, and increased brain ANG II could be involved, possibly in synergy with the elevated blood levels of aldosterone. In addition, decreased cerebral [Na^+] may increase sodium intake by acting on sodium sensors or by increasing aldosterone secretion. In addition, ANG II acting as a neurotransmitter may have a role in these effects also. Reconciliation between these pathways and mechanisms will be challenging.

The data indicate that an increase in sodium intake occurs during stress and during pregnancy and lactation. In these situations, the sodium appetite appears to be determined by the hormones secreted under such conditions. Some of the hormones associated with the stress response (e.g., ACTH and adrenocortical hormones) could cause sodium appetite. Although there may be some exceptions, increased sodium intake appears to be mediated by the action of the adrenocortical hormones acting on receptors located in the amygdala and BNST. At present, however, a role for adrenocortical hormones acting on receptors located in the lamina terminalis cannot be ruled out. A stress-related increase in activity of the SNS and systemic ANG II also may stimulate sodium intake. The mechanism by which the SNS stimulates sodium appetite is unknown, but could be via ANG II.

In pregnancy and lactation, sodium appetite is stimulated. Interestingly, in rats, like humans, the plasma volume is maintained above normal level and plasma [Na^+] is maintained below normal level, yet intake of sodium in increased.

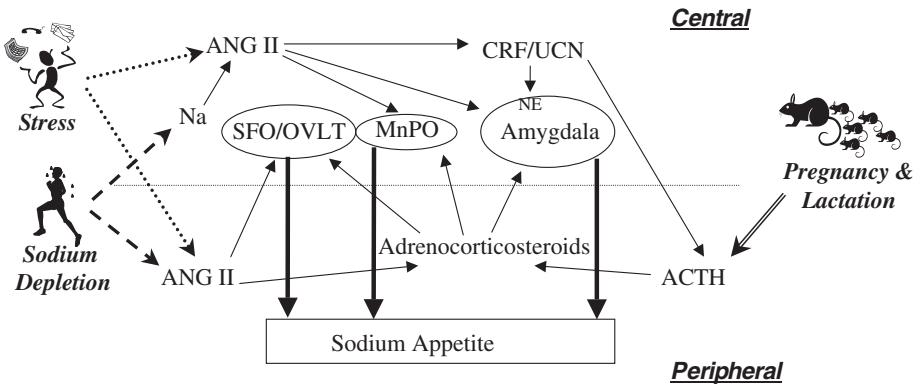

Figure 5. Schematic diagram of inter-relationship of factors stimulating salt appetite during sodium depletion, stress, and pregnancy. In response to sodium depletion, both central (possibly due to decreased cerebral Na) and blood levels of ANG II are increased. Sodium appetite in response to sodium depletion is due to stimulation by circulating ANG II of AT1 receptors in the neurons subserving sodium appetite, which are located in the CVOs and the action of adrenal hormones. The adrenal hormones act on neurons subserving sodium appetite located in brain areas with a blood–brain barrier (e.g., the MnPO and amygdala). The adrenal hormones also could act directly on neurons subserving sodium appetite or by enhancing the action of ANG II at the CVOs (e.g., by increasing the number of AT1 receptors).

In response to stress, increased brain ANG II could stimulate the release of CRF/UCN, which then stimulate sodium appetite by actions on brain SNS (adrenergic/noradrenergic systems; NE) to increase blood ANG II, as well as on ACTH. The latter effect increases sodium appetite directly, via unknown mechanisms, or by stimulation of the adrenocorticosteroids.

Finally, during pregnancy and lactation, ACTH levels are increased and could stimulate sodium appetite via mechanisms described above. Thus, sodium depletion, stress, pregnancy, and lactation could stimulate sodium appetite via common central mechanisms subserving sodium appetite.

This increase in sodium intake has been studied in the rabbit and appears to depend on the hormonal consequences of pregnancy and lactation including ACTH. Thus, the sodium appetite could be generated, at least in part, by adrenocortical hormones utilizing mechanisms thought to contribute to the sodium appetite observed during stress or sodium depletion.

The anatomical organization of the neural systems subserving sodium appetite is scarcely defined. While several hormones and neurotransmitters involved in sodium appetite have been identified (e.g., ANG II, ACTH, ANP, SOM, OT), their roles are still to be fully elucidated.

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